

**Phenotypic and genotypic characterization of Extended
Spectrum Beta-Lactamases in *Escherichia coli* and *Klebsiella
pneumoniae* isolated across Karnataka**



By

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Doctor of Philosophy (Ph.D.)

(faculty of medicine)

in Microbiology

Under the guidance of

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February 2015**

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ABBREVIATIONS

Bla: β -Lactamase coding gene	FASTA format: text-based format for representing either nucleotide sequences or peptide sequences
APB: Amino-phenylboronic acid	HP: Himachal Pradesh
ATCC: American Type Culture Collection	IPD: Inhibitor-potentiated disk diffusion
BLAST: Basic Local Alignment Search Tool	IRT: Inhibitor-Resistant TEMs
BEL: Bellary	KOL: Kolar
CLSI: Clinical and Laboratory Standards Institute	KPC: <i>Klebsiella pneumoniae</i> carbapenemase
CMT: Complex Mutant Types	LCR: Ligase chain reaction
CSF: Cerebrospinal fluid	MALDI-TOF MS: Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry
CTX-M: Active on Cefotaxime, First Isolated at Munich	MBL: Metallo-Beta-Lactamase
DVG: Davangere	MIC: Minimal Inhibitory Concentration
DDST: Double Disk Synergy Test	MNG: Mangalore
DDDT: Double Disk Diffusion test	MYSTIC: Meropenem Yearly Susceptibility Test Information Collection
DNA: Deoxyribonucleic acid	NCBI: National Center for Biotechnology Information
dNTP: deoxynucleotide triphosphates	Omp: "Outer Membrane Protein"
DWD: Dharwad	OXA: Active on Oxacillin
ESBL: Extended-Spectrum β -Lactamase	PBP: Penicillin-Binding Protein
ESBL-EC: ESBL producing <i>E. coli</i>	PCR: Polymerase Chain Reaction
ESBL-KP: ESBL producing <i>K. pneumoniae</i>	PCR-RFLP: Restriction Fragment Length Polymorphism - Polymerase Chain Reaction
ESC: Extended spectrum cephalosporin	PCR-SSCP: Single Strand Conformation Polymorphism - Polymerase Chain
EUCAST: European Committee on Antimicrobial Susceptibility Testing	
ET tube: Endotracheal tube	

Reaction

PCT: Phenotypic Confirmatory Test

PER: Pseudomonas Extended Resistant

RSI-PCR: Restriction Site Insertion-PCR

SHV: Sulphydryl Reagent Variable

SDD: susceptible-dose dependent

SDN: Serine130, Aspartic Acid131 and Asparagine132

SMART: Study for Monitoring Antimicrobial Resistance Trends

ST: Sequence Type

TBE: Tris/Borate/EDTA, is a buffer solution

TEM: Named After Patient Temoniera

TEST: Tigecycline Evaluation and Surveillance Trial

UK: United Kingdom

USA: United States of America

UTI: Urinary Tract Infection

Antibiotics:

AO: Aztreonam

CTX: Cefotaxime

CTR: Ceftriaxone

CAZ: Ceftazidime

CAC: Ceftazidime + clavulanic acid

CEC: Cefotaxime+clavulanic acid

CN: Cefoxitin

CPM: Cefepime

CFC: Cefepime+clavulanic acid

CPT: Cefepime+tazobactam

CMC: cefixime+clavulanic acid

CA: clavulanic acid

IMP: Imipenem

Amino Acid Abbreviations:

Ala Alanine

Arg Arginine

Asn Asparagine

Asp Aspartic Acid

Cys Cysteine

Gln Glutamine

Glu Glutamic Acid

Gly Glycine

His Histidine

Ile Isoleucine

Leu Leucine

Lys Lysine

Met Methionine

Phe Phenylalanine

Pro Proline

Ser Serine

Thr Threonine

Trp Tryptophan

Tyr Tyrosine

Val Valine

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Abstract

Extended-Spectrum Beta-Lactamases (ESBL) confer resistance to oxyimino-cephalosporins and monobactams and treatment failures have been associated with infections caused by ESBL producers.

A multi-centric study involving five centers across Karnataka state was undertaken to determine the prevalence of ESBL producing clinical isolates of *E. coli* and *K. pneumoniae* and to characterize their ESBL genes. One thousand clinical isolates each of *E. coli* and *K. pneumoniae* isolates were collected from Mangalore, Dharwad, Bellary, Davangere and Kolar. They were tested for ESBL production by phenotypic methods as recommended by CLSI. ESBL positive isolates were tested for susceptibility to few beta-lactams and beta-lactamase inhibitor combinations. Two hundred isolates each of ESBL producing *E. coli* and *K. pneumoniae* were studied for *bla*TEM, *bla*SHV and *bla*CTX-M genes.

By phenotypic tests, 61.5% *E. coli* and 46.2% *K. pneumoniae* were identified as ESBL producers across Karnataka. The prevalence of ESBL producing *E. coli* varied across the centers but the distribution of *K. pneumoniae* was fairly uniform. All ESBL producers were resistant to ceftazidime, cefotaxime and ceftazidime. Resistance of ESBL producers was higher towards cefotaxime and ceftriaxone than ceftazidime and ceftazidime resistance was higher among *K. pneumoniae* than *E. coli*. Cefepime+tazobactam and imipenem exhibited good in-vitro activity against ESBL producers.

Of the 725 *bla* genes characterized from 400 isolates, only 372 (51.3%) were identified as ESBL types. ESBL genes were detected in 94.5% of *E. coli* and 86% of *K. pneumoniae* isolates. Five different CTX-M types, 17 SHV types and 2 TEM types were detected among ESBL producers. All of the *bla*CTX-M, only 29.4% of the

*bla*SHV and none of the *bla*TEM types were confirmed as ESBL types. Although CTX-M-15 ESBLs predominated among both *E. coli* and *K. pneumoniae*, significantly higher numbers of *E. coli* possessed *bla*CTX-M-15 gene. SHV type ESBLs detected were SHV-2a, -12, -18, -27 and -31. CTX-M-155, -156, -157; SHV-185, -186 and TEM-219 were the novel beta-lactamases discovered in this study. ESBL producing *K. pneumoniae* harboured higher numbers of *bla*TEM and *bla*SHV than *E. coli*. Multiple *bla* types were common among *K. pneumoniae* whereas single *bla* types were common among *E. coli*.

For identification of ESBLs, complete sequencing of *bla* gene is necessary. Further studies may be performed to characterize the novel *bla* types. Regional and national-level multi-centric surveillance programs are required to track the evolution and spread of beta-lactamase mediated resistance.

Introduction

Antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae* pose problems in the treatment of infections caused by them. A family of bacterial enzymes called beta-lactamases confer resistance to beta-lactam antibiotics. Extended-spectrum beta-lactamases (ESBLs) are variants of beta-lactamases that confer resistance to the extended-spectrum cephalosporin antibiotics such as cefotaxime, ceftazidime, ceftriaxone and monobactams such as aztreonam. These enzymes are derived either from broad-spectrum beta-lactamases following mutations in the beta-lactamase genes or have been derived from chromosomal beta-lactamases of certain environmental bacteria. Being present on plasmids and aided by transferable genetic elements, these genes have spread within and across the bacterial species. Once confined to the hospital environment, ESBL producing bacteria are now found in healthy individuals in the community as well as the environment. Carriage of ESBL producing bacteria is a risk factor for acquisition of infection in future. Expression of ESBLs among *E. coli* and *K. pneumoniae* isolates is a big concern as they are responsible for resistance to the commonly used oxymino-cephalosporins, thus contributing to treatment failure.

Accurate detection of ESBL types helps in selection of appropriate antibiotics, institution of antibiotic usage policies as well as in tracking trends and evolution of beta-lactamases. Estimation of prevalence and distribution of ESBL producers helps in determining the extent of ESBL mediated drug resistance. Detection and identification of ESBL types by molecular techniques is important in understanding their epidemiology. Global incidence of ESBL production among Enterobacteriaceae members is on the rise ever since their discovery. In the absence of regional or national-level surveillance programs, accurate prevalence rates of ESBL producing *E. coli* or

K. pneumoniae is currently unavailable. Isolated reports that vary in research methodologies can only provide sketchy data.

There are several published reports on the detection of ESBLs among Enterobacteriaceae from India but not many among them have characterized ESBL genes by molecular biological techniques. Phenotypic detection of ESBLs are not complete as these methods suffer from false positive and false negative results. Therefore, it becomes essential to undertake molecular techniques in the detection of ESBLs. Although detection of beta-lactamase genes by PCR is a sensitive method of detection, it may not be entirely specific as some of them may not code for the ESBL phenotype at all. Hence, PCR amplification of the beta-lactamase gene must be followed by sequencing the entire gene to accurately identify the ESBL type.

Only a study that is conducted across geographically distributed locations in a region, which is uniform in sampling, time scale and methodology would give a better picture on the prevalence of ESBL producers. Such studies from Karnataka are currently unavailable, hence this study was undertaken to detect the prevalence of ESBL producing clinical isolates of *E. coli* and *K. pneumoniae* across five different centres and to characterize their beta-lactamase (*bla*) genes by molecular techniques, including gene sequencing.

*Review of
Literature*

1. Beta-lactam antibiotics

1. 1 Discovery of beta-lactam antibiotics:

Globally bacterial infectious diseases have been responsible for a very large number of deaths and mankind has been in a constant state of conflict with bacteria since time immemorial for the diseases they produce.

The earlier attempts to cure bacterial infections involved the use of plant extracts or crude preparations of heavy metals. One of the earliest synthetic compounds with specific antibacterial activity was sulphonamide. Antibiotic was discovered in 1928 when Alexander Fleming published his famous discovery of Staphylococcal colonies being inhibited by a contaminating *Penicillium* mould. Upon subsequent experiments on selected bacterial cultures, he noted that the extract of fungal culture possessed “inhibitory, bactericidal and bacteriolytic properties” against most gram positive bacteria such *Staphylococcus aureus*, *Bacillus anthracis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Corynebacterium diphtheria* but not against Gram negative bacteria such *Escherichia coli*, *Klebsiella pneumoniae*, *Vibrio cholerae*, and *Hemophilus influenzae*. He coined the termed “penicillin” to refer to the “mould broth filtrate” that had antibacterial properties, since it was derived from *Penicillium* sps[1].

Although Fleming’s discovery was a matter of providence, investigation into the antibacterial properties of fungi had begun much earlier. The observation that fungus can inhibit bacteria was first documented by a French physician, Ernest Duchesne in 1897. In his dissertation, he described the antibacterial properties of the fungus *Penicillium glaucum* [2, 3]. Unfortunately, he did not pursue on his discovery and such a significant work went unnoticed for a very long time. In fact, more than 25 earlier to this discovery, Joseph Lister is believed to have noted antibacterial effects of

Penicillium glaucum on bacteria in 1871 and in 1884, he had successfully treated a case of abscess with this fungus [3].

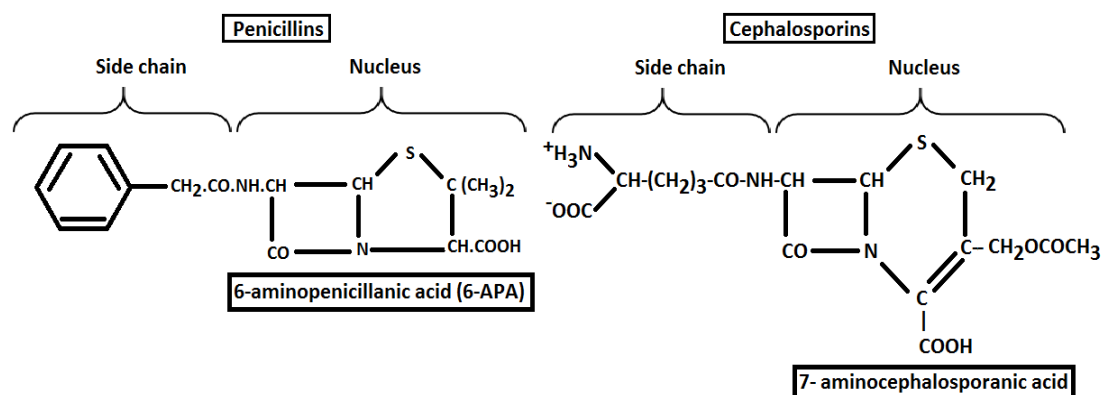
Fleming did not undertake any experiments to evaluate the clinical utility of his discovery. But, his work was continued by Howard W. Florey and Ernst Boris Chain in 1940, who successfully demonstrated the utility of purified penicillin by conducting in-vivo studies using mice deliberately infected with *Streptococcus* [4]. This was followed by successful clinical trials in 1941 and the drug was then made available for therapeutic use [5]. At the same time other biologically derived compounds with antibacterial property were being discovered from actinomycetes. In 1940, Selman A. Waksman, along with his students, discovered Streptomycin from *Streptomyces griseus* [6]. In order to differentiate such biologically derived compounds from other chemotherapeutic agents, he coined the term “antibiotic” [7].

These initial discoveries led to vigorous interest in searching for other antibiotics and one such attempt was made by Giuseppe Brotzu in 1948, who discovered antibacterial activity in crude extracts of the mould *Cephalosporium acremonium* (now known as *Acremonium chrysogenum*). Of the three compounds characterized in the culture extract, Cephalosporin C possessed most useful antibacterial property [8]. Thus, a new class of antibiotics called cephalosporins came into existence. Unlike penicillins, whose activities were restricted to Gram positive bacteria only, the cephalosporins had extended spectrum of activity to include Gram negative bacteria. In order to understand the mechanism of bacterial inhibition, studies were then undertaken to unravel their structure.

1.2 Structure of beta-lactam antibiotics

Following chemical and X-ray crystallographic studies by the pioneering effort of Chain and Hodgkin, the penicillin was shown to contain a unique structure; the beta-lactam nucleus. Beta (β)-lactam ring is a four-membered cyclic amide and ' β ' represents the position of Nitrogen (N) atom relative to the carbonyl (C=O) group. In penicillin, the β -lactam ring is fused to a five membered thiazolidine ring. Thus, penicillin is structurally 6-amino-penicillanic acid 'nucleus' along with a side chain (Figure 1). Cephalosporins too contain a beta-lactam ring, which is fused to a six-membered dihydrothiazine ring. Chemically, it is 7-aminocephalosporanic acid nucleus with an attached side chain (Figure 1).

Figure 1: Structure of penicillin and cephalosporin molecules



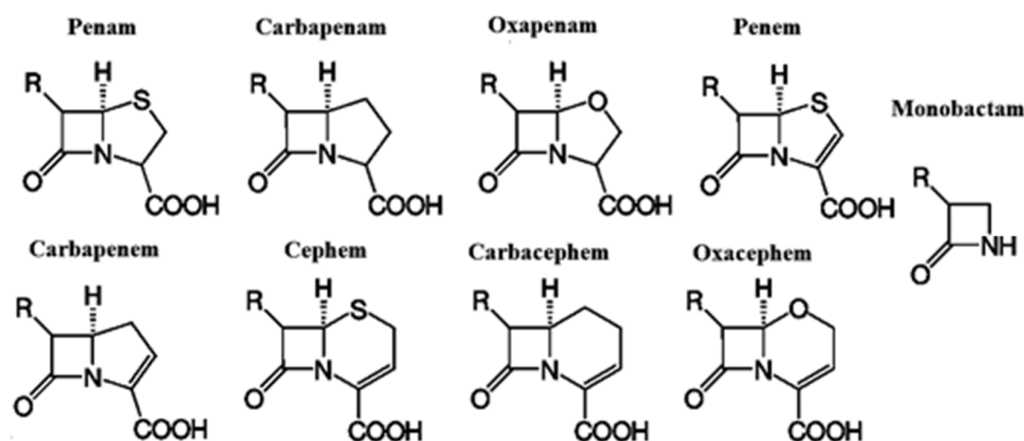
Between 1959 and 1970, a large numbers of semi-synthetic penicillins were created by modifying the side chains attached to the β -lactam ring [9]. Early penicillins were active only against Gram positive bacteria but subsequent generations had expanded spectrum of activity to include Gram negative bacteria. Unlike penicillins, most cephalosporins have good antibacterial activity against Gram negative bacteria. Several modifications in the side chain have made cephalosporin the largest group of beta-lactam antibiotics, comprising of five generations, each with distinct spectrum of

activity. Resistance exhibited by the bacteria to these antibiotics necessitated these modifications. Both, the naturally and semi-synthetically derived beta-lactam compounds are currently the largest family of antimicrobial drugs in use.

1.3 Classification of beta-lactam antibiotics

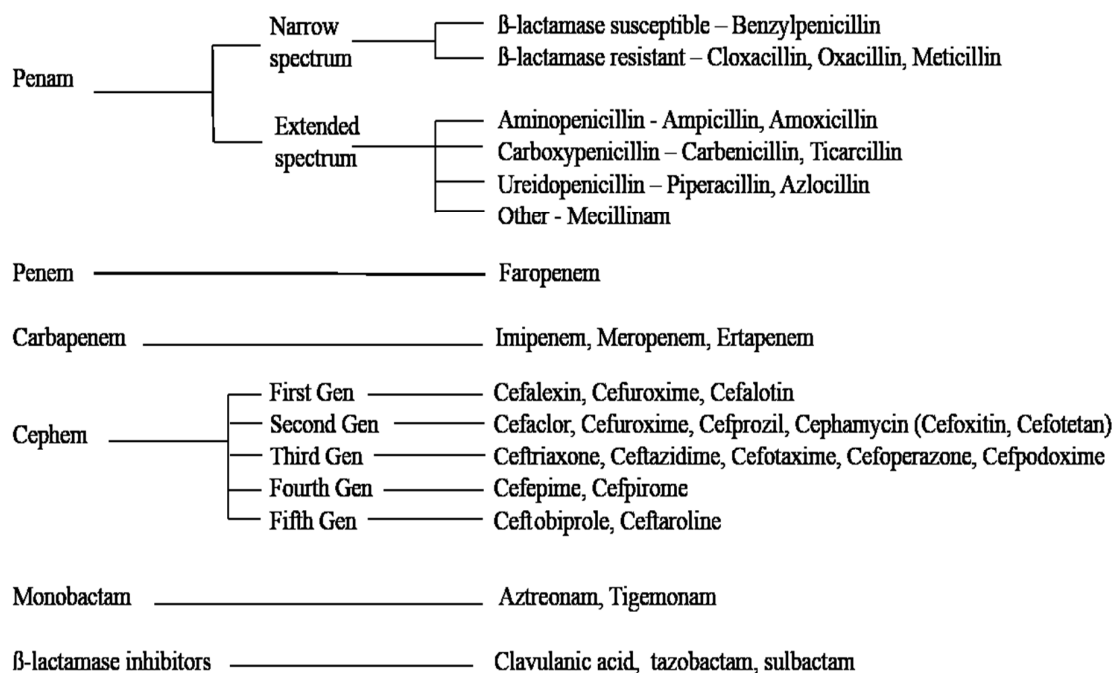
Beta-lactam groups are named after the type of rings that are fused to beta-lactam rings. The groups include penams, clavams (oxapenams), penems, carbapenems, cephems, carbacephems, oxacephems, monobactams and cephamycins. The core structure of various beta-lactam compounds are similar and is displayed in Figure 2.

Figure 2. Core structures of various beta-lactam compounds



A broad consensus on the classification of generations of cephalosporin and the position of cephamycins in that classification is yet to form. Figure 3 shows classification of beta-lactam antibiotics along with examples. The fifth generation cephalosporin such as ceftaroline and ceftobiprole are considered to be the latest generation of cephalosporins with enhanced activity against both Gram positive and Gram negative bacteria. Although beta-lactam antibiotics have slightly different pharmacologic properties and spectrum of activities, all of them act by inhibiting bacterial cell wall synthesis.

Figure 3: Classification of beta-lactam antibiotics

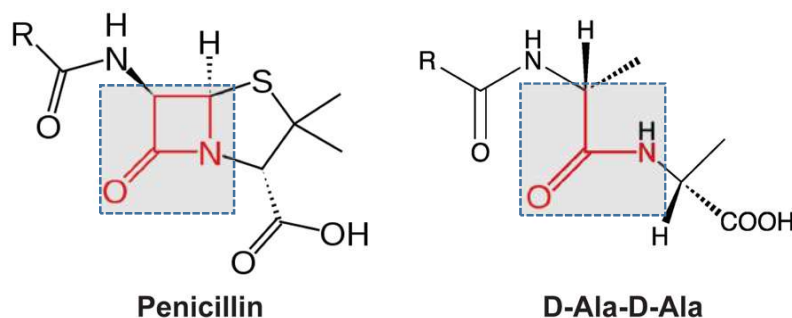


1.4 Mechanism of action of beta-lactam antibiotics

During the course of bacterial growth, the cell wall is continuously remodeled as the old ones are broken down and new ones are formed. Transglycosylase, transpeptidase, carboxypeptidase and endopeptidase are the bacterial enzymes that are involved in remodeling and synthesis of cell walls. These are a family of acyl serine transferases that catalyze the polymerization (transglycosylation) and cross-linking (transpeptidation) of the glycan strands of peptidoglycan. D-alanyl-D-alanine, a natural substrate of these enzymes is structurally similar to beta-lactam antibiotic (Figure 4). This similarity tricks the bacterial enzymes into binding with beta-lactam antibiotics in their active sites. It is for this reason that the transpeptidase enzymes are also known as Penicillin Binding Proteins (PBPs). By blocking the active site of these enzymes, beta-lactam antibiotics deprive them of their natural substrates. This prevents the cross linking of cell wall and ultimately the cell dies due to osmotic instability [10]. Bacteria

evolved quickly to counter the beta-lactam antibiotics and resistance appeared in *S. aureus* within a year of penicillin's use [11].

Figure 4: Structural similarity of beta-lactam ring and D-alanyl-D-alanine



1.5 Resistance to beta-lactam antibiotics:

Resistance to beta-lactam antibiotics can be intrinsic or acquired; most gram negative bacteria are intrinsically resistant to penicillin. Bacteria have evolved to counter the adverse effects of beta-lactam antibiotics in the following four diverse ways: a) mutations leading to loss or under-expression of porins that disallow entry of beta-lactams, b) production of new penicillin binding proteins that have low affinity to beta-lactams, c) expulsion of beta-lactams from periplasmic space mediated by efflux pumps and d) production of enzymes that hydrolyze beta-lactam rings.

Of all these methods, the enzymatic inactivation by beta-lactamases is the most common strategy adopted by the bacteria. The first evidence of enzymatic inactivation of penicillin came in 1940, even before the antibiotic was used in therapeutics. Abraham and Chain were able to demonstrate an enzyme in *E. coli* that hydrolyzed penicillin; they named it “penicillinase” [12]. Beta-lactamase is a broader name given to bacterial enzymes that hydrolyze various beta-lactam antibiotics. In case of Gram positive bacteria, these enzymes are excreted outside the cell whereas in Gram negative bacteria, they are present in the periplasmic space.

2. Beta-lactamases

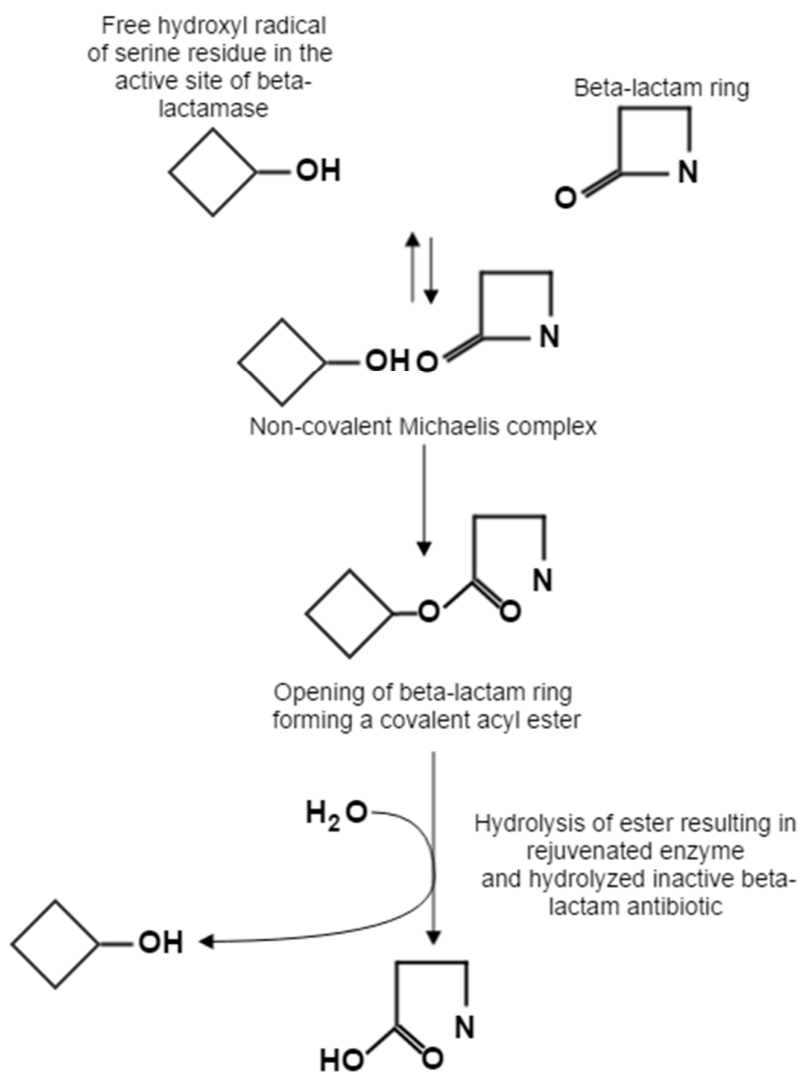
Beta-lactamases are classified as serine beta-lactamase when they have a serine radical or as metallo-beta-lactamases (MBLs) when they have zinc ion at the enzyme's active site. Typically, the inactivation of beta-lactam antibiotic involves acylation and deacylation steps. In the acylation step, the beta-lactam ring is opened forming an enzyme-acyl complex, which is then deacylated from serine following hydrolysis. While the acylation step requires nucleophilic serine, deacylation requires hydrolytic water molecule.

2.1. Mechanism of action of beta-lactamases

Following binding of the beta-lactam substrate in the active site of the beta-lactamase, a non-covalent (Henri-Michaelis) complex is formed. This step is reversible. The serine radical in the active site mounts a nucleophilic attack on the carbonyl leading to high-energy tetrahedral acylation intermediate. Protonation of the beta-lactam Nitrogen and cleavage of C-N bond results in opening up of the beta lactam ring and the intermediate then transitions into a lower-energy covalent acyl-enzyme complex.

An activated water molecule then attacks the covalent complex leading to high-energy tetrahedral deacylation intermediate. Hydrolysis of the bond between the beta-lactam carbonyl and the oxygen of the serine is then hydrolyzed, which regenerates the enzyme and releases the inactive beta-lactam molecule [13]. While the beta-lactam molecule gets destroyed, the enzyme is rejuvenated and becomes fully functional. Hydrolysis of beta-lactam molecule by serine beta-lactamase is depicted in Figure 5.

Figure 5: Mechanism of hydrolysis of beta-lactam molecule by beta-lactamase



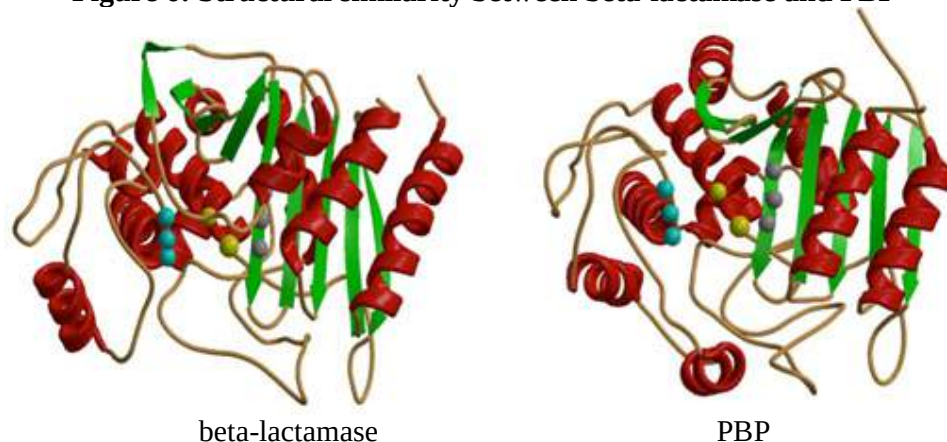
2.2 Molecular structure of beta-lactamases

Both the X-Ray crystallography and amino acid sequencing were pivotal in elucidating the molecular structure of beta-lactamases. In 1975, Ambler sequenced the entire beta-lactamase (*bla*) gene of penicillinase produced by *S. aureus* PC1 strain and that original numbering scheme of amino acids still remains the benchmark for numbering other serine beta-lactamases [14].

Beta-lactamases vary in several properties but the basic structure and amino-acid homology at the conserved regions suggests that they are phylogenetically related and that they have common evolutionary origin. Serine beta-lactamases and Penicillin Binding Proteins (PBPs) have similar tertiary folding, active-site topology and catalytic mechanism [15]. Hence, it has been proposed that serine beta-lactamases might have evolved from some ancestral D-D peptidases involved in synthesis and maintenance of peptidoglycan cell wall. Recently, a new family of PBPs with significant sequence similarity to serine beta-lactamases was discovered in a cyanobacterium, suggesting evolutionary relatedness between PBP and beta-lactamases [16]. The structural similarity between *E. cloacae* AmpC beta-lactamase P99 and PBP R61 of *Streptomyces sps* is shown in Figure 6.

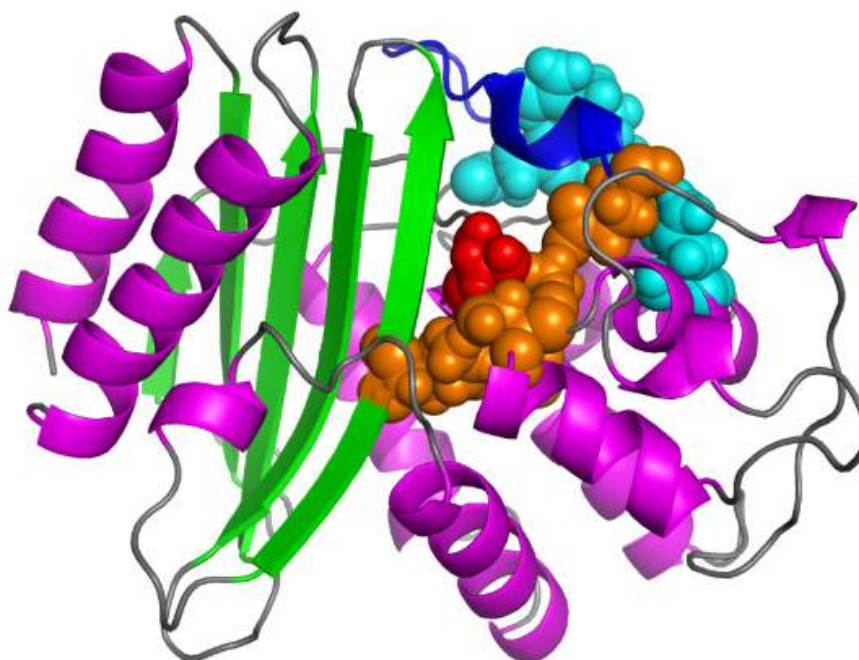
X-ray crystallography have revealed the three-dimensional structure of beta-lactamases. The serine beta-lactamases are composed of two domains: α/β domains and α domain. The α/β domains is formed by five anti-parallel β -sheet surrounded by three α -helices on one face and one α -helix on the other. The α domain is formed entirely by α -helices. The catalytic activity of the active site is formed between these two domains [17]. A three dimensional structure of serine beta-lactamase is displayed in Figure 7. The characteristic arrangement of amino acids, which may also be found on other proteins is called a motif.

Figure 6: Structural similarity between beta-lactamase and PBP



Similarity in tertiary folding and active-site topology is apparent. Coloured spheres represent amino acid residues that are involved in active sites.
(Image credit: Jean-Marie Frère. Nova Science Pub Incorporated)

Figure 7: Three dimensional structure of serine beta-lactamase



The catalytic residue Serine-70 is shown in red. Other catalytic residues are shown in orange, whereas the Ω -loop is shown in blue and the residues that maintain the structural integrity are shown in cyan. (Image credit: Verma D, *et al.* PLoS Comput Biol 9(7): e1003155.)

The highly conserved structural motifs in serine beta-lactamases include four polar regions:

1. Active-site pocket: Ser70 – X – X - Lys73 (where X can represent any amino acid)
2. "Ω-loop": residues 163 to 178
3. "SDN loop": Ser/Tyr130 - Asp131 - Asn132 (on a loop in the all α -domain)
4. Lys234 - Thr/Ser235 - Gly236 (on a β -sheet forming opposite wall of active site)

The "SDN loop" is believed to participate in maintaining the structure of the active site cavity and stability of the enzyme. The beta-lactam antibiotic in the active site cavity of beta-lactamase interacts with the dense hydrogen-bond network formed by the amino acid residues of the conserved structural motifs. The nucleophilic attack of the beta-lactam ring is initiated by the hydroxyl group of Serine at position 70. The strategically placed water molecule, which hydrolyses the acyl-enzyme bond is activated by glutamic acid, tyrosine or an unusual carbamylated lysine at position 166 located in the Ω-loop. [17]

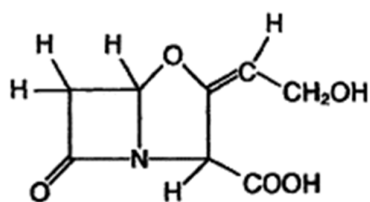
Point mutations in the nucleotide sequences of *bla* gene leading to single amino acid substitution can have varying effect on the enzyme property. While silent mutations confer no change, other mutations may result in the transformation of the enzyme with enhanced hydrolytic capacity or even acquire resistance to inhibitors. By performing site-direct mutagenesis studies, scientists have been able to identify the significance of specific amino acids at specific locations.

The knowledge of molecular structure, especially the active site, is crucial in drug discovery and development. The various molecular interactions occurring between

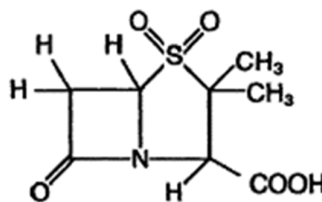
the drug and the active site residues help scientists in the pharmaceutical industry to develop or modify drugs that can inhibit the activity of these enzymes.

2.3 Beta-lactamase inhibitors:

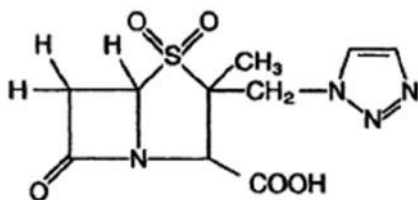
In order to counter the growing menace of enzyme mediated resistance to beta-lactam antibiotics, an earnest search was made to find suitable inhibitors. Beta-lactamases are inhibited by certain beta-lactam antibiotics as well as by beta-lactamase inhibitors, which also mimic beta-lactam structure. Reversible inhibitors (such as extended-spectrum cephalosporins, monobactams, and carbapenems) form acyl-enzymes and are able to bind to the active site of beta-lactamases with high affinity, but have the limitation of getting hydrolyzed very slowly. Irreversible inhibitors too act as substrates for beta-lactamases, but after hydrolysis, they persist in the active sites and inactivate the enzymes. Hence, irreversible inhibitors are also known as “suicide inhibitors” or “suicide inactivators”. [18]



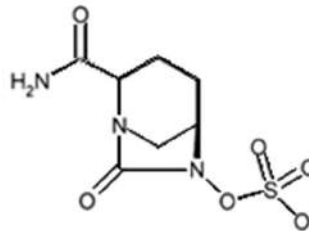
Clavulanic acid



Sulbactam



Tazobactam



Avibactam

The first natural beta-lactamase inhibitor was obtained from a soil bacterium- *Streptomyces clavuligerus*.

This organism was isolated from South

Figure 8: Structures of beta lactamase inhibitors in use

American soil sample in 1971 while investigating potential beta-lactam antibiotic

producers. [19] Although it was found to produce a few antibacterial substances similar to cephalosporin, it was the discovery of a compound with anti-beta-lactamase property that caught the attention of researchers. This novel beta-lactamase inhibitor was named as clavulanic acid to indicate the source of this compound [20]. Subsequently, semi-synthetic beta-lactamase inhibitors such as sulbactam and tazobactam were introduced. Avibactam is a member of a new class of synthetic, non-beta-lactam inhibitors called the diazabicyclooctanes. Previously known as NXL104, avibactam is known to inhibit several beta-lactamases. It forms covalent enzyme-substrate complex in the enzyme's active site, leading to its deacylation but does not undergo hydrolysis and therefore, is a slow reversible inhibitor. It has been combined with ceftazidime and ceftaroline (a new fifth generation cephalosporin) [21]. The molecular structure of the common beta-lactamase inhibitors in use is displayed in Figure 8.

Like beta-lactam antibiotics, these inhibitors also bind to the active site of the beta-lactamase enzyme and undergo acylation. Following acylation, the beta-lactam ring is broken and forms a transient imine intermediate. The imine intermediate may then rearrange to form enamine intermediate in either cis- or trans- conformation. While tazobactam forms stable trans-enamine intermediates, clavulanic acid and sulbactam form a mixture of trans-enamine and the less stable cis-enamine intermediates. Prolonged inhibition of the enzyme occurs when this intermediate is stabilized. The sulphone and triazolyl moieties of tazobactam stabilizes the intermediate in the enzyme's active site. These properties make tazobactam a better inhibitor than the others. Depending on the nature of the enzyme and the inhibitor, the reaction may either proceed to deacylation or complete inhibition of the enzyme. In case of reversible inhibitors, the enamine intermediate undergoes decarboxylation and ester bond hydrolysis, which ultimately results in regeneration of the enzyme. However, this

reaction is very slow and may last beyond the generation time of the bacterium. Irreversible inhibitors permanently inactive the enzyme. [17]

Although these three inhibitors have similar mechanism of action, they have different pharmacological and inhibitory properties. Unlike sulbactam and tazobactam, clavulanic acid is a potent inducer of AmpC beta-lactamase. Compared to clavulanic acid, sulbactam is stable in solutions, lasts longer in body fluids and has intrinsic activity against *Bacteroides spp*, *Acinetobacter spp*, and *N. gonorrhoeae*. Tazobactam is more active against CTX-M type beta-lactamases than clavulanic acid or sulbactam. OXA-2 and OXA-32 beta-lactamases are inhibited by tazobactam but not by clavulanic acid or sulbactam. [17] The difference in the number of molecules required to inhibit different enzymes is displayed in Table 1. [17]

Table 1: Comparison of activities of three beta-lactamase inhibitors

	Number of molecules of inhibitor required to inactivate beta-lactamases		
	<i>S. aureus</i> PC1	TEM-1	SHV-1
Clavulanic acid	1	160	60
Sulbactam	Not Available	10,000	13,000
Tazobactam	1	140	5

The knowledge of these properties will enable physicians to select appropriate beta-lactamase inhibitor depending on the type of beta-lactamase produced by the infecting organism. Since beta-lactamases vary in their susceptibility to inhibition by beta-lactamase inhibitors, this property is a key factor used in the functional classification and characterization of beta-lactamases.

2.4 Characterization of beta-lactamases:

Characterization of beta-lactamases on various properties not only helps scientists in detecting new variants or types but also allow researchers to identify the existing types. Several methodologies have been used to characterize these enzymes, each with its own advantages and limitations. These methods include determination of substrate profile, inhibition of enzyme activity, determination of isoelectric point by isoelectric focusing, immunological reactivity to antisera, estimation of molecular weight, inducibility of enzyme, determination of MIC, determination of gene location, detection of specific sequences by labeled probes, and determination of nucleotide and amino acid sequences by molecular techniques.

Substrate profile indicates the hydrolytic activity of a beta-lactamase against a number of different beta-lactam substrates. An enzyme is considered as a penicillinase if benzylpenicillin is hydrolyzed at rate >30% that of cephalosporin. An enzyme is considered as a cephalosporinase if it hydrolyzes cephaloridine at a rate of >30% than that of benzylpenicillin. For penicillinases, benzylpenicillin has been used as the historical reference for hydrolysis, whereas cephaloridine was the chosen reference for cephalosporinases. For complete characterization, the enzymes are often tested against a panel consisting of benzylpenicillin, ampicillin, carbenicillin, cloxacillin, cefotaxime, ceftazidime, aztreonam and imipenem. Early studies used ratio of hydrolysis of the substrate against a chosen standard, such as penicillin G, but in subsequent studies, rate of hydrolysis (V_{max}) and binding affinity (K_m) for a number of substrates were measured [22]. Substrate profile provides a valuable functional property of the enzymes and since not many laboratories are not equipped undertake these studies, most of the recently discovered enzymes remain uncharacterized.

Inhibition profiles were built using susceptibility of enzymes to inhibition by oxacillin, methicillin, aztreonam or non-beta-lactam compounds such as chloride ions and p- chloromercuribenzoate. Since the discovery of beta-lactamase inhibitors, clavulanic acid and sulbactam are used for comparing inhibition profiles of these enzymes [22]. The results of these methods are often unreliable when isolates express multiple beta-lactamase types. In order to obtain the inhibition profile of the enzyme, it must be made available in pure form, which is beyond the scope of many laboratories.

Isoelectric focusing is used to characterize and identify beta-lactamases based on their isoelectric points (pI). This is based on the principle that positively or negatively charged proteins move accordingly under electric field during electrophoresis. In this technique, the proteins are made to move in a gradient of pH and the pH at which the protein has no net charge is identified as its isoelectric point (pI). At its isoelectric point, the protein is focused as a narrow band, which can be visualized by detecting its enzymatic activity using chromogenic nitrocefin as the substrate. Enzymes, which are identical in their substrate profiles can be differentiated based on their pI values [23]. This technique is useful in differentiating multiple beta-lactamases produced by an isolate and also distinguishes chromosomal enzymes from plasmid mediated enzymes. Isoelectric points has been shown to vary from one laboratory to another and unrelated beta-lactamases may have similar pI values [22, 24]. Despite the advantages, not many laboratories are equipped to perform isoelectric focusing.

Immunological reactivity by specific antisera raised against beta-lactamase types were used to characterize beta-lactamases in early studies [25]. Beta-lactamases were identified by gel precipitation methods or by inhibition of substrate hydrolysis using specific antisera. As large number of new variants were being frequently reported,

it became impossible to raise antisera against all the types. High costs and frequent cross-reactions rendered this method obsolete.

Initially, the determination of molecular weight was a popular method of characterizing beta-lactamases. Molecular weight were determined by various techniques such as gel filtration, dodecyl sulphate-polyacrylamide gel electrophoresis, equilibrium centrifugation, or amino acid analysis. Estimation involved laborious process of purification as a preliminary step. Molecular weight estimation varied according to the method employed. Sometimes, beta-lactamases that differed in many aspects had identical molecular weight and molecular weights of different beta-lactamases were found to be similar when estimated by amino acid composition [22]. Currently, amino acid composition is used to deduce the molecular weight of a protein.

Mating assays for conjugal transfer of plasmids were used to determine the location of *bla* gene. Successful transfer of *bla* gene to the standard recipient strain indicated that the gene was located on a plasmid. This method is not always reliable as some *bla* genes may be present on non-conjugative plasmids. In some cases multiple attempts may have to be performed for successful conjugation. In order to avoid these potential problems, the plasmids are separated, cut with restriction enzyme, cloned into a vector and then transformed into a recipient strain. Expression of beta-lactamase in the recipient strain confirms the presence of *bla* gene on the plasmid [26]. These methods are labour intensive and expensive.

Induction tests are useful to determine if the enzyme is produced constitutively or can be induced by any other beta-lactam antibiotic. AmpC beta-lactamases are often inducible in gram negative bacilli such as *Enterobacter* *sps*, *Citrobacter* *sps*, *Proteus* *sps* and *Morganella* *sps*. Broth culture of beta-lactamase producing isolate is exposed

to the potential inducer at varied concentrations for two hours. After the protein synthesis is arrested by the addition of 8-hydroxyquinoline, the cells are harvested by centrifugation, washed in buffer and the pellet is sonicated to extract the enzymes. Beta-lactamase activity contained in the sonicate is determined against a suitable substrate such as nitrocefin or cephalothin. A positive result indicates that the enzyme is inducible [27].

Estimation of MIC levels of various beta-lactam antibiotics against beta-lactamase producing bacteria is a useful marker of the enzyme activity. MICs can be estimated by microbroth dilution, agar dilution, E-test strips or by automated systems. MIC values are affected by several factors such as inoculum density, co-production of other beta-lactamases, loss of porins and the nature of beta-lactamase expression. In order to avoid these pitfalls, the plasmid encoding *bla* are cloned in standard recipient strains and then the MICs are estimated.

DNA hybridization with labelled probes were used in early studies to detect specific beta-lactamases. This method could also detect chromosomal location of *bla* gene. The procedure involves extraction of plasmids, digestion by restriction endonuclease enzymes, electrophoresis on gel, Southern blot transfer to nylon/nitrocellulose membranes, hybridization by labeled probes followed by visualization [28]. As more and more mutant forms of these enzymes were detected it was not possible to design probes for all of them. In order to attain accurate hybridization, high levels of stringency are required to be maintained. Since silent mutations can code for the same amino acid, mere nucleotide substitutions are not helpful in characterizing a beta-lactamase. After the advent of PCR, probe hybridization has fallen into disuse.

Currently, many laboratories clone the entire *bla* gene by PCR and obtain its complete nucleotide sequence. Both the nucleotide sequences and their translated amino acid sequences are maintained in public databases (GenBank). Using appropriate bioinformatics tools, researchers can now easily identify and characterize beta-lactamases. A big disadvantage of this method is that it provides no clue on the functional properties of the enzyme.

The methods to characterize the beta-lactamases are many; but it is beyond the means of most laboratories to undertake all of them and are thus restricted only to research laboratories. As more and more beta-lactamases were discovered and characterized by the aforementioned methods, it is only logical that these enzymes had be grouped or classified.

2.5 Classification of beta-lactamases:

In 1968, Sawai and Mitshashi attempted classification of beta-lactamases for the first time. Thereafter, few other schemes were proposed by Jack and Richmond, Richmond and Sykes and Sykes and Matthew [29-32]. These schemes were based on physiological properties of the enzymes such as inducibility, hydrolytic prolife, inhibitory profile, isoelectric point and molecular weight. The key features of these initial classification schemes is summarized in the Table 2.

Presently, the two popular classification schemes are derived from the works of Ambler RP and Karen Bush. In 1980, Ambler proposed the “phylogenetic” or “molecular” classification based on the amino-acid sequences of the beta-lactamases. In his classification, he divided beta-lactamases into two groups: Class A (serine beta-lactamases) and Class B (metallo-beta-lactamases) [33]. Class C, consisting of AmpC beta-lactamases was added subsequently by Jaurin and Grundstrom in 1981 [34]. In

1988, Huovinen P *et al* expanded this classification by including Class D, which encompasses oxacillinases (OXA-type) [35].

Table 2: Key features of the initial classifications of beta-lactamases

Year	Authors	Key features
1968	Sawai T & Mitsuhashi S	a) inducible cephalosporinase that hydrolyzes cephalexin efficiently but not penicillins b) inducible cephalosporinase that also had penicillinase property c) Constitutive penicillinase
1970	Jack GW & Richmond MH	Enzymes were grouped into eight types (Enzyme types 1 to 8) on the basis of hydrolysis to benzylpenicillin, ampicillin, cephaloridine and susceptibility to anti-TEM serum, p-chloromercuribenzoate and cloxacillin.
1973	Richmond MH & Sykes RB	Enzymes were grouped into classes I to V based on substrate profile and inhibition of beta-lactamases; classified as penicillinases, cephalosporinases or broad-spectrum; location of <i>bla</i> gene on chromosome or plasmid was also considered
1976	Sykes RB & Matthew M	Additional parameters such as isoelectric point (pI values) and molecular weight of beta-lactamases were included

Building on the foundations laid by Richmond and Sykes, Karen Bush proposed another classification based on physiological properties of beta-lactamases in 1988 [36]. After a minor update in 1989, it was re-launched as the “functional” classification by the team of Bush K, Jacoby GA and Medeiros AA [37- 39]. It was updated again in 2010 [40]. Based on the substrate and inhibitory profiles, beta-lactamases were divided into three groups: 1, 2 and 3. Based on the differences among the enzymes in these

groups, they were further divided into several subgroups. Among the three groups, group 2 has the most number of subgroups.

Despite being based on physiological properties, this classification is in good agreement with Ambler's molecular classification. AmpC beta-lactamases fall under Bush's group 1 and Ambler's class C, whereas metallo-beta-lactamases fall under Bush's group 3 and Ambler's class B. Rest of the serine beta-lactamases were included in Ambler class A, whereas Bush divided them into 11 subgroups under group 2. Bush's functional classification in relation to Ambler's molecular classification is displayed in Table 3.

Among the various subgroups listed in Bush's classification, most enzymes that are encountered in clinical isolates of Gram negative bacilli belong to the subgroups of group 2. In the functional classification, the letter "e" in the subgroup names is used to denote extended spectrum of activity. The subgroup 2b comprises of enzymes that hydrolyze penicillins and early cephalosporins and are inhibited by clavulanic acid. These enzymes are sometimes termed as "broad-spectrum" or "classical" beta-lactamases as they exhibit better activity against first and second generation cephalosporins.

Subgroup 2be includes enzymes that are able to hydrolyze penicillins, early cephalosporins and one or more of the third generation cephalosporins or monobactams. Because of their extend activity against third generation cephalosporins, they are called Extended Spectrum Beta-Lactamases (ESBLs).

Table 3. Functional classification by Bush, Jacoby and Medeiros

Group and subgroup	Molecular class (Ambler)	Preferred substrate	Inhibited by		Representative enzymes
			CA/TZB	EDTA	
1	C	Cephalosporins	-	-	MIR-1, ACT-1, CMY-2, FOX-1
1e	C	Cephalosporins	-	-	GC-1, CMY-37
2a	A	Penicillins	+	-	PC1
2b	A	Penicillins, early cephalosporins	+	-	TEM-1, TEM-2, SHV-1
2be	A	ESC, monobactams	+	-	TEM-3, SHV-2, CTX-M-15, K1, PER-1, VEB-1
2br	A	Penicillins	-	-	TEM-30, SHV-10
2ber	A	ESC, monobactams	-	-	TEM-50
2c	A	Carbenicillin	+	-	PSE-1, CARB-3
2d	D	Cloxacillin	±	-	OX-1, OXA-10
2de	D	ESC	±	-	OXA-11, OXA-15
2df	D	Carbapenems	±	-	OXA-23, OXA-48
2e	A	ESC	+	-	CepA
2f	A	Carbapenems	±	-	KPC-2, IMI-1, SME-1
3a	B	Carbapenems	-	+	IMP-1, L1, NDM- 1, VIM-1
3b	B	Carbapenems	-	+	CphA, Sfh-1

CA: clavulanic acid, TZB: tazobactam, ESC: Extended spectrum cephalosporin

Inhibition by clavulanic acid is a characteristic property of ESBLs. Subgroup 2br includes broad-spectrum beta-lactamases having hydrolytic properties similar to those

in group 2b, but are characteristically resistant to inhibition by clavulanic acid. These enzymes are also referred to as Inhibitor-Resistant TEMs (IRTs), as they were initially recognized among TEM types. Subgroup 2ber includes mutant forms of enzymes that have inhibitor resistant property of group 2br as well as extended activity against third generation cephalosporins. These are sometimes referred to as Complex Mutant Types (CMT) [40].

The Ambler's classification is convenient to group the enzymes based on molecular relatedness but Bush's classification seems to be more practical as the subgroups correlate well with their functional properties. Bush is of the opinion that functional properties of the enzymes will allow the clinicians to make informed selections of appropriate antibiotics for therapy [41]. The system of classification is essential to group the related members together and at the same time, also brings an order in their nomenclature.

2.6 Nomenclature of beta-lactamases

The absence of any naming convention in the past led the discoverers of beta-lactamases to name the enzymes in a variety of ways. Beta-lactamases were named after the places of discovery, names of the bacterial species or strain, gene, plasmid, patient, hospital, the discoverer or some property of the enzyme [42]. Nomenclature of some beta-lactamases has since, undergone some revisions. Some enzymes were renamed, as they turned out to be identical to the enzymes previously described. The names of a few variants have been withdrawn for the same reason. As per the present naming convention, once the name of an enzyme gets accepted, subsequent new variants are named by incrementing the number. (TEM-1, TEM-2, TEM-3 etc.). The disadvantage of this naming scheme is that even if a new variant possess a considerably different

property, its name doesn't does not give any clue about the new property. Of all the names that have been given to a class or a family of beta-lactamases, none gives more significance to its property than the name "Extended Spectrum Beta-Lactamases".

3. Extended Spectrum Beta-Lactamases (ESBL):

In 1988 Jarlier *et al* used the term "extended broad-spectrum beta-lactamases" to refer those enzymes which had 'extended' activity compared to the 'broad spectrum activity' of classical TEM or SHV enzymes [43]. In subsequent publications, the term 'broad' was dropped and the name "Extended-Spectrum Beta-Lactamase" became established [44].

3.1 Definition and properties

ESBLs are enzymes whose rates of hydrolysis of the extended-spectrum beta-lactam antibiotics such as ceftazidime, cefotaxime, or aztreonam are >10 % than that for benzylpenicillin. These are susceptible to inhibition by beta-lactam inhibitors such as clavulanic acid, tazobactam, or sulbactam but have no hydrolytic activity against cephamycins and carbapenems [41]. In the light of discovery of newer beta-lactamases and their hydrolytic profiles, the existing definition of ESBL is being strongly contested. Livermore insisted that all beta-lactamases conferring resistance to extended-spectrum cephalosporins should be considered as ESBLs without taking into account an arbitrary cut-off value of >10% hydrolysis rate [45]. Giske *et al* proposed that the definition of ESBLs should include AmpC beta-lactamases and carbapenemases [46]. The debate over the two alternate proposals by Livermore and Giske, respectively, shows that the definition and classification of ESBLs might undergo some changes in future [47]. Despite the ongoing debate, there is global acceptance of the current definition.

Using the current definition, ESBLs have been detected among several bacteria, although members of Enterobacteriaceae remain their chief hosts. They have also been encountered among a few non-Enterobacteriaceae bacteria such as *Pseudomonas spp*, *Stenotrophomonas spp*, *Acinetobacter spp*, *Vibrio spp*, and *Haemophilus spp*, among others [48-50]. Despite the diversity in their hosts, the ESBLs predominantly belong to a few limited types.

Depending on the general prevalence, ESBLs are broadly grouped into major and minor ESBL types. Major ESBL are commonly expressed by many clinical isolates and are detected in many parts of the world whereas the minor types are rarely encountered or are restricted to certain geographical locations only. Currently, the three major ESBL types are TEM, SHV and CTX-M.

3.2 TEM type ESBLs:

All the beta-lactamases among Gram negative bacteria known to scientists until 1965, were chromosomally encoded. The first report of a plasmid encoded beta-lactamase in Gram negative bacilli came in 1965 from Athens, Greece [51]. The transferable plasmid, named RTEM1, encoding resistance to ampicillin and streptomycin, was detected in an *E. coli* TEM strain cultured from the blood of a female patient by the name Temoniera. In the subsequent literature, this beta-lactamase came to be known as TEM-1. TEM-2, a variant of TEM-1 that differed by one amino-acid substitution was detected in 1979, but its hydrolytic properties were similar to that of TEM-1 enzyme [52].

In 1985, strains of *K. pneumoniae* with transferable resistance to higher cephalosporins were reported from French hospitals. The beta-lactamase detected in those strains was named CTX-1 to denote its hydrolytic activity against cefotaxime

[53]. Amino acid sequencing revealed that it differed from TEM-2 beta-lactamase by two amino acid substitutions; Lys104Glu and Ser238Gly, which conferred it the ability to hydrolyze extended spectrum cephalosporins [54]. It was ultimately renamed as TEM-3 and is the first ESBL of the TEM type to be discovered.

The *bla*TEM gene codes for a peptide of 286 amino acids, but first 23 amino acids form the signal sequence at the N-terminal, and is cleaved to form the mature enzyme. ESBL phenotype among TEM enzymes is associated with amino acid substitutions at positions 104, 164, 238, or 240. The prominent substitutions include Glu104Lys, Arg164Ser, Arg164His, Gly238Ser, and Glu240Lys. Substitutions occurring in the Ω -loop (positions 164, 179) and β 3 sheet (position 238) are critical for change in the hydrolytic spectrum of these enzymes [55]. Random mutations in the nucleotide sequences of *bla* gene are responsible for these substitutions, but further selections of these mutants result from the selective pressures exerted by exposure to antibiotics. At least 90 mutations occurring at different locations are documented among *bla*TEM genes (<http://www.lahey.org/studies/temtable.asp>). Saturation mutagenesis study reveal that 220 out of 263 amino-acid positions of the mature TEM-1 protein can tolerate substitutions [56]. This suggests that further evolution of TEM beta-lactamases is possible.

Currently there are 219 TEM type beta-lactamases documented in the website- <http://www.lahey.org/studies/temtable.asp>. Of these, only 92 (42%) are ESBLs belonging to 2be subgroup, 16 (7.3%) are non-ESBLs belonging to 2b subgroup, 38 (17.4%) are inhibitor resistant type belonging to 2br subgroup, 11 (5%) are beta-lactamases with both extended-spectrum activity and inhibitor resistant property belonging to 2ber subgroup (CMT) and the rest 62 (28.3%) are currently unclassified.

Globally, the most commonly reported TEM type ESBLs include TEM-3, TEM-5, TEM-10, TEM-12, TEM-26 and TEM-52. Prevalence of some of the TEM ESBLs appear to be restricted to certain areas whereas few others are globally distributed. Reports of TEM-10 from USA and Europe, TEM-3 from France, TEM-47 from Poland and TEM-52 from South Korea indicate their restrictive distributions [24].

3.3 SHV type ESBLs:

First account of a plasmid mediated beta-lactamase in *Klebsiella* sps was made by Pitton in 1972 [57]. O'Callaghan *et al* named the enzyme as Pit-2 to honor Pitton. In 1979, Matthew M renamed it as SHV-1 (for sulphhydryl variable) as he believed that the sulphhydryl group of the enzyme was inhibited by p-mercuricholorobenzoate [58]. Despite the fact that the active site contained hydroxyl group instead of the assumed sulphhydryl group, the name SHV gained global acceptance. Complete amino acid sequence of SHV-1 revealed that it shared only 68% amino acid homology with TEM-1 enzyme [59]. SHV type beta-lactamases are often chromosomally encoded in *K. pneumoniae*, which have since escaped on to the plasmids.

In 1983, plasmid mediated resistance to cefotaxime in clinical isolates of *K. pneumoniae* and *S. marcescens* was described from Germany [60]. Kliebe *et al* took one strain of *Klebsiella ozaenae* from that collection for further studies and after confirming the ESBL phenotype, it was designated as SHV-2 [61]. This became the first ESBL to be discovered. Amino acid sequencing of SHV-2 revealed that it differed from SHV-1 by a single amino acid substitution- Gly213Ser [62]. As per Ambler's present numbering scheme, that position is now identified as 238. Substitutions of amino acids at positions 146, 156, 169, 179, 205, 238 or 240 are responsible for ESBL

phenotype in SHV enzymes [55]. Globally SHV-2, SHV-2a, SHV-5, and SHV-12 are the common SHV type ESBLs.

Currently there are 185 SHV types documented in the website- <http://www.lahey.org/studies/>. Of these, only 47 (25.4%) are ESBLs belonging to 2be subgroup, 37(20%) are non-ESBL belonging to 2b subgroup, seven (3.8%) are non-ESBL inhibitor resistant types belonging to 2br subgroup and the rest 94 (50.8%) are yet to be classified.

3.4 CTX-M type ESBLs

In 1988, a new type of non-TEM, non-SHV plasmid mediated ESBL was reported in fecal *E. coli* obtained from laboratory dogs from Japan [63]. Named FEC-1, this ESBL had prominent cefotaximase activity as it conferred high-level resistance to cefotaxime and ceftriaxone but not to ceftazidime. Although this was the first description of a new class of ESBL, it remained obscure for several years. In 1990, a similar type of ESBL was discovered in an *E. coli* strain isolated from the ear exudate of a neonate in Munich, Germany [64]. Because of its predominant cefotaximase activity and the location of its isolation (Munich), the enzyme was named CTX-M. Around the same time, an *E. coli* MEN strain producing a similar enzyme, designated as MEN-1, was reported from France [65] Amino acid sequencing revealed that both CTX-M and MEN-1 enzymes were identical and the name CTX-M-1 gained acceptance.

Thereafter, several variants of CTX-M enzymes were discovered from the various parts of the world; those with amino acid homology of $\leq 90\%$ formed separate groups and those with $>94\%$ homology were grouped together as members of a group. Presently, CTX-M ESBLs are divided into five clusters or groups where members of

each group share >94% amino acid homology and members across the groups have $\leq 90\%$ homology [66]. The five groups of CTX-M are CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25. By convention, each group is named after the first member of the group that was described. Recent studies suggest that CTX-M-45 might represent a new group and that CTX-M group 1 can be subdivided into two subgroups, A and B [67, 68]. The discoveries of major CTX-M groups and their members is displayed in Table 4. As of now, 158 allelic variants of CTX-M enzymes have been catalogued. Among the five CTX-M groups, CTX-M-1 and CTX-M-9 groups are the most diverse. While CTX-M-8 group is the smallest with only three members, CTX-M-9 group is the largest group with over 40 variants.

In 2013, Zhao and Hu used the aligned amino acid sequences of 124 CTX-M ESBLs to derive a phylogenetic tree [69]. By comparing the amino acid homology, they identified seven clusters, namely cluster 2, cluster 3, cluster 8, cluster 14, cluster 25, cluster 45 and cluster 64 (Figure 9). Based on the central positions in the tree, CTX-M-2, CTX-M-3, CTX-M-8, CTX-M-14, CTX-M-25, CTX-M-45 and CTX-M-64 were chosen as the representative enzymes in each cluster.

Phylogenetic studies suggest that *bla*CTX-M genes have evolved from chromosomal *bla* genes of *Kluyvera* spp. The *bla*CTX-M gene encodes a 291 amino acid enzyme although insertions and deletions in a few variants have sequences ranging from 282 to 292 amino acids. Even a single amino acid change in the gene constitutes a new CTXM type. After the first 28 amino acids at the N-terminal are cleaved, the mature enzyme contains 263 amino acids.

Table 4: CTX-M groups along with their year and place of detection

Year	Country	Source	Old/present name	Group	Reference
1998	Japan	<i>E. coli</i>	FEC-1	CTX-M-1	[63]
1990	Germany	<i>E. coli</i>	CTX-M/ CTX-M- 1	CTX-M-1	[64]
1992	France	<i>E. coli</i>	MEN-1/ CTX-M- 1	CTX-M-1	[65]
1992	Argentina	<i>S. typhimurium</i>	CTX-M-2	CTX-M-2	[70]
1995	Japan	<i>E. coli</i>	Toho-1 CTX-M-44	CTX-M-2	[71]
1998	Japan	<i>E. coli</i>	Toho-2/CTX-M- 45	?CTX-M- 45	[72]
1998	Poland	<i>C. freundii</i> , <i>E. coli</i>	CTX-M-3	CTX-M-1	[73]
2000	Brazil	<i>E. cloacae</i> , <i>E. aerogenes</i> , <i>C. amalonaticus</i>	CTX-M-8	CTX-M-8	[74]
2000	Spain	<i>E. coli</i>	CTX-M-9	CTX-M-9	[75]
2001	India	<i>E. coli</i> , <i>K. pneumoniae</i> , <i>E. aerogenes</i>	CTX-M-15	CTX-M-1	[76]
2004	Canada	<i>E. coli</i>	CTX-M-25	CTX-M- 25	[77]

The ribbon picture of TEM-1 beta-lactamase showing locations of key amino acids and the comparative sequences of TEM, SHV and CTX-M ESBLs in relation to beta-lactamase of *S. aureus* PC1 strain is shown in Figure 10 and 11, respectively.

CTX-M beta-lactamases share less than 40% identity with TEM and SHV-types. While only some TEM and SHV types are ESBLs, all CTX-M enzymes express ESBL phenotype. In TEM and SHV ESBLs, the increased activity towards oxyimino-cephalosporins is due to increased active site volume, which serves to accommodate bulkier side chains. In case of CTX-M enzymes, the flexibility of the β 3 strand and Ω loop allows it to accommodate the bulky cephalosporins [78, 79]. CTX-M-9 and CTX-M14, which are characteristic of the overall family, possess extraordinarily high hydrolytic activity (>1000 folds) against cefotaxime than ceftazidime [79]. The CTX-M ESBLs discovered initially were chiefly cefotaximases and the first CTX-M ESBL to have significant hydrolytic activity against ceftazidime was CTX-M-15, which was reported from India [76]. Besides CTX-M-15, CTX-M-16, CTX-M-19 and CTX-M-27 too have significant activity against ceftazidime [78]. In fact, CTX-M-16 and CTX-M-27 are at least four times more active against ceftazidime than cefotaxime. The substitution Asp240Gly is responsible for the enzymes' enhanced activity against ceftazidime molecule in CTX-M-15, CTX-M-16, and CTX-M-27, whereas the substitution Pro167Ser serves the same purpose in CTX-M-19.

While some CTX-M enzymes have remained endemic in the region where they originated, few others have spread all over the world. CTX-M-1 in Italy, CTX-M-2 in Argentina, CTX-M-3 in Poland, CTX-M-9 Spain, and CTX-M-14 in Spain, Canada, and China are examples of endemicity. CTX-M-15, which is endemic in India is now the most dominant CTX-M type worldwide. [68, 80, 81] In almost every country, CTX-M ESBLs are being reported and therefore the term "CTX-M β -lactamase pandemic" coined by Canton *et al.* seems more befitting [81].

Figure 10. Key positions of amino acids of TEM-1 beta-lactamase

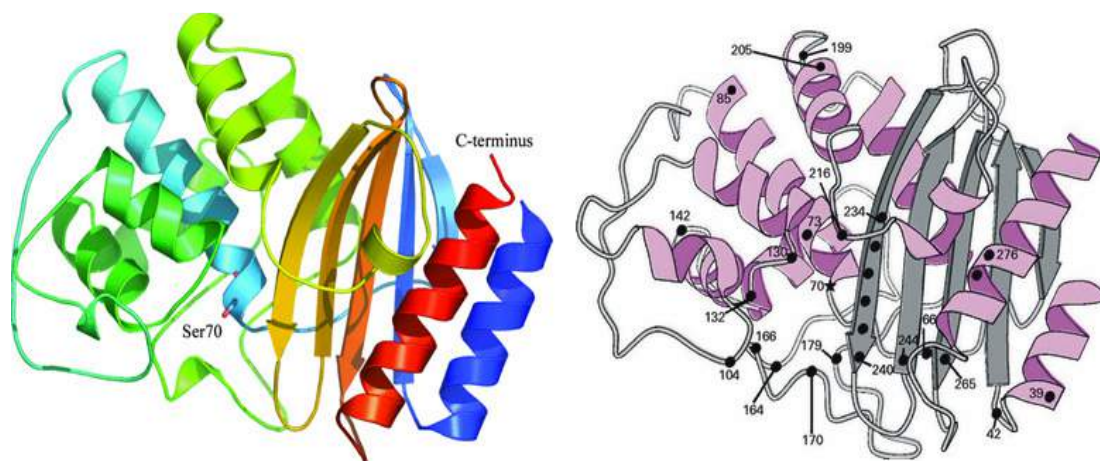


Figure 11: Amino acid sequences of PC1, TEM-3, SHV-12 and CTX-M-15

	10	20	30	40	50	60	70	80
PC1	-----MKKLIF	LIVIALVLSACNSNSSHAKELND	LEKKYNAHIGVYALDTKSGKEV	-KFNSDKRFAYASTSKAIN				
TEM_3	---MSIQHFRVALIPFFAAFC	LPVFAHPETL---	VKVKDAEDKLGARVGYIELDLNSGKILESFRPEERFPM	STFKVLL				
SHV_12	-----MRYIRLCIISLLATLPLAVHASPQPL---	EQIKQSESQLSGRVGMIEMDLASGRTLTAWRADERFPM	STFKVVL					
CTX_M_15	MVKKSLRQFTLMATATVTLLGSVPLAQTADVQQLAE	LERQSGGRLGVALINTADNSQI	-LYRADERFAMCSTSKVMA					
	90	100	110	120	130	140	150	160
PC1	SAILLEQVPY--	NKLNKKVHINKDDIVAYSP	ILEKYVGKDITL	KALIEASMTYS	SDNTANNKIIKE	IGGKIKVKQRLKELG		
TEM_3	CGAVLSRVDAGQQLGRR	IHYSQNDLVKYS	PVTEKHLTDGMTVRE	LCSAAITMSDN	TANALLTTIGGPKEL	TAFLHNMG		
SHV_12	CGAVLARVDAGDQLERK	IHYRQDDLVDYSP	VEKHLADGMTVGE	LCAAITMSDN	SAANLLLATVGGPAGL	TAFLRQIG		
CTX_M_15	AAAVLKKSESEP	NLNQRVEIKKSDLVN	YNPIAEKHVNGTMSLAEL	SAAALQYSDNVAMN	KLIAHVGGP	PASVTAFARQLG		
	170	180	190	200	210	220	230	240
PC1	DKVTNPVRYEIE	LNYYSPKSKKDTST	PAAF	GKTLNKL	LIANGKLSKENKFL	LDLMLN	NKSGDTLIKDGVPKDYKVADKSG	
TEM_3	DHVT	RLDRWEPELNEAIP	NDERDTTMPAAMAT	TLRKLLTGELL	TLASRQQLIDWMEADK	VAGPLLR	SALPAGWFIADKSG	
SHV_12	DNVT	RLDRWETELNEALPGDARDTT	PASMAATLRKLLTSQRL	SARSQRQLQWMVDDR	VAGPLIRSVLPAGWFIADKTG			
CTX_M_15	DETF	RLDRTEPTLNTAIPGDPRD	TSPRAMAQTLRNLTLGKALGDSQRAQLVTWMK	NTTGAASIQAGLPASWVVGDKTG				
	250	260	270	280	290			
PC1	QAITYASRNDVAFVYP	KGQSEPIVLVIFTN	KNKDNKSDKPN	DKLISE	TAKSV	MKEF--	281	
TEM_3	AS-ERGS	RGI	IAALGPDGKPSRI	VVIYTTGSQATMDERNRQ	-IAEIGAS	LIKH	--	286
SHV_12	AS-KRG	ARGI	VALLGPNNKAERI	VVIYLRDTPASMAERNQ	-IAGIGAAL	IEHWQR		286
CTX_M_15	SG-GY	TTNDIAVIWPKDRAP	LILVTYFTQPQPKAES	RRDV-LASAAK	IVTDGL--			291

Note: numbering is not as per Ambler's scheme. Red colour indicates similarity and likely indicates conserved region.

Key: G-Glycine-Gly, P-Proline-Pro, A-Alanine-Ala, V-Valine-Val, L-Leucine-Leu, I-Isoleucine-Ile, M-Methionine-Met, C-Cysteine-Cys, F-Phenylalanine-Phe, Y-Tyrosine-Tyr, W-Tryptophan-Trp, H-Histidine-His, K-Lysine-Lys, R-Arginine-Arg, Q-Glutamine-Gln, N-Asparagine-Asn, E-Glutamic Acid-Glu, D-Aspartic Acid-Asp, S-Serine-Ser, T-Threonine-Thr

Until the 1990s, TEM and SHV types were the predominant ESBLs but now, CTX-M ESBL seems to have displaced them. Once regarded as minor ESBLs, CTX-

M are now the major ESBLs [82]. At this rate, it can be speculated that in future the TEM and SHV ESBLs might become one among the minor ESBLs.

3.5 Minor ESBLs

The minor ESBLs are rarely encountered types that are not related to any of the three major ESBL types. Currently these include OXA, PER, VEB, SFO, BES, BEL, TLA, and GES types. The origins of many of these enzymes are currently unknown and many of them are currently classified under class A or D of Ambler's classification. These ESBLs can be either chromosomal or plasmid encoded. Chromosomal ESBLs have been observed in bacteria like *K. oxytoca* (K1/KOXY), *Proteus vulgaris* (CumA), *Proteus penneri* (HUGA), *Erwinia persicina* (ERP-1), *Citrobacter sedlakii* (SED-1), *Kluyvera spp* (KLUA, KLUC, KLUG), *Serratia fonticola* (FONA), and *Rahnella aquatilis* (RAHN-1) [83].

Presence of at least 430 allelic variants makes OXA type, the most common plasmid mediated minor ESBL. These are basically oxacillinases, some of which have extended spectrum of activity against oxyimino-cephalosporins or carbapenems. Derivatives of OXA-2, such as OXA-15 and OXA-18 and the derivatives of OXA-10 such as OXA-11, OXA-14, OXA-16, and OXA-19 show ESBL like property, although they are not well inhibited by clavulanic acid. Initially detected among *P. aeruginosa* in Turkey, these enzymes are now also being detected among Enterobacteriaceae members. The other two frequently reported minor ESBLs include VEB and PER types, which too are reported more commonly among *Pseudomonas spp* than Enterobacteriaceae members [48]. Other types such as SFO, GES, BES, BEL and TLA types are rarely encountered or reported only in countries where they are endemic [83].

In the last two decades, the world has witnessed an explosive increase in the numbers and types of beta-lactamases. In this period, one minor ESBL has transformed into major type and few major types are on the verge of becoming minor types. It is intriguing to note that the combined effects of selective pressure from the antibiotic abuse and the roles played by the mobile genetic elements have contributed immensely in the evolution of beta-lactamases.

3.6 Evolution of beta-lactamases

Since the discovery of plasmid (R factor) mediated antibiotic resistance in the 1940s, the scientific community has witnessed different strategies adopted by the bacteria to acquire and disseminate antibiotic resistance. The SHV type ESBLs have originated from the chromosomal *bla*SHV of *K. pneumoniae*. The fact that first *bla* gene in *Klebsiella* *sps* was found on a transposon suggests that this mobile genetic element has played a role in the escape of genes from the chromosome into the plasmid [57]. The origin of TEM enzymes is less certain, but they are almost always found encoded on plasmids with exceptions of TEM-12 and TEM-134, whose genes were found on chromosomes [84]. The origin of CTX-M genes have been traced to the chromosomal *bla* genes of *Kluyvera* *spp*. Phylogenetic studies suggest that *bla*CTX-M genes of CTX-M-1 cluster has evolved from chromosomal *bla* genes of *K. cryocrescens*, CTX-M-2 cluster from *K. ascorbata* whereas CTX-M-8, CTX-M-9 and CTX-M-25 clusters have evolved from *K georgiana* [80]. Mobile genetic elements such as transposon (Tn1, Tn2, Tn3, Tn21), integrons (Class 1, 2), insertion sequences (*ISEcp1*, *IS26*, *ISCR1*) and even bacteriophages are believed to have helped these genes cross the genus barrier [85]. While mutations are the primary reason for the large numbers of allelic variants, recombination events too have played a role [86].

4. Epidemiology of ESBL producers

4.1 Dissemination of ESBL producers

ESBL producing organisms have been detected from almost all the continents of this planet, including Antarctica [87]. CTX-M type ESBLs have been isolated from the tribal population in an Amazonian village, which had very little exposure to the modern world [88]. In that study, TEM-52 was the only ESBL detected when samples were taken in 2001 but the scene had changed completely when the study team revisited the place in 2006. By then, the intestinal carriage rates had jumped from 3.2% to 8% and CTX-M-2 and SHV-2 were the only ESBL types found among them. It is not clear how the change happened, but is likely that these bacteria acquired some resistance plasmids from non-resident visitors.

Antibiotic resistance genes are transferred horizontally among bacteria mainly by conjugation. While plasmids of narrow host range such as (IncFI, IncFII, IncHI2 and IncI) serve to disseminate gene within the species or very closely related species, the broad host-range (IncN, IncP-1- α , IncL/M and IncA/C) plasmids disseminate genes across different species. Dissemination of *bla*CTX-M-15 in bacteria across the world seems to be associated with IncFII plasmids [89]. Transfer of promiscuous plasmids alone does not explain the spread of ESBL producers. In 2008, simultaneous emergence of *E. coli* O25:H4-ST131 clone in samples collected across three continents was reported [90]. This clone was multi-drug resistant, possessed IncFII plasmid encoding multiple drug resistance (including *bla*CTX-M) and belonged to the virulent phylogenetic group B2. This clone is now being increasingly reported from many parts of the world, including India [91]. This pandemic clone has been isolated from healthy persons, patients, companion animals, food as well as the environment. Travel to

countries such as India and Pakistan has been shown to be associated with infection by *E. coli* ST 131 strains producing CTX-M-15 ESBLs. Hence, the clone *E. coli* ST131 is also believed to have played a role in worldwide dissemination of CTX-M-15 ESBLs [92].

4.2 Risk factor for acquisition of ESBL producers:

Several studies have been undertaken to find the association between several well characterized risk factors and colonization or infection by the ESBL producers. Many studies have reported isolation of ESBL producing organisms from raw vegetables, fruits as well as food of animal origins [93-95]. It is most likely that these animals might have acquired the ESBL producers from human contacts, at the same time, origin among themselves cannot be entirely ruled out. Although there are not many reports indicting human acquisition of ESBL producers following consumption of contaminated animal food, the risk is always there [96].

Some of the risk factors that have been positively associated with increased colonization or infection by ESBL producers include Asian/African country of birth, travel to Asian/Middle-East countries, hospital stay of more than seven days, nursing home residence, transfer from another health care facility, stay in surgical ward, low birth weight, intra-abdominal surgery, invasive procedures, presence of central venous catheter, hemodialysis, diabetes mellitus, and antibiotic use (especially third-generation cephalosporins and quinolones) for more than a week [97-102].

4.3 Global distribution of ESBL producers:

Several national and international collaborative studies such as SENTRY Antimicrobial Surveillance Program, Meropenem Yearly Susceptibility Test Information Collection (MYSTIC), and Study for Monitoring Antimicrobial Resistance

Trends (SMART) among others are monitoring the trends and emergence of antibiotic resistance in many parts of the world [103]. The compiled data from the several international collaborative studies from 1994 to 2013 along with the references is presented in Annexure 1.

Although the studies differed in time period, geography and sample sizes, they give a broad picture on the changing pattern of prevalence of ESBL producers across time and place. By compiling data from several studies, the bar charts for ESBL producing *E. coli* (ESBL-EC) and ESBL producing *K. pneumoniae* (ESBL-KP) is presented in Figure 12 and 13, respectively. In the studies reviewed here, the prevalence of ESBLs was notably higher among *K. pneumoniae* than *E.coli* until 2008-09, thereafter the prevalence among *E. coli* increased significantly and in many cases, exceeded that of *K. pneumoniae*.

The prevalence of ESBL-EC in North America (Canada and United States of America) had generally remained consistently low at the range of 2-4% until 2005. Thereafter, it rose from 4.8% in 2007 to 18.4% in 2012. The prevalence of ESBL-KP in North America had been stably low until 2004, where it ranged between 4.9% and 7%. Since 2005, the rates have risen from 8% to 12.7% with a peak of 17.2% in the year 2011. The reason for their comparatively low prevalence of ESBL producers appears to be due to strict antibiotic usage and robust infection control measures.

Figure 12. Patten of global prevalence of ESBL-EC

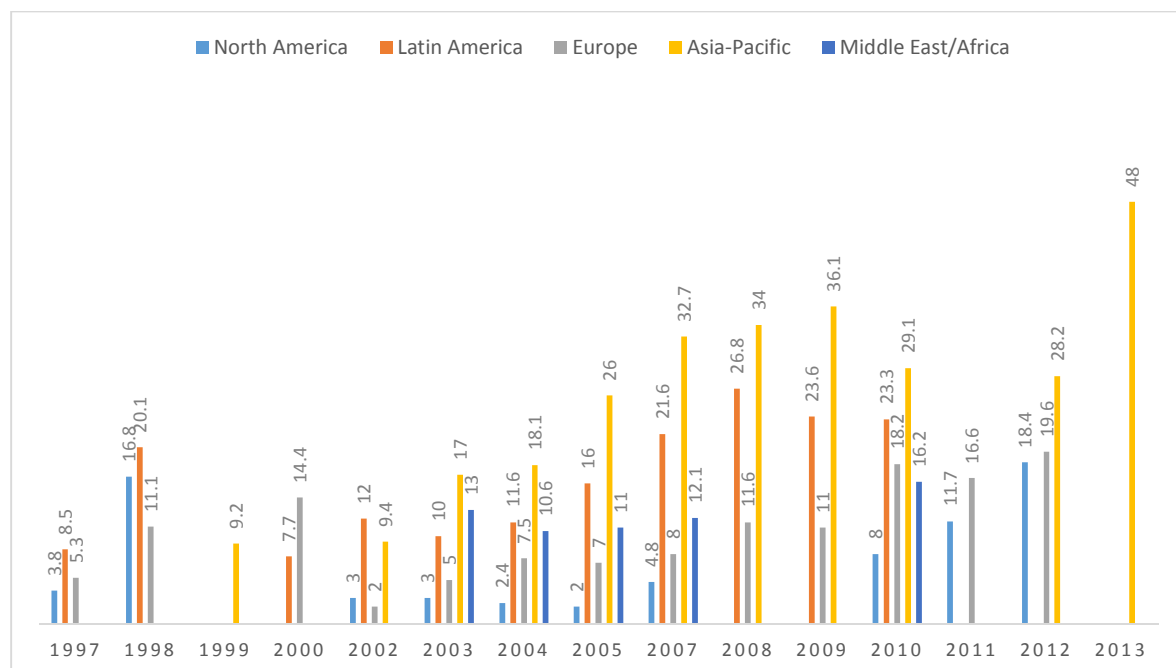
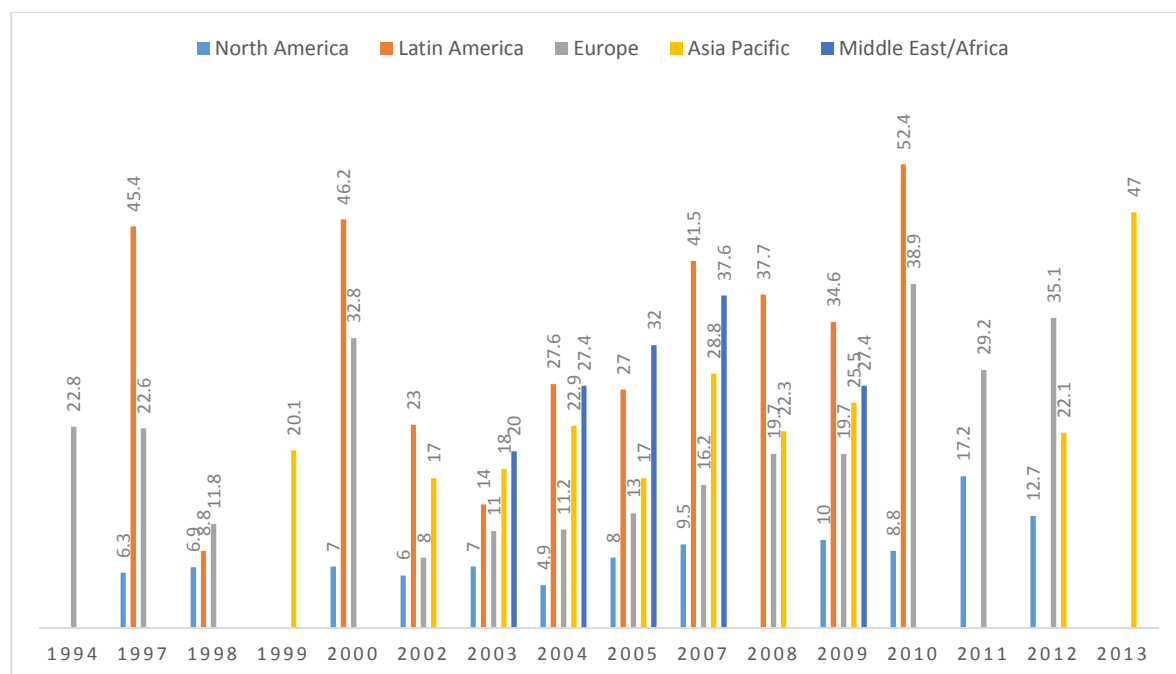


Figure 13. Patten of global prevalence of ESBL-KP



In Latin America, the prevalence of ESBL-EC and ESBL-KP seems to be much higher than North America. The prevalence of ESBL-EC remained more or less stable

at an average of 10.3% between 2000 and 2004. Since then, the rates peaked to 26.8% in 2008 and remained stable at an average of 23.3% for the next two years. The prevalence of ESBL-KP in Latin America were considerably high during 1997 and 2000, with an average of 45.8%. The rates declined by half to 23% in 2002 and thereafter rose steadily to 41.5% in 2007. After a minor drop to 34.6% in 2009, it rose spectacularly to 52.4% in 2010. Compared to ESBL-EC, the prevalence rates of ESBL-KP fluctuated in these regions.

The prevalence of ESBL producers were higher in the European countries compared to North America. While Scandinavian countries have the least prevalence rates (2-8%), those on Eastern bloc such as Poland, Bulgaria, Turkey have very high prevalence rates (40%) [104, 105]. The prevalence rates of ESBL-EC in Europe rose briefly from 5.3% in 1997 to 14.4% in 2000 and then fell sharply to 2% in 2002. Thereafter, the rates gradually increased from 5% in 2003 to 19.6% in 2012. As with Latin America, fluctuations in the prevalence rates of ESBL-KP were noted. The rates averaged 22.7% during 1994-96. After a brief fall to 11.8% in 1998, it rose sharply to 32.8% in 2000. Thereafter, the rate plummeted to 8% in 2002 and then witnessed a gradual increase over the next six years to 19.7% in 2008. In the 2009, the rate almost doubled to 38.9% and in the next two years, it averaged at 32.2%.

Asia-Pacific is a very diverse region and therefore the prevalence rates are bound to contrast across regions. The prevalence rates of ESBL-EC rose sharply from 9.2% to 36.1% in a decade (1999-2009). The rates dipped to an average of 28.7% for the next 2-3 years and then rose sharply to 48% in 2013. The prevalence rates of ESBL-KP in Asia Pacific region fluctuated without major spikes at a rate between 17% and 28.8% during the period 1999-2012. In the recent 2013 study, the rate has almost doubled to 47%. In contrast to the Asian regions, the prevalence rates in the Pacific

countries (Australia and New Zealand) are disproportionately low. The prevalence rates in Australia rose from 0.2-12% among ESBL-EC and 5.9-15% among ESBL-KP between 1998-99 and 2013.

Data from surveillance of the Middle East and African countries are scarce and appear in the literature from the year 2003 onwards. The prevalence rates of ESBL-EC have risen gradually from 13% in 2003 to 16.2% in 2010. With respect to ESBL-KP, the rates rose gradually from 18% in 2003 to 37.6% in 2007, thereafter the rate fell to 25.5% in 2009.

4.5 Global distribution of ESBL types

The distribution of ESBL types among the ESBL producers vary considerably. While some ESBL types have remained endemic in the region of their origin, few others have spread across continents. At the same time, some ESBL types such as CTX-M are found to be replacing the TEM or SHV type ESBLs.

In the North American countries of Canada and USA, CTX-M-15 and CTX-M-14 are the predominant ESBL types among Enterobacteriaceae members. In recent studies from USA majority of ESBLs were found to be of CTX-M type [106, 107]. Other CTX-M types that are common to both these countries include CTX-M-2, CTX-M-3 and CTX-M-27. CTX-M-9 and CTX-M-24 have been reported additionally, from Canada [108]. SHV-12 and SHV-2 are commonly reported from both these countries. Apart from these two SHV types, SHV-2a occur in Canada, whereas SHV-5 and SHV-7 are reported from USA. Among the TEM type ESBLs, TEM-10 and TEM-12 were predominantly seen in USA, followed infrequently by TEM-26 and TEM-155. TEM-11, TEM-12 and TEM-52 types have been reported from Canada [106-108].

The Latin American countries too are dominated by the CTX-M ESBLs; CTX-M-2 is frequently reported from Argentina, Brazil, Peru and Bolivia. Apart from this, CTX-M-8 has been observed in isolates from Brazil and Bolivia whereas CTX-M-14 is reported both from Bolivia and Peru. CTX-M-15, which is the predominant type in Mexico has also been reported from Brazil, Peru and Bolivia. Among the SHV type ESBLs, SHV-2 and SHV-5 are the ones that are reported from these countries. In addition to these major ESBL types, PER-2 from Argentina and TLA-1 from Mexico are the minor ESBLs that too are often reported [109-111].

Presently, TEM type ESBLs are infrequently reported from Europe, but earlier studies reported presence of TEM-3, TEM-5, TEM-7, TEM-15, TEM-24, TEM-26, TEM-47 and TEM-52. Among the SHV type ESBLs, SHV-2, SHV-3, SHV-5 and SHV-12 have been reported from several parts of Europe. CTX-M-15 is now reported from almost every part of Europe. Among the Scandinavian countries, CTX-M-1, CTX-M-2 and CTX-M-9 are often reported. In the Eastern countries of Europe, such as Russia and Poland CTX-M-1, and CTX-M-3 are endemic. In the western European countries such as Spain and Portugal, CTX-M-9 and its derivative, CTX-M-14 are endemic. Nine different types of CTX-M enzymes have been reported from studies conducted at United Kingdom. CTX-M-2 is reported from Belgium, Italy, France, Spain and Russia. CTX-M-32 has been reported from Spain, Portugal, Italy and UK [105, 112, 113].

Among the Asian countries, China seems to have maximum variants of CTX-M enzymes among the Enterobacteriaceae members. While CTX-M-3, CTX-M-14, CTX-M-15 and CTX-M-9 are the most commonly reported types, CTX-M-22, CTX-M-24, CTX-M-27, CTX-M-55 and CTX-M-79 are found less commonly. SHV-12, SHV-5, and SHV-13 are the predominant SHV types reported from China [114, 115].

Studies from Japan too reveal predominant presence of CTX-M enzymes among their isolates: CTX-M-2, CTX-M-9, CTX-M-1, CTX-M-15 and CTX-M-14 are the commonly reported types. Apart from these, CTX-M-8, CTX-M-27, CTX-M-3, and CTX-M-25 are also reported from different parts of Japan. Reports of TEM and SHV type ESBLs are scarce from Japan; only SHV-2, SHV-12, SHV-24, SHV-27 and TEM-28 have been reported [116, 117]. Among the South Korean isolates, CTX-M-14 is clearly the dominant type, although the prevalence of CTX-M-15 producers is rapidly increasing. Besides these two types, CTX-M-3, CTX-M-27, CTX-M-57, CTX-M-9, CTX-M-12, CTX-M-22 and CTX-M-24 are also reported in the literature. Among the SHV type ESBLs, SHV-12 and SHV-2a are the only types that are frequently reported. Among the TEM type ESBLs, TEM-52 is widely reported among Enterobacteriaceae isolates [118, 119]. In the rest of South Asian countries, CTX-M-14, CTX-M-15 and CTX-M-3 are the commonly reported ESBL types. Besides these, the less commonly reported types include CTX-M-9, CTX-M-22, CTX-M-24, CTX-M-27 and CTX-M-55. Among the SHV types, SHV-2, SHV-2a, SHV-5, SHV-12 and SHV-27 ESBLs are often encountered among Enterobacteriaceae isolates. VEB-1 type minor ESBLs have been reported in isolates from Thailand and Vietnam [120].

Enterobacteriaceae isolates from Australia are dominated by CTX-M-1 group enzymes, mainly belonging to the CTX-M-15 type. Other infrequently detected CTX-M types include CTX-M-3 and CTX-M-14. Among the SHV type ESBLs, SHV-12 is the most commonly reported type among the isolates [120]. Presence of various ESBL types across the continents is depicted in Figure 14.

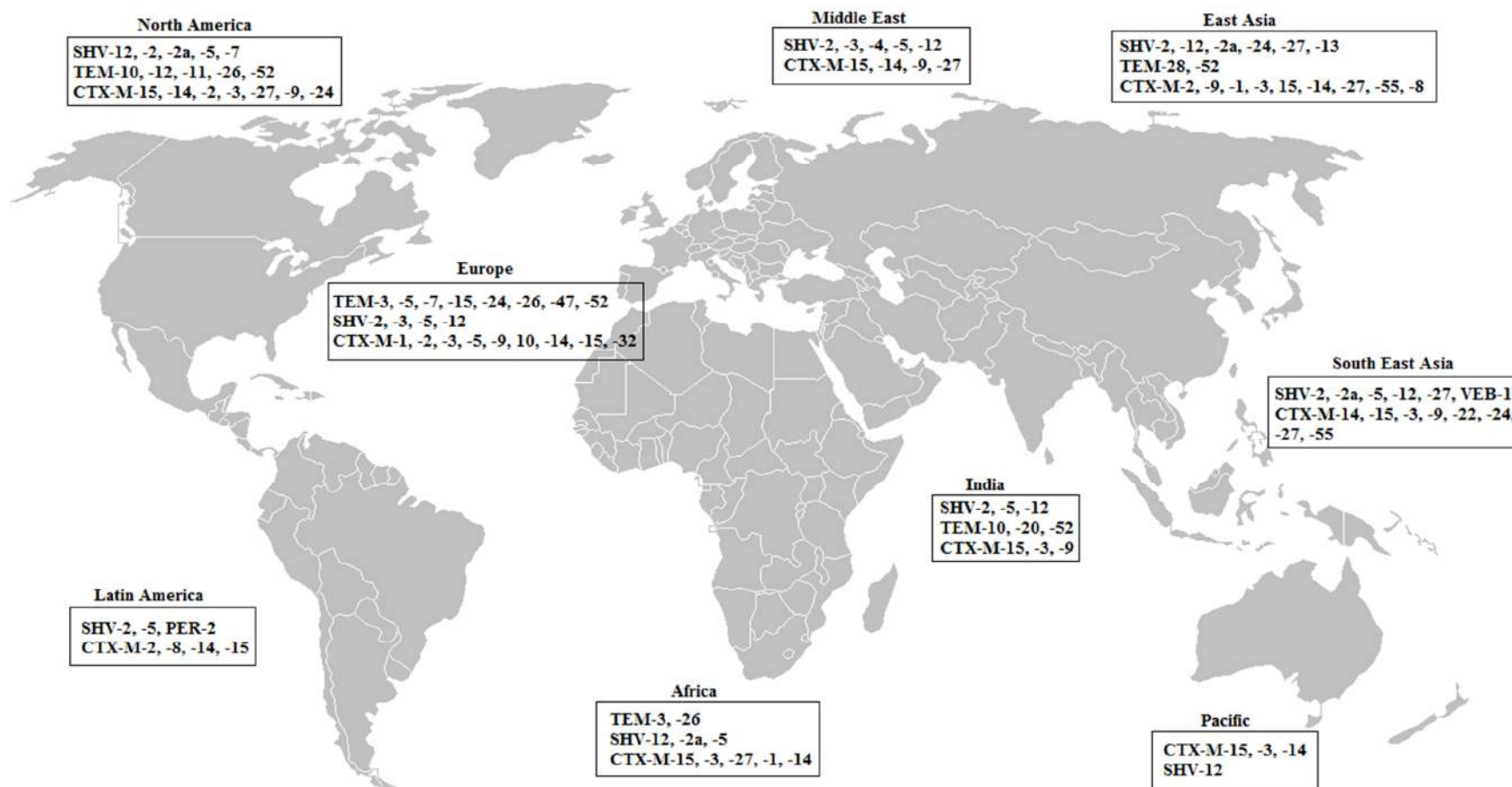
4.6 Prevalence of ESBL producers in India

There are no national surveillance program to monitor the prevalence of ESBL producers in India, hence data was collected from international surveillance studies, which included multiple centers from India. As a part of the MYSTIC surveillance programme, bacterial samples were collected from six cities (New Delhi, Lucknow, Indore, Mumbai, Bangalore and Vellore) across India prior to 2000. The prevalence of ESBL producers in that study was very high; 92% of *E. coli* and 96% of *K. pneumoniae* were found to be ESBL producers [121]. Interestingly, another publication of the same study reported ESBL production in >61% and >55% among *E. coli* and *Klebsiella spp*, respectively [122].

India was included in the Asia-Pacific SMART study in 2007 and samples collected from nine centers in that period showed prevalence of ESBLs in 79% of *E. coli* and 69.4% of *K. pneumoniae* isolates [123]. In one of the centers included in that study, a prevalence rate of 94.1% was observed among *E. coli* isolates. ESBL producers were found responsible for 78.9% of both nosocomial and community acquired infections in that study.

In the 2008 Asia-Pacific SMART study, samples collected from seven centers across India reported a prevalence of ESBLs in 61.2% of *E. coli* and 46.8% *K. pneumoniae* isolates [124]. The prevalence rates increased marginally in the subsequent study. The 2009 Asia-Pacific SMART study, reported a prevalence rate of 67.1% among *E. coli* and 56.8% among *K. pneumoniae* isolates [125].

Figure 14: World map showing distribution of various ESBLs



In the same period, multinational Asian Network for Surveillance of Resistant Pathogens (ANSORP) surveillance study reported similar prevalence (57.1%) of ESBL-KP from Indian centers [126]. The regional Resistance Surveillance (RRS) program in the Asia-Pacific (APAC) region reported a slightly increased prevalence of ESBLs; 78% of *E. coli* and 64% of *K. pneumoniae* isolates were found to be ESBL producers [127].

Although there are numerous studies from various parts of India, they differ widely in patient population, sample type and size, detection methodology and the study period. Hence, even with a vast amount of data, meaningful interpretation is difficult to achieve. In the period 2013-14, the rates of ESBL detection among *E. coli* ranged from 24-90% and that of *K. pneumoniae* ranged from 9-80% in the Northern parts of India [128,129]. In the Eastern parts, the rates ranged from 12-89% among *E. coli* and 26-93% among *K. pneumoniae* isolates [130-132]. In the central parts of India, the rates ranged from 41-50% among *E. coli* and 26-48% among *K. pneumoniae* isolates [133, 134]. In the western India, the rates ranged from 20-62% among *E. coli* and 15-67% among *K. pneumoniae* isolates [135-137]. In the southern India, the rates ranged from 18-73% among *E. coli* and 33-63% among *K. pneumoniae* isolates [138-140]. The ESBL detection rates in India during the 2013-14 period is displayed in Figure 15. Compilation of several studies across India from 1995 to 2014 along with references is presented in Annexure 2.

4.7 ESBL types in India

The first report of an ESBL producer from India came in 1998, when SHV-5 ESBL was detected among *Salmonella senftenberg* isolated from patients during an outbreak in a burns ward at Delhi [141]. In 2001, Karim *et al* reported detection of

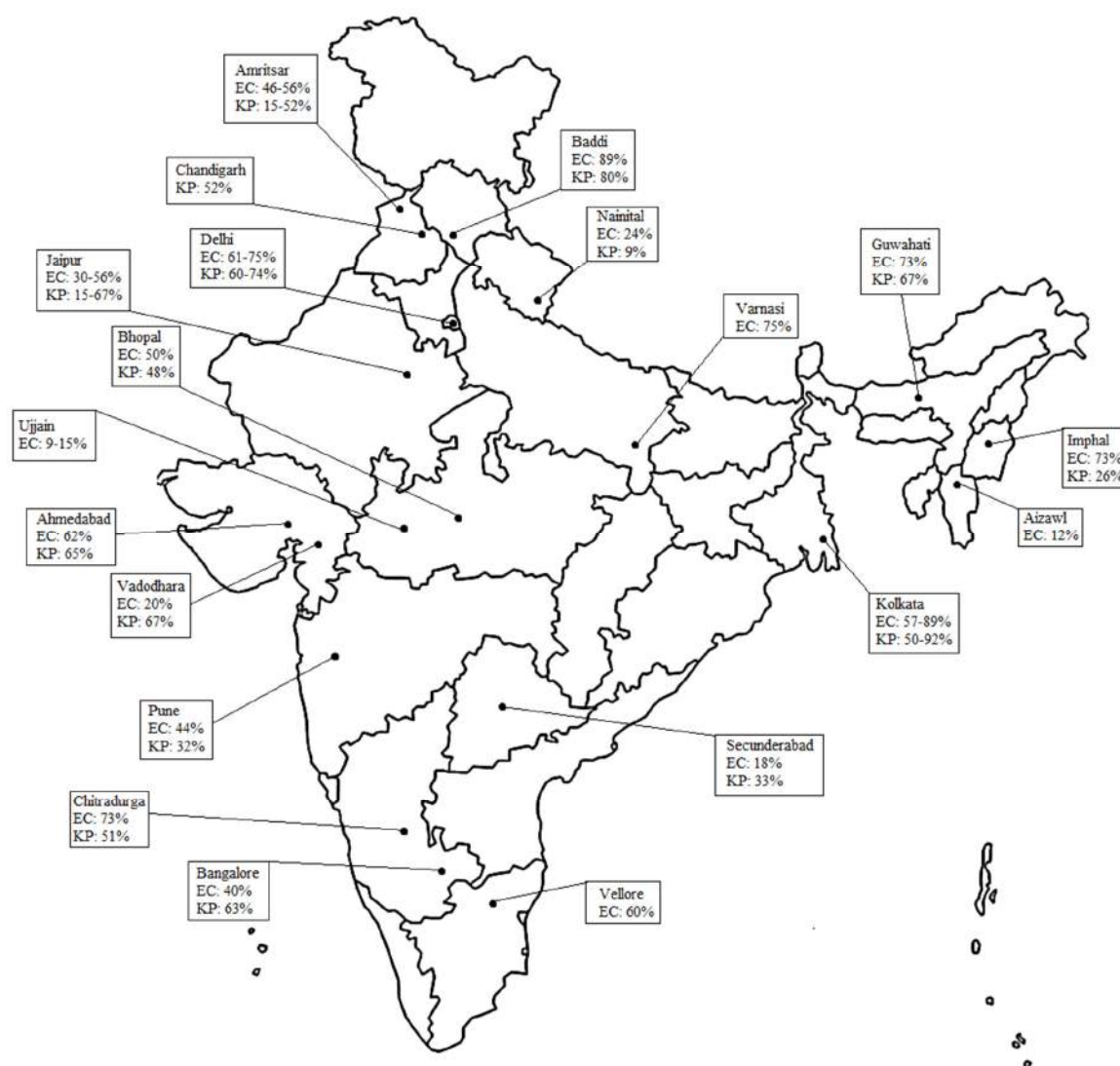
CTX-M-15 in clinical isolates of *E. coli*, *K. pneumoniae*, and *E. aerogenes* collected in 1999 at a hospital in Delhi [76]. This was the first account of a CTX-M enzyme that was also capable of hydrolyzing ceftazidime.

In 2006, Ensor *et al* reported detection of CTX-M-15 in 73% of *E. coli* and *K. pneumoniae* isolates collected from centers located in Aligarh, Varanasi and Hubli [142]. In response to this publication, Walsh *et al* reported that their study team, which had collected samples from New Delhi, Mumbai, Indore, Lucknow, Bangalore and Vellore prior to 2000, had detected CTX-M-15 in 83% of *E. coli* and 75% of *Klebsiella* spp [121]. CTX-M-15 still continues to be the dominant CTX-M type in India. Several studies from India have detected *bla*CTX-M genes among the clinical isolates by amplifying only a part of the *bla*CTX-M gene. Many of these studies had used multiplex PCR protocols, which allow the researchers to detect only the CTX-M groups but not their individual members.

In 2008, a study from Chennai reported detection of CTX-M-1 ESBL among Enterobacteriaceae members [143]. Authors of that study, used the same primer pairs for another study and reported detection of CTX-M-28 [144]. CTX-M-1, CTX-M-15 and CTX-M-28 belong to the CTX-M-1 cluster; CTX-M-15 differs from CTX-M-1 by four amino acids but CTX-M-28 differs from CTX-M-15 by one amino acid at position 289. The primers used by the authors of those two studies are more suitable for detecting CTX-M-15 ESBLs than CTX-M-1 or CTX-M-28. The possibility of misidentification of CTX-M-28 by using primers designed for CTX-M-15 has been pointed out by Menezes *et al* [145].

As a part of the Tigecycline Phase 3 Clinical Trials conducted during 2002-06, *E. coli* and *K. pneumoniae* strains collected from India were reported to possess CTX-M-2, CTX-M-5, CTX-M-15 and CTX-M-28 genes [146].

Figure 15: Prevalence of ESBL producers in parts of India (2013-14)



Until this report, CTX-M-15 was the only CTX-M type reported in India. First report of CTX-M-9 group ESBL in India came from Kolkata in 2011; the CTX-M-9 group genes were detected in isolates of *E. coli* and *K. pneumoniae* from the feces of infants [147]. Authors of that study also reported presence of CTX-M-14-like and CTX-

M-27-like sequences in those isolates. A pharmaceutical company based in Baddi, Himachal Pradesh, which undertook a study involving multiple clinical samples from several hospitals, reported CTX-M-9 group gene in an *E. coli* isolate [129]. In a study on diabetic foot ulcers conducted at Varanasi, 70% of *E. coli* were positive for *bla*CTX-M-15, and one isolate each possessed *bla*CTX-M-1, *bla*CTX-M-3 and *bla*CTX-M-9 [148]. Tripathi *et al* from Kolkata reported detection of CTX-M-72-like enzyme from *K. pneumoniae* isolate [149].

In 2009, a report from Chennai detected SHV-28 in a clinical isolate of *K. pneumoniae* and claimed it to be an ESBL since the isolate gave positive ESBL phenotypic test and isoelectric focusing revealed only a single band [150]. As of now, SHV-28 is not confirmed to be an ESBL. Among the few reports on SHV type ESBLs, SHV-12, followed by SHV-2, SHV-4 and SHV-5 and are the only ones reported from India [129-131, 148, 151]. There are two reports of TEM type ESBLs from India, both of which were found in *E. coli* isolates. While TEM-10, TEM-20 and TEM-52 were detected at Varanasi, TEM-3 was detected at Baddi (HP) [148, 129].

5. Laboratory detection of ESBLs

In 1999, the National Committee for Clinical Laboratory Standards (NCCLS), which is now known as CLSI, published methods for screening and confirming the presence of ESBLs in *K. pneumoniae*, *K. oxytoca*, and *E. coli* (M100-S9) [152]. The detection involved two steps; a screening test to detect resistance to oxyimino-cephalosporins and a confirmatory test based on inhibition of ESBL by clavulanic acid. Since then, the guidelines on the performance and interpretation of ESBL detection methods have been revised by CLSI. In 2010, CLSI recommended in its document M100-S20 that "routine ESBL testing is no longer necessary before reporting results"

and that it was no longer necessary to edit results for cephalosporins, aztreonam, or penicillins to resistant if the isolate was identified as an ESBL producer [153]. However, CLSI also maintains that “until laboratories implement the new interpretive criteria, ESBL testing should be performed as described”. Over the years, several rapid methods including chromogenic media, automated systems and molecular methods have been devised to decrease the reporting period and to increase the sensitivity as well as the specificity of ESBL detection.

5.1 Screening methods

Screening methods involve detection of resistance to any of the third generation cephalosporin antibiotics such as cefotaxime, ceftazidime, ceftriaxone, cefpodoxime or aztreonam by disk diffusion method or by MIC estimation. The current breakpoints for resistance to antibiotics are shown below [154]:

Antibiotic	Disk diffusion			MIC determination	
	μg	<i>E. coli</i> , <i>K. pneumoniae</i> & <i>K. oxytoca</i>	<i>P. mirabilis</i>	<i>E. coli</i> , <i>K. pneumoniae</i> & <i>K. oxytoca</i>	<i>P. mirabilis</i>
aztreonam	30	≤ 27 mm	-	≥ 2 $\mu\text{g/ml}$	-
ceftriaxone	30	≤ 25 mm	-	≥ 2 $\mu\text{g/ml}$	-
ceftazidime	30	≤ 22 mm	≤ 22 mm	≥ 2 $\mu\text{g/ml}$	≥ 2 $\mu\text{g/ml}$
cefotaxime	30	≤ 27 mm	≤ 27 mm	≥ 2 $\mu\text{g/ml}$	≥ 2 $\mu\text{g/ml}$
cefpodoxime	10	≤ 17 mm	≤ 22 mm	≥ 8 $\mu\text{g/ml}$	≥ 2 $\mu\text{g/ml}$

CLSI recommends use of multiple antibiotics to increase the sensitivity of screening. Breakpoint MIC levels of the screening agents can also be obtained by automated antibiotic susceptibility systems such as MicroScan Walk-Away (Dade Behring Inc, Phoenix) and VITEK-2 (bioMerieux, France). These methods provide accurate results in a short period of time, but are expensive.

Commercial screening media incorporate third-generation cephalosporins and resistance exhibited by the test isolates are detected by either growth on those media or a change in colour of colonies. While BLSE agar (AES Laboratory, France) is a non-chromogenic medium, ChromID ESBL (BioMérieux, Lyon, France), Brilliance ESBL (Oxoid, Basingstoke, United Kingdom), ESBL-Bx (bioMérieux, France) and CHROMagar ESBL (CHROMagar, Paris, France) are chromogenic media [155, 156]. The chromogenic substrates may differ among the various chromogenic agars, but ESBL producing bacteria are identified by the colour of their colonies as recommended by the manufacturer. The advantage of chromogenic ESBL screening media is that by directly culturing the clinical samples on these media, ESBL-producing Enterobacteriaceae members can be detected in a short period of time. In a comparative study, non-chromogenic media were found to possess sensitivity ranging from 74.6 to 86.5% and specificity ranging from 82 to 96.8%. The sensitivity of ChromID ranged from 88.2 to 97.3% and specificity ranged from 90.4 to 95.5%. The sensitivity of Brilliance ESBL ranged from 94.9 to 100% and specificity ranged from 93.3-95.7% [157]. Quicolor agar (QC ES) (Salubris Inc., Massachusetts, USA) is a chromogenic medium, which changes colour within 4-6 hours due to the metabolic activity of growing bacteria. This medium can be used instead of Mueller Hinton agar. Upon growth, the red colour of this medium turns to yellow whereas the inhibition zones remain red [158]. While these media have high sensitivities, they often have lower specificities.

5.2 Detection of ESBL production by phenotypic methods

The methods to detect ESBLs rely on a) inactivation of a chromogenic substrate by a rapid test, b) inactivation of indicator antibiotic by the extracts of the cells by three-

dimensional tests, or more specifically by c) inhibition of ESBLs by clavulanic acid (clavulanate).

5.2.1 Rapid tests:

Commercially available rapid tests such as β -Lacta test (Bio-Rad, France) and Cica- β -Test (Kanto Chemical, Tokyo, Japan) employ HMRZ-86, a novel chromogenic cephalosporin to detect beta-lactamase activity [159-161]. The former test gives positive results with ESBLs, MBLs, and de-repressed AmpC beta-lactamases, but not by broad-spectrum beta-lactamases such as TEM-1 or SHV-1. The latter test is more specific as it uses specific beta-lactamase inhibitors for each class of beta-lactamases. While the appearance of red colour in the former test is considered positive for the presence of beta-lactamases, failure to develop red colour is considered positive in the latter test. With the former test, it is not possible to differentiate between the three beta-lactamase types. The sensitivity and specificity of the β -Lacta test were reported to be 96% and 100%, respectively when tested for *E. coli* and *K. pneumoniae* [159]. Another study, which investigated its use in the detection of ESBL producers directly in urine samples, reported sensitivity of 94% and specificity of 100% [160]. The sensitivity and specificity of Cica- β -Test was found to range from 74 to 96% and from 57 to 98%, respectively [157, 161]. Further tests are required to establish their utility in detection and differentiation of the prevalent beta-lactamases.

5.2.2 Three dimensional tests:

Three-dimensional (3D) tests are employed to detect hydrolytic activity of ESBLs against indicator antibiotics. In case of direct 3D tests, the extracts of test isolates are tested against itself, whereas in indirect 3D test, the extracts of test isolates are tested against a standard susceptible strain [162, 163]. In direct 3D test, the extracts

of the test isolate is prepared first and lawn culture of the same isolate is made on a Mueller Hinton agar plate. A disk containing indicator antibiotic (e.g., cefotaxime) is placed at the center. A small volume of the extract is dispensed close to the antibiotic disk in a variety of ways, such as a narrow trough, a slit or a well. If the extract contains ESBL, it would inactivate the antibiotic that has diffused in the medium and following incubation, the zone of inhibition would get distorted. This method works only if the isolate produces some zone of inhibition around the indicator disc. This limitation can be overcome in the indirect 3D test by using a fully susceptible standard strain (*E. coli* ATCC 25922) to make lawn culture. Both these methods are labour intensive and may yield non-specific or false positive results.

5.2.3 Tests based on inhibition by clavulanic acid

Since inhibition by clavulanic acid is a cardinal property of ESBLs, tests that use this inhibitor to reverse ESBL mediated resistance are naturally preferred. Clavulanate based tests can be performed as disk diffusion test, agar screen test, agar or broth dilution test, or E-tests. Even the commercially available automated systems work on this principle.

5.2.3.1 Double Disk Synergy Test (DDST)

The test, which is also known as Double Disk Diffusion test (DDDT) was introduced by Jarlier *et al* in 1988 [43]. It relies on the action of clavulanic acid to augment the zone of inhibition. In this test, following the lawn culture of the test isolate, a clavulanate containing disk (such as amoxycillin+clavulanic acid 30/10 µg) is placed at the center of the plate and disks of cefotaxime (30 µg), ceftazidime (30 µg), aztreonam (30 µg) or cefpodoxime (10 µg) are placed around it at a variable distance of 20-30 mm and incubated. Augmentation of zone of inhibition towards the centrally

place disk is considered as positive for ESBL production. The test suffers from poor sensitivity due to variations in the distance of disk placement, indeterminate results and subjective reading by the researcher. The sensitivity of the test is also affected by the choice of antibiotic, the type of ESBL produced by the organism and the distance of placement of disks. The sensitivity has been shown to vary from 55-100% but the specificity vary from 72-100% [164-166].

5.2.3.2 Inhibitor-potentiated disk diffusion (IPD)

Limitation of DDST test can be overcome by this method as it gives a quantitative result. This method involves use of two plates of Mueller Hinton agar; one with 4 µg/ml clavulanic acid and the other without. Standardized inoculum of the test isolate is swabbed on the surface of both agar plates and disks of cefotaxime, ceftazidime, ceftriaxone and aztreonam are placed on both of them and incubated overnight at 37°C. A difference in the zone diameter of ≥ 10 mm around disc(s) in plates with clavulanic acid versus disks on plain Mueller Hinton agar is considered positive for ESBL production [167]. The sensitivity of this method is also affected by the choice of antibiotic and type of ESBL produced by the organism. The sensitivity has been shown to vary from 55 - 100% whereas the specificity is generally high (~100%). When the interpretative zone size difference is changed from 10 to 5 mm, the sensitivity has been shown to increase but with a fall in specificity [164, 167]. This method gives good results but the limitation of this method is the requirement of a stock solution of clavulanic acid, which is highly temperature sensitive and has short shelf-life.

5.2.3.3 CLSI Phenotypic Confirmatory Test (PCT)

Also known as Combined Disk Method, this test is recommended by the CLSI for confirmation of ESBL production in isolates of *E. coli*, *K. pneumoniae*, *K. oxytoca*

and *P. mirabilis* [154]. The test can be performed either as disk diffusion or by MIC estimation and both these methods are mostly in agreement with each other. Since CLSI PCT method is quantitative, it eliminates the subjectivity in interpretations.

In the disk diffusion method, a standardized inoculum of the test isolate is swabbed on the surface of a Mueller Hinton agar plate. Ceftazidime (30 µg), ceftazidime+clavulanic acid (30/10 µg), cefotaxime (30 µg), cefotaxime+clavulanic acid (30/10µg) disks are placed on the plate and incubated overnight at 37°C. Confirmatory testing requires use of both cefotaxime and ceftazidime, alone and in combination with clavulanic acid. An increase in the zone diameter by ≥ 5 mm around the disks with clavulanic acid over the disks with cephalosporins alone confirms ESBL production.

In microbroth dilution tests, the organism is tested against different dilutions of cefotaxime and ceftazidime alone as well as in combination with 4 µg/ml of clavulanic acid. The dilutions are- ceftazidime 0.25–128 µg/ml, ceftazidime+clavulanic acid 0.25/4–128/4 µg/ml, cefotaxime 0.25–64 µg/ml and cefotaxime+clavulanic acid 0.25/4–64/4 µg/ml. A reduction in MIC by ≥ 3 two-fold dilutions for cephalosporin with clavulanic acid versus the MIC of cephalosporins alone confirms ESBL production.

Even though the two methods yield similar results, disk diffusion method is preferred as it is easier to perform and is cheaper than the microbroth dilution method. Both the sensitivity and specificity of this method shown to be $>90\%$ [158, 160]. False positive results may occur with isolates producing KPC-beta-lactamases or chromosomal K1 beta-lactamases. False negative results may occur in isolates that also produce high levels of AmpC beta-lactamases.

5.2.3.4 E-test

ESBL E-test (bioMérieux, France) strips are thin, inert and non-porous plastic carriers measuring 5 x 60 mm. One side of the strip is calibrated with MIC reading scales in $\mu\text{g/ml}$ while the reverse surface carries two predefined antibiotic gradients. There are two E-test strips for ESBL detection; one strip (CT/CTL) contains cefotaxime gradient at one end and cefotaxime+clavulanic acid gradient at the other end. The other strip (TZ/TZL) contains ceftazidime gradient at one end and ceftazidime+clavulanic acid at the other end. Testing for ESBL must be performed with both the strips. The MIC is read as the point where the inhibition ellipse intersects the edge of the E-test strip. Following concentration gradients are used with these strips: Cefotaxime (0.25-16 $\mu\text{g/ml}$) at one end and cefotaxime+clavulanic acid (0.016 - 1 $\mu\text{g/ml}$) plus 4 $\mu\text{g/ml}$ of clavulanic acid at the other end. Ceftazidime (0.5 - 32 $\mu\text{g/ml}$) at one end and ceftazidime+clavulanic acid (0.064 - 4 $\mu\text{g/ml}$) plus 4 $\mu\text{g/ml}$ clavulanic acid at the other end. The presence of an ESBL is confirmed if MIC of cefotaxime or ceftazidime is reduced by ≥ 3 two-fold dilutions in the presence of clavulanic acid. Production of ESBL is also inferred by the appearance of a phantom zone or deformation in the ellipse. E-test confirmatory strips are convenient but are expensive. The sensitivity of E-test has been shown to vary from 94-98% and the susceptibility vary from 59-84% [166, 168, 169]. Since the concentration gradient in E-test strip is not as extensive as recommended by the CLSI, it yields more indeterminate results than CLSI phenotypic confirmatory test. Indeterminate result can also occur due to co-production of AmpC beta-lactamases.

5.2.3.5 Automated systems:

Automated ESBL detection tests such as Vitek Legacy and Vitek 2 (BioMérieux), MicroScan (Siemens Medical Solutions Diagnostics), Sensititre (TREK Diagnostic Systems), and Phoenix (BD Diagnostic Systems) detect ESBLs on the principle of inhibition by clavulanic acid. Studies have shown that these systems have sensitivities ranging from 84-100% and specificities ranging from 52-98% [166, 169-171]. Automated systems offer the advantage of rapid detection of ESBLs in clinical isolates but are also prohibitively expensive.

5.3 Detection of ESBL production in isolates also producing AmpC beta-lactamases

Various strategies have been developed to overcome the false negative results in clavulanate based tests due to co-expression of AmpC beta-lactamases in the test isolates. AmpC beta-lactamases are able to interfere in these tests, as they are not inhibited by clavulanic acid. Hence, the activity of clavulanic acid can be unmasked by selectively inhibiting AmpC beta-lactamases. Amino-phenylboronic acid (APB) and cloxacillin are good inhibitors of AmpC beta-lactamases. Disk diffusion tests involving clavulanate can be modified by incorporating 200 µg/ml of cloxacillin in the Mueller Hinton agar [172]. CLSI PCT can be modified by supplementing the antibiotic disks with 400 µg/ml of APB [173]. Isolates co-producing ESBLs and AmpC beta-lactamase would produce a zone of ≥ 5 mm around disk having cefotaxime+ clavulanic acid+APB over disk with cefotaxime+ clavulanic acid only. Another approach to detect ESBLs in isolates co-producing both these enzymes is to use cefepime, as this fourth generation cephalosporin is unaffected by AmpC beta-lactamases. A 30 µg disk of cefepime has been used in addition to the other cephalosporin disks in DDST test [174]. CLSI-PCT

has been modified by including cefepime disk and cefepime+ clavulanic acid disk [175]. Similarly, additional E-test strips with cefepime at one end and cefepime+ clavulanic acid at the other end has also been introduced [176]. In all these tests, clavulanic acid based inhibition of ESBL becomes apparent since cefepime suppresses AmpC beta-lactamase activity.

5.4. CLSI vs EUCAST guidelines

Currently, there are two widely popular guidelines on antimicrobial susceptibility testing, the CLSI and EUCAST (European Committee on Antimicrobial Susceptibility Testing) [154]. CLSI guidelines originating from USA are more popular and are followed in many countries across the globe. The EUCAST guidelines are used in the European countries (available at http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Consultation/EUCAST_guidelines_detection_of_resistance_mechanisms_121222.pdf). The two guidelines are not in complete agreement with each other and there are few differences between the two regarding screening and detection of ESBL. For screening purposes, CLSI recommends disk diffusion or MIC microbroth dilution method whereas EUCAST additionally recommends MIC agar dilution method. CLSI recommends use of cefpodoxime, cefotaxime, ceftriaxone, ceftazidime or aztreonam for screening by disk diffusion or MIC estimation. EUCAST recommends ceftazidime, ceftriaxone and cefotaxime for both MIC estimation and disk diffusion. EUCAST guidelines mentions that although cefpodoxime is a sensitive indicator for detection of ESBL production, it is less specific than the combination of cefotaxime (or ceftriaxone) and ceftazidime. While CLSI guidelines are restricted to be applied only on *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis*, the EUCAST has developed algorithm applicable for several Enterobacteriaceae members. As per EUCAST guidelines MIC value of ≥ 1 µg/ml of

cefotaxime, ceftriaxone or ceftazidime is considered as a positive screening test. According to CLSI guidelines, MIC ≥ 8 $\mu\text{g/ml}$ for cefpodoxime or MIC ≥ 2 $\mu\text{g/ml}$ for ceftazidime, aztreonam, cefotaxime, or ceftriaxone; and for *P. mirabilis*, MIC ≥ 2 $\mu\text{g/ml}$ for cefpodoxime, ceftazidime, or cefotaxime is considered as a positive screening test. There are differences between EUCAST and CLSI guidelines in the prescribed zone diameters for disk diffusion testing. While the diameter of zone of inhibition of ceftazidime is common to both CLSI and EUCAST at 22 mm, they are different for cefotaxime and ceftriaxone. It is 21 mm and 27 mm for cefotaxime whereas 23 mm and 25 mm for ceftriaxone according to EUCAST and CLSI, respectively. Unlike CLSI guidelines, which recommend 30 μg disk for cefotaxime, ceftriaxone and ceftazidime antibiotics, EUCAST recommends 5 μg cefotaxime disk, 10 μg ceftazidime disk and 30 μg ceftriaxone disk. There are no guidelines from CLSI on detection of ESBLs in other Enterobacteriaceae members or in cases where isolates may also co-produce AmpC beta-lactamases.

For phenotypic detection of ESBLs, both CLSI and EUCAST recommend testing with ceftazidime, cefotaxime alone and in combination with clavulanic acid. While CLSI recommends the combination disk method and microbroth dilution method for detection of ESBLs, EUCAST also permits two other detection methods including Double Disk Synergy Test (DDST) and E-test method. CLSI guidelines on ESBL detection applies only to *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis*, the EUCAST guidelines extend this recommendation to *Shigella spp* and *Salmonella spp* as well. Additionally, EUCAST guidelines recommend usage of cefepime/cefepime+clavulanic acid when *E.coli*, *Klebsiella spp.*, *P. mirabilis*, *Salmonella spp.*, and *Shigella spp* test negative by ceftazidime and cefotaxime based test. Cefepime/cefepime+clavulanic acid combination has been recommended by

EUCAST for detection of ESBL among *Enterobacter spp.*, *Citrobacter freundii*, *Morganella morganii*, *Providencia stuartii*, *Serratia spp.*, and *Hafnia alvei* whereas CLSI offers no such guidelines for these organisms.

Because of the disagreements in the guidelines and protocol, there is bound to be variation in the screening and detection of ESBLs depending on which of the two protocols is followed by the researcher.

5.5 Molecular techniques in the detection and identification of ESBLs

Detection and identification of beta-lactamase (*bla*) gene became accurate when nucleotide sequencing techniques were introduced. Earlier, the detection of *bla* genes was undertaken by DNA hybridization assay using labeled probes, but with the advent of Polymerase Chain Reaction (PCR) technology, detection and identification of *bla* genes have become more convenient.

5.5.1 Nucleic acid based molecular techniques

By designing suitable primers, entire *bla* gene can be amplified by PCR technique. By detecting specific parts of the *bla* gene, beta-lactamases can be identified to a certain extent but for complete accuracy, the entire *bla* gene must be amplified and sequenced. Conventional PCR can be run either as uniplex or multiplex reactions; the latter offers the convenience of detecting multiple *bla* genes at the same time and at a lesser cost [177, 178]. In order to skip the laborious post-PCR detection and validation methods, real-time PCR has been developed. Using well designed primers and probes, real-time multiplex PCR can detect multiple gene types in a much shorter time [179]. However, reproducibility of the results seems to be a limitation of this technique [180].

Conventional DNA sequencing methods such Sanger's dideoxy chain termination involves purification of PCR product, which adds to the delay in obtaining

results. Sequencing reactions are often outsourced to specialist service providers, which also contributes to the delay. New generation sequencing technologies such as pyrosequencing and ion semiconductor sequencing promises to provide better results at much shorter time [181, 182]. Some of the new generation sequencing technologies have been made as bench top instruments that can be used in diagnostic laboratories [183]. Despite these advancements, DNA sequencing is beyond the means of many researchers due to its prohibitive costs.

Alternatives to DNA sequencing for the identification of *bla* gene have been developed; these include PCR-RFLP (Restriction fragment length polymorphism) and Restriction Site Insertion-PCR (RSI-PCR). In PCR-RFLP, the PCR amplicons are digested with specific restriction endonuclease and then subjected to electrophoresis, which are then compared with the profiles of known *bla* genes [184]. For this technique, a thorough knowledge of nucleotide sequences, expected position of mutations and appropriate selection of endonuclease is necessary. Using this technique, only some of the already known *bla* types can be identified. A major limitation of this method is the absence of natural restriction site at the point of interest. This limitation has been overcome by introducing an artificial restriction site of choice by a technique called the Restriction Site Insertion-PCR (RSI-PCR). In this technique, the primers are designed to bind to areas where mutations are expected. These primers are modified at their 3' end so that they contain additional one or three nucleotides that are not complementary to the target, but their presence creates an artificial restriction site when the target gets amplified [185]. The restriction site would be created in the amplicon only when the PCR amplification is successful. Following PCR reaction, the amplicons are subjected to restriction digestion using specific endonuclease. By using a series of different primers and endonucleases, it is possible to accurately identify the point mutations. By

suitably modifying the restriction sites, new variants can also be detected. This method has been used to detect several variants of *bla*SHV genes [185]. A major limitation of this technique is that detection of mutations is possible only where it is feasible to introduce a restriction site.

Other nucleic acid based technologies such as Ligase chain reaction (LCR), PCR-Single-Strand Conformational Polymorphism (PCR-SSCP) and Real-Time PCR - Melting Curve Analysis (MCMD) have been explored by a few researchers to identify the *bla* types. Although many of these new technologies have good discriminatory power to differentiate single nucleotide changes, they are restricted to the detection of existing types only. PCR-SSCP has been used to detect a limited SHV mutant forms and discrimination of inhibitor resistant TEM types [186, 187]. RT-PCR MCMD has been used to detect SHV genes and to differentiate non-ESBL from ESBL genes but this technique cannot identify individual SHV types [188, 189].

Microarray technology relies on the binding of specific sequences in the organisms with pre-designed probes that are immobilized on the array matrix. This way, a very large number of genes can be detected simultaneously. Check KPC/ESBL (Check-Points HealthBV, Netherlands) is a commercially available, microarray-based diagnostic test system that can rapidly detect and identify *bla* genes belonging to the TEM, SHV, CTX-M, and KPC types [190]. This technology has also been shown to detect genes coding for antibiotic resistance in bacteria from processed blood samples, thereby reducing the time involved in detection of specific genes [191]. Most of these systems take 6-8 hours for results to be available but their high costs make them beyond reach for many diagnostic establishments.

5.5.2 Non-nucleic acid based molecular techniques

Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) is new technique to analyze biological molecules. The substance to be evaluated is subjected to a laser beam, which ionizes the components. These ions are accelerated into a tube, which drift towards the detector at a rate proportional to their molecular weight. The time taken for the particles to reach the detector is measured, from which the mass-to-charge ratio of the ions is calculated. The generated mass spectra are analyzed by dedicated software and compared with stored profiles. This technology has been used in the identification of β -lactams and β -lactam degradation products. Bacterial cultures are incubated with β -lactam antibiotic, centrifuged, and then MALDI-TOF MS is used to analyze the supernatants for the presence of β -lactam degradation products [192]. This technology has been shown to discriminate ESBL-producing from non-ESBL producing clinical isolates of *E. coli*, *K. pneumoniae* and *P. aeruginosa* [193]. This technology is still in the nascent stage and requires thorough evaluation of its specificity.

6. Clinical significance of ESBLs

When the ESBLs were detected during late 1980s and early 1990s, they were often seen in *K. pneumoniae* isolates causing hospital infections. ESBL-KP have been incriminated in several outbreaks of nosocomial infections [194]. Thereafter, ESBLs began to be detected in *E. coli* isolates especially those causing community acquired infections [195]. In many studies CTX-M ESBLs have been detected predominately among *E. coli* causing community acquired infections, especially the urinary tract infection [86].

In early studies, the intestinal colonization by ESBL producers were noticed in hospitalized patients or those living in long-term health care facilities. Now, intestinal colonization is known to occur in healthy individuals living in the community [86]. Prior colonization by ESBL producers at the time of hospital admission has been shown to be a significant risk factor for developing serious infections [195]. Thus, colonized individuals serve as a source for their own infection. At the same time, they can be a potential source of colonization or infection in other patients in the hospital. For this reason, some hospitals mandatorily screen the patients on admission for ESBL producers and quarantine them, if found positive [196]. Those patients who become carriers of ESBL producers in the hospital, remain so for a long time and then disseminate them in the community [197]. Therefore, it becomes important to undertake de-colonization measures [198].

Third generation cephalosporins are the antibiotics that are used commonly to treat a variety of infections produced by *E. coli* and *K. pneumoniae*. These are also used in the empirical treatment of serious infections such as intra-abdominal infection or sepsis. By inactivating the cephalosporin antibiotics, ESBL producing bacteria can result in treatment failure. Several controlled studies have been carried to determine relationship with infection by ESBL producers and the outcome of such infections. The results of these studies have been conflicting; while some studies have shown positive association, some others have not found significant association between ESBL production and the outcome of treatment [199-206]. However, in most of these studies infection by ESBL producing bacteria have been shown to be significantly associated with prolonged hospital stay and increased morbidity.

Treatment of infections by ESBL producers has been complicated by the fact that many of these bacteria produce multiple beta-lactamases, which confer resistance

to many class of antibiotics [207]. In some bacteria, resistance to carbapenems occur due to combination of ESBL production, low level production of AmpC beta-lactamases and loss of porin proteins [208]. It must also be borne in mind that resistant strains can emerge during the course of treatment, therefore repeated susceptibility testing during the course of treatment may be warranted in severe infections [209, 210]. In many hospitals, carbapenems have replaced cephalosporins as the preferred antibiotic. Just as excessive use of cephalosporins are thought to have led to widespread resistance, resistance to carbapenems is also on the rise.

7. Treatment of infections produced by ESBL producers

Until 2010, CLSI had suggested to report resistance to all cephalosporins if the organism was found to be an ESBL producer. Subsequently, this recommendation was withdrawn [152, 153]. Yet, not much enthusiasm is seen in using the cephalosporins as there are reported cases of treatment failure with cephalosporins even when the MIC values were in the susceptible range [199, 211]. Prior exposure to third generation cephalosporin antibiotics for treatment has been shown to be a risk factor for acquiring subsequent bacteremia by ESBL producing bacteria, hence these antibiotics must be used with caution [212]. Treatment failure can occur despite low MIC values because of the so-called inoculum effect, where a high microbial load leads to rise in MIC levels [213, 214]. In intra-abdominal infections, the microbial loads are often high and the level of antibiotic attained at site is often not sufficient, which may lead to treatment failure.

Cefepime, a fourth generation oxyimino-cephalosporin, is variably hydrolyzed by ESBLs. It is resistant to hydrolysis by stably de-repressed AmpC beta-lactamase but is hydrolyzed by CTX-M ESBLs including CTX-M-15. Susceptibility to cefepime from Asia-Pacific area has been reported to range from 33 to 93% in *E. coli* and from

with the use of ceftiofur in treatment of infections by *K. pneumoniae* is the emergence of resistance due to the selection of porin mutants [221]. A study conducted in Taiwan on treatment of bacteremic patients with ESBL producing *K. pneumoniae* suggested that flomoxef was similar to carbapenem in clinical efficacy [222]. Doi A *et al* successfully used cefmetazole for the treatment of UTIs caused by ESBL-producing organisms and found no difference in clinical cure rate in the group treated with cefmetazole versus the group treated with carbapenem [223]. The authors opined that cefmetazole can be used for the treatment of UTIs as long as the organisms do not produce AmpC beta-lactamase.

Several combinations of beta-lactam and beta-lactamase inhibitors are available for clinical use. These include amoxicillin + clavulanic acid, ampicillin + sulbactam, cefoperazone + sulbactam, ticarcillin + clavulanic acid, piperacillin + tazobactam and cefepime + tazobactam. The combinations have not been very effective in the treatment of ESBL-producing Enterobacteriaceae because of poor drug concentrations achieved at site of infection, co-production of other beta-lactamases, hyperproduction of classical beta-lactamases such as TEM-1 or SHV-1 and induction of AmpC beta-lactamases by clavulanic acid [13, 224].

Despite the encouraging in-vitro results, there not many clinical studies on these combinations. Rodriguez-Bano J *et al* and Park SH *et al* have found amoxicillin + clavulanic acid and piperacillin + tazobactam (PCT) to be suitable alternatives to carbapenems for treating bloodstream infection and community acquired acute pyelonephritis caused by ESBL-producing *E. coli*, respectively, provided the in-vitro results displayed susceptibility [225, 226]. A Spanish study, which investigated 30-day mortality in patients with bacteremia by ESBL-EC suggested that PCT may be used in the treatment of bacteremia with origins from urinary tract despite elevated MIC levels,

but if the source is other than urinary tract, PCT may be used only if MIC is $\leq 2 \mu\text{g}/\mu\text{l}$ [227]. As a part of hospital policy, PCT was reportedly used for six years in a Korean hospital in lieu of oxyimino-cephalosporins. Their study revealed that not only the incidence of infection by ESBL producers came down but also no increase in resistance to PCT was observed in that period [228]. There are very few studies on the clinical efficacy of cefepime + tazobactam. A study by Ghafur *et al*, showed that this combination can be safely used in the treatment of bacteremia by Gram negative bacteria [229]. Cefoperazone, a second generation cephalosporin has been combined with tazobactam or sulbactam. A study from Mumbai reported susceptibility to cefoperazone + sulbactam and cefoperazone + tazobactam in 89% of ESBL-producing *E. coli* [230]. In that study cefoperazone + tazobactam was found to be more active than cefoperazone + sulbactam (83% vs 67%) against ESBL-KP. In an in-vitro study from Mumbai, cefoperazone + sulbactam was shown to be significantly more effective than PCT against ESBL producing Enterobacteriaceae [231].

Mecillinam, is an extended-spectrum oral penicillin with good in-vitro activity against Enterobacteriaceae members. Since the concentration of this drug is achieved at good levels in the urine, it is used in the treatment of UTIs by *E. coli* isolates. Mecillinam is not stable to hydrolysis by beta-lactamases and also suffers from the inoculum effect. Both the resistance and the inoculum effect have been shown to be reversed when it was combined with clavulanic acid. Thomas K *et al* and Lampri N *et al* have suggested that it may be useful in the treatment of uncomplicated lower UTIs caused by ESBL-EC with low MIC values [232, 233].

Temocillin, a derivative of ticarcillin is active only against Enterobacteriaceae members, with no significant activity against non-fermenters, anaerobes or Gram positive organisms [234]. It is more stable than the parent drug to hydrolysis by ESBLs

and AmpC beta-lactamases. The susceptibility breakpoint for temocillin is not available in CLSI document S100-S24. In a study by Livermore *et al* in UK, >88% of the isolates producing ESBL or AmpC beta-lactamases were susceptible to temocillin at ≤ 16 $\mu\text{g/ml}$ and 99% of them were susceptible at ≤ 32 $\mu\text{g/ml}$ [235]. In a clinical study from UK, temocillin use was associated with clinical cure in 93% of cases with UTI and 83% of cases with bloodstream infection by ESBL or AmpC producing Enterobacteriaceae members [236]. Because good levels of this antibiotic is achieved in the urine, this antibiotic has been suggested as an alternative to carbapenem in the treatment of UTI by ESBL/AmpC beta-lactamase producers.

Carbapenems such as imipenem, meropenem, doripenem and ertapenem are widely used as antibiotics of first choice for the treatment of serious infections due to ESBL producing Enterobacteriaceae members. These antibiotics are preferred since they are resistant to hydrolysis by different types of beta-lactamases, achieve good tissue concentrations, do not suffer from inoculum effect and are not potent inducers of AmpC beta-lactamases. A retrospective cohort study on bloodstream infections by ESBL producing *E. coli* and *K. pneumoniae* found similar outcomes when treated with ertapenem, imipenem or meropenem [237]. Use of ertapenem as a first-line antibiotic in treating different infections by ESBL producers has been associated with good clinical response [238, 239]. Vardakas KZ *et al* reported that use of carbapenems as empirical antibiotic in the treatment of bloodstream infection by ESBL producers was associated with lower mortality than those treated with combinations of beta-lactam + beta-lactamase inhibitors but the difference was not found to be significant [240]. Clinical failures have been reported to occur during the course of treatment with carbapenems, which have been attributed to selection of porin mutants and co-

production of AmpC beta-lactamase or KPC enzymes [241, 242]. Other important causes of carbapenem resistance is the production of metallo-beta-lactamases.

Ceftaroline fosamil, a fifth generation cephalosporin, is active against Enterobacteriaceae members but is ineffective against isolates producing ESBLs, cephalosporinases, and carbapenemases. Avibactam (previously known as NXL104) is a new non-beta-lactam beta-lactamase inhibitor that inhibits Ambler classes A (e.g., ESBL, KPC), C (AmpC), and D (OXA-like) beta-lactamases [20]. The combination of ceftaroline and avibactam is known to have expanded spectrum of activity. At a fixed concentration of 4 µg/ml, ceftaroline + avibactam combination was shown to be active against Enterobacteriaceae strains producing ESBLs, plasmid-mediated AmpC, carbapenemases, and a combination of multiple enzymes [243]. In that study, the ceftaroline + avibactam MICs for ESBL producers ranged from 0.015/4 – 1/4 µg/ml. In another study, 98.2% of ESBL producing isolates were reportedly inhibited by ceftaroline + avibactam at MIC of < 0.5/4 µg/µl [244]. This combination was shown to be effective against producers of CTX-M, SHV as well as KPC beta-lactamases with activity comparable to that of imipenem. In another in-vitro study, the combination of ceftaroline + avibactam was shown to be active against 100% of ESBL-EC and 96% of ESBL-KP [245].

Ceftolozane + tazobactam is a novel cephalosporin and beta-lactamase inhibitor combination that has improved activity against *P. aeruginosa* and several Enterobacteriaceae members. Although interpretive criteria for ceftolozane + tazobactam susceptibility has not been established, an American study has shown that it was active against most ESBL-EC isolates at MIC of 0.5/4 µg/ml [246]. However, its potency was much lower against ESBL-KP, which was probably due to co-production

of carbapenemases in those isolates. Further clinical trials are awaited for its approval as a therapeutic agent.

Tigecycline, is a novel glycylcycline antibiotic, which inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit of the bacteria. Report from Tigecycline Evaluation and Surveillance Trial (TEST) conducted in USA reported a susceptibility rate of 100% to ESBL-EC and 92% to ESBL-KP [247]. A study from Argentina reported susceptibility of 99.8% against ESBL-EC and 93.7% against ESBL-KP [248]. Roy *et al* reported that all the ESBL-EC in their study were susceptible to tigecycline but 3.5% of ESBL-KP were resistant to it [131]. The authors also observed that over a period of four years (2007-10), the MIC levels of tigecycline increased by two-folds, which was comparatively higher among *K. pneumoniae* than *E. coli*.

Fosfomycin, which inhibits an enzyme that catalyzes the first step in bacterial cell-wall synthesis, has a broad spectrum of antimicrobial activity. The MIC breakpoints for susceptibility against urinary isolates is 64 µg/ml. A study on ESBL producing urinary isolates in USA showed that 91.3% of CTX-M and 100% of SHV or TEM type ESBL-EC were susceptible to fosfomycin [249]. In a study from India, all the ESBL producing urinary isolates of *E. coli* tested by them were susceptible to fosfomycin [250]. Clinical cure with fosfomycin in patients with UTI produced by ESBL-EC has been shown to vary from 78.8% to 93.8% [251, 252].

Apart from the aforementioned antibiotics, others such as aminoglycosides, fluoroquinolones and trimethoprim + sulfamethoxazole may be used if the ESBL producers are in the susceptible range. Park SH *et al*, reported that the efficacy of antibiotics such as amikacin, ciprofloxacin and trimethoprim + sulfamethoxazole were similar to that of carbapenems in treating community acquired acute pyelonephritis

caused by ESBL-EC [226]. Hsueh PR reported that aminoglycosides and polymyxins (colistin or polymyxin B) can be used for the treatment of multidrug-resistant organisms or serious UTIs when treatment with first line antibiotics fail [253]. However, many CTX-M ESBL producing *E. coli* possess plasmids that also code for resistance to fluoroquinolones and aminoglycoside [254, 255].

Thus, there are multiple approaches to treat infections by ESBL producing Enterobacteriaceae members such as beta-lactam + beta-lactamase inhibitor combinations, mecillinam, temocillin, fosfomycin and tigecycline. These antibiotics may be encouraged to prevent overuse of carbapenems.

Current interest in ESBL

A timeline in the history of significant discoveries is displayed in Figure 17. Ever since the discovery of the ESBL in 1983, these enzymes have been increasingly reported from all parts of the world. Due to the increased awareness, many researchers are taking interest in its detection and identification.

Both EUCAST and CLSI do not recommend testing for ESBLs in clinical isolates on a routine basis anymore. Despite that studies on ESBL continue to be published. By the first week of January 2015, 28 articles have been indexed in PubMed with keyword “ESBL”. A search on PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>) with query of “ESBL” OR "extended spectrum beta-lactamase" OR "extended spectrum β -lactamase" showed up over 5400 results. The actual number would be even more as several journals are not indexed in PubMed. Year wise data on number of literature published with the aforementioned search terms has shown sharp increase since 1989.

Figure 16: Number of publications indexed in PubMed citing ESBL

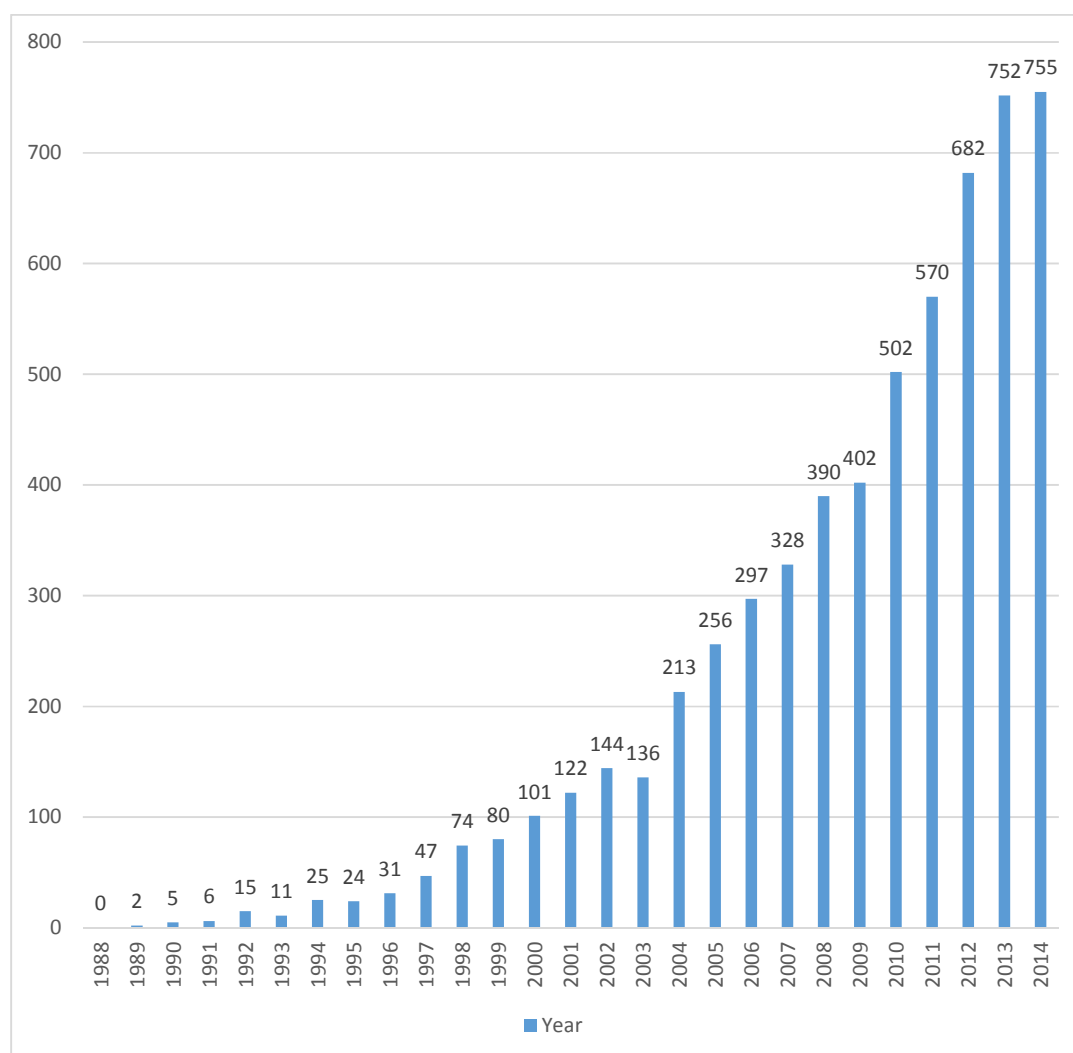
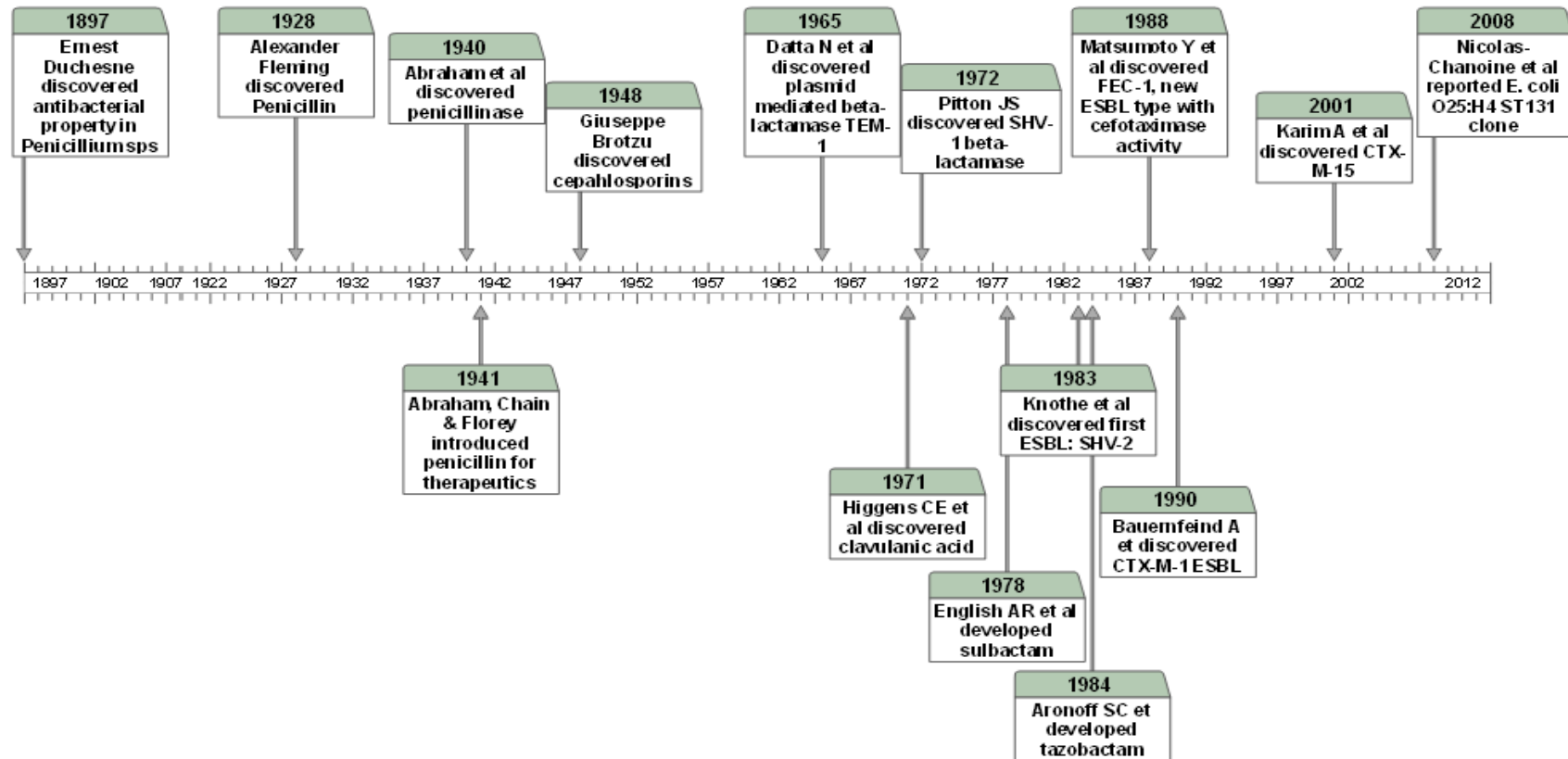


Figure 17: Timeline of significant milestones in the history of beta-lactamase mediated resistance and its inhibition



Aims & Objectives

1. To screen and detect ESBL production by phenotypic methods among the clinical isolates of *E. coli* and *K. pneumoniae* collected from five different centers across Karnataka, namely- Mangalore, Dharwad, Bellary, Davangere and Kolar.
2. To detect *bla*TEM, *bla*SHV and *bla*CTX-M genes in 200 isolates each of ESBL producing *E. coli* and *K. pneumoniae* by PCR technique.
3. To identify beta-lactamase (*bla*) type by complete nucleotide and amino acid sequences of the *bla* gene.
4. To find out the prevalence of ESBL types among *E. coli* and *K. pneumoniae* with respect to five centers studied.
5. To determine the antibiotic susceptibility pattern of ESBL producers to beta-lactam antibiotics and combinations of beta-lactam + beta-lactamase inhibitors.
6. To correlate the antibiotic susceptibility pattern of ESBL producers with ESBL gene carried by them.

*Materials and
Methods -
Phenotypic*

Duration of the study:

The study spanned a period of five years, from May 2009 to May 2014.

Sample size:

The formula $n = (Z_{1-\alpha})^2 (P (1 - P)/D^2)$ was used to calculate the sample size, where P is the estimated proportion and D is the desired precision [256]. The power of study was set at 80%. Sample size was calculated using an approximate prevalence rate of 50%, confidence interval of 95% and precision of 5%. The value of $Z_{1-\alpha}$ at 5% level of significance is 1.96. Using this formula, a sample size of 385 was calculated, which was rounded off to 400 samples per center including 200 isolates of *E. coli* and 200 isolates of *K. pneumoniae*.

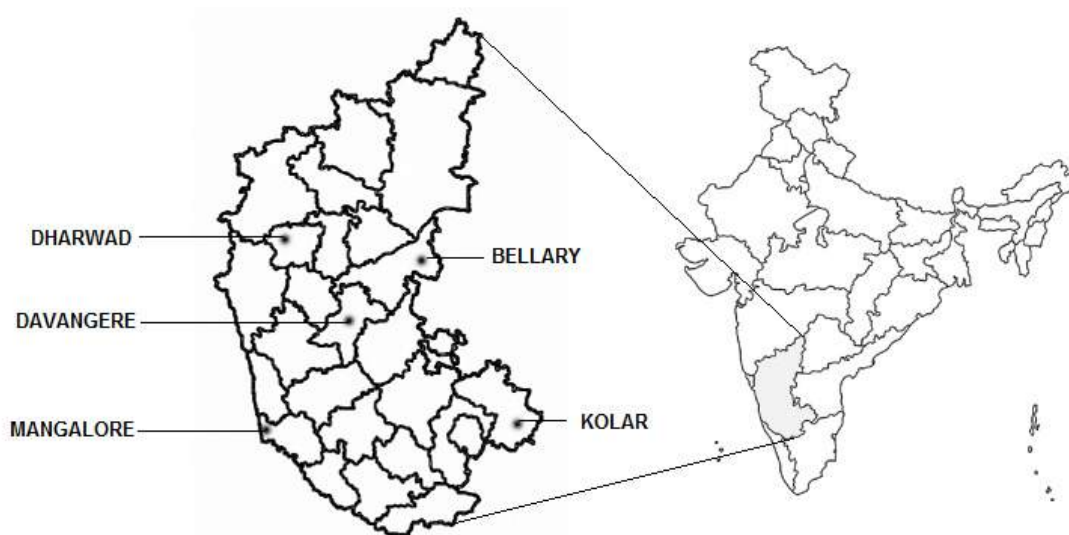
Sample collection:

One thousand isolates each of *E. coli* and *K. pneumoniae* were collected from the five centers across Karnataka. The contributing centers were the departments of Microbiology of the following medical colleges:

1. Kasturba Medical College, Mangalore
2. SDM college of Medical Science and Hospital, Dharwad
3. Vijayanagara Institute of Medical Sciences, Bellary
4. Jagadguru Jayadeva Murugarajendra Medical College, Davangere
5. Sri Devaraj Urs Medical College, Kolar

The isolates were obtained from the cultures of various clinical samples submitted by both out-patients and in-patients from these centers. Figure 18 shows the location of these centers in the map of Karnataka. The flowchart of methodology followed here is represented in Figure 19.

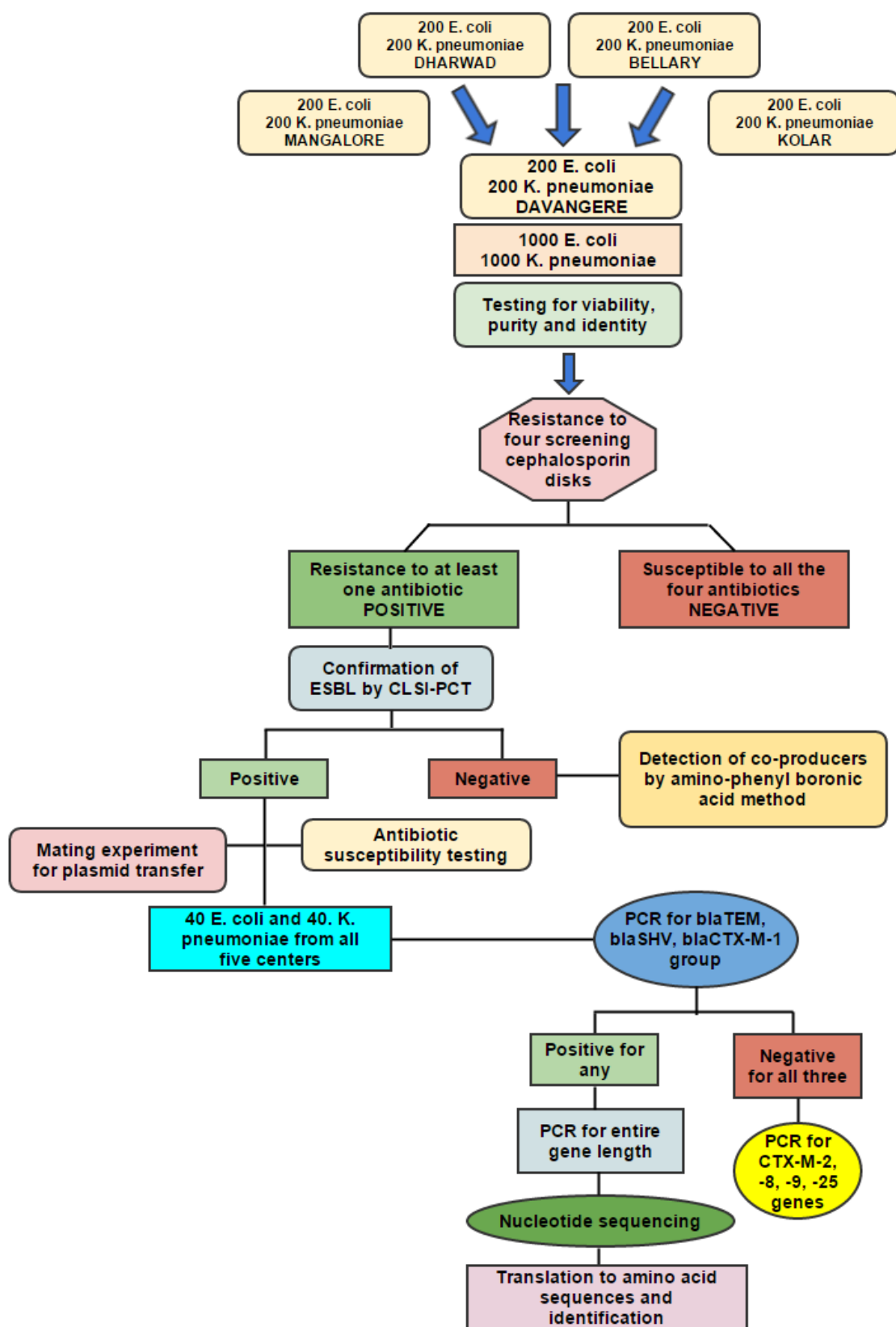
Figure 18: Locations of the five centers in the Karnataka map



Pure cultures of both *E. coli* and *K. pneumoniae* were received at the Department of Microbiology, JJM Medical College in Davangere. All the bacterial cultures were tested for viability and purity once again by sub-culturing on MacConkey agar. The identification of the isolates was re-confirmed by performing indole test, citrate utilization test, urease test, motility test, lysine decarboxylase and ornithine decarboxylase test. Properties used to characterize *E. coli* and *K. pneumoniae* are shown below:

Property	<i>E. coli</i>	<i>K. pneumoniae</i>
Motility test	Positive	Negative
Colonies	Smooth but dry, flat	Moist and mucoid, dome
Indole test	Positive	Negative
Citrate utilization test	Negative	Positive
Urease test	Negative	Positive
Ornithine decarboxylase test	Variable	Negative
Lysine decarboxylase test	Positive	Positive

Figure 19: Flowchart of methodology followed in this study



Phenotypic tests for the detection of ESBLs

Screening for ESBL production by disk diffusion test

Resistance to cefotaxime, ceftazidime, ceftriaxone and aztreonam was detected by disk diffusion test as recommended by CLSI. From the pure cultures of bacteria grown overnight on MacConkey agar, a suspension matching 0.5 McFarland standard (1.5×10^8 CFU/ml) was made in nutrient broth. Using sterile cotton swab, the bacteria were spread on Mueller Hinton agar to obtain a lawn culture. After allowing the plate to dry, the antibiotic disks were placed on the surface and the plates were incubated in air at 37°C for 18-24 hours. Following growth, the diameter of the zone of inhibition around the disks were measured and recorded. The disk potency and inhibition zone diameters used for inferring resistance is displayed in Table 5. Resistance to at least one of the antibiotics was considered as positive in the screening test for possible ESBL production as per 2010 CLSI guidelines [153].

Table 5: Disk potency and zone diameters for inferring resistance in the screening test

Antibiotic disk	Resistant, if zone diameter was
aztreonam (30 µg)	≤ 27 mm
ceftriaxone (30 µg)	≤ 25 mm
ceftazidime (30 µg)	≤ 22 mm
cefotaxime (30 µg)	≤ 27 mm

Confirmation of ESBL detection by Phenotypic Confirmatory Test (PCT)

Isolates of *E. coli* and *K. pneumoniae* that were considered to be positive for ESBL production by the screening test were subjected to the Phenotypic Confirmatory Test (CLSI-PCT) as recommended by 2010 CLSI guidelines [153]. From the pure

cultures of bacteria grown overnight on MacConkey agar, a suspension matching 0.5 McFarland standard (1.5×10^8 CFU/ml) was made in nutrient broth. Using sterile cotton swab, the bacteria were spread on Mueller Hinton agar to obtain a lawn culture. After allowing the plate to dry, disks of ceftazidime (30 µg) (CAZ), ceftazidime + clavulanic acid (30/10 µg) (CAC), cefotaxime (30 µg) (CTX), cefotaxime + clavulanic acid (30/10µg) (CEC) were placed on the surface and the plates were incubated in air at 37°C for 18-24 hours. Following growth, the diameter of the zones around the disks were measured and recorded. An increase in the zone diameter by ≥ 5 mm around the disks containing cephalosporin with clavulanic over the disks containing cephalosporin alone indicated ESBL production.

Controls: *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative and positive controls, respectively.

Detection of ESBL in isolates also producing AmpC beta-lactamase:

Isolates that were positive in the initial screening test but negative in the subsequent ESBL phenotypic confirmatory test were tested for co-production of AmpC beta-lactamases as described by Coudron [257]. Inhibition of AmpC beta-lactamase was achieved by adding 400 µg of amino-phenylboronic acid to antibiotic disks. The stock solution of amino-phenylboronic acid was prepared by dissolving 120 mg of amino-phenylboronic acid in 3 ml of dimethyl sulfoxide. Three milliliters of sterile distilled water was added to this solution. Twenty microliters of the stock solution was dispensed onto disks containing cefotaxime + clavulanic acid (30/10µg) and cefotaxime (30µg). Plain filter paper disks treated with amino-phenylboronic acid were used as controls. Disks were allowed to dry for 30 minutes and stored in airtight vials with desiccant at 4°C.

From the pure cultures of bacteria grown overnight on MacConkey agar, a suspension matching 0.5 McFarland standard (1.5×10^8 CFU/ml) was made in nutrient broth. Using sterile cotton swab, the bacteria were spread on Mueller Hinton agar to obtain a lawn culture. Cefotaxime, cefotaxime + clavulanic acid, cefotaxime + clavulanic acid + amino-phenylboronic acid, cefotaxime + amino-phenylboronic acid disks were placed on the surface of the plates and incubated in air at 37°C for 18-24 hours. Interpretation of the test was made as displayed below:

CTX	CTX + CLA	CTX + CLA+ PBA	CTX+ PBA	Interpretation
R	+	NA	NA	ESBL only
R	-	+	+	AmpC only
R	-	+	-	ESBL & AmpC

CTX = cefotaxime, CLA = clavulanic acid, PBA = amino-phenylboronic acid,

R = resistant, NA= Not applicable, (+) indicates an increase in inhibition zone diameter by ≥ 5 mm over CTX disk and (-) indicates increase in inhibition zone diameter by < 5 mm

Antibiotic susceptibility testing by disk diffusion:

Isolates confirmed as ESBL producers by CLSI-PCT were tested for susceptibility against cefixime + clavulanic acid (CMC), cefotaxime (CTX), cefotaxime + clavulanic acid (CEC), ceftazidime (CAZ), ceftazidime + clavulanic acid (CAC), cefepime (CPM), cefepime + clavulanic acid (CFC), cefepime + tazobactam (CPT) and imipenem (IMP). The disk potency and their interpretative zone diameter breakpoints as per 2010 CLSI guidelines are displayed in the Table 6 [153]. From the pure cultures of bacteria grown overnight on MacConkey agar, a suspension matching 0.5 McFarland standard (1.5×10^8 CFU/ml) was made in nutrient broth. Using sterile cotton swab, the bacteria were spread on Mueller Hinton agar to obtain a lawn culture. After allowing the plate to dry, the above mentioned disks were placed on the surface

and the plates were incubated in air at 37°C for 18-24 hours. Following growth, the diameter of the zone of inhibition around the disks were measured and recorded [363].

Table 6: Susceptibility breakpoints of antibiotics tested by disk diffusion method

Antibiotic	Disk potency	Susceptible	Intermediate	Resistant
	in µg		in mm	
*Cefixime + clavulanic acid	5/10	≥ 19	16-18	≤ 15
Ceftazidime	30	≥ 21	18-20	≤ 17
*Ceftazidime + clavulanic acid	30/10	≥ 21	18-20	≤ 17
Cefotaxime	30	≥ 26	23-25	≤ 22
*Cefotaxime + clavulanic acid	30/10	≥ 26	23-25	≤ 22
Cefepime	30	≥ 18	15-17	≤ 14
*Cefepime + clavulanic acid	30/10	≥ 18	15-17	≤ 14
*Cefepime + tazobactam	30/10	≥ 18	15-17	≤ 14
Aztreonam	30	≥ 21	18-20	≤ 21
Imipenem	10	≥ 16	14-15	≤ 16

* Interpretative criteria are not available in CLSI. Hence, breakpoints of the cephalosporin was applied to the combination

Estimation of Minimum Inhibitory Concentration (MIC) by agar dilution assay:

MIC of ceftazidime, cefotaxime, ceftriaxone and ceftioxin were estimated by agar dilution method against isolates identified as ESBL producers [258, 259]. Stock solutions at the concentration of 10,000 µg/ml, 1,000 µg/ml and 100 µg /ml of these antibiotics were made using the formula shown below.

$$\text{Weight (mg)} = \frac{\text{Volume (ml)} \times \text{concentration } (\mu\text{g/ml})}{\text{Antibiotic potency } (\mu\text{g/mg})}$$

Sterile distilled water was used both as solvent and diluent for cefotaxime, ceftriaxone and ceftazidime, whereas 0.1 N NaOH was the solvent and distilled water was the diluent for ceftazidime. Several aliquots of the stock solutions were dispensed in vials and held at -20°C until use. A dilution range of 128 to 0.25 µg/ml in Mueller Hinton agar was prepared for each antibiotic. Pre-determined volume of antibiotic stock solution was added to 50 ml of molten Muller Hinton agar per plate that was cooled to 45-50°C. Immediately, the molten agar was dispensed into sterile petri plates measuring 150 mm in diameter. The dilutions achieved in each plate for a total volume of 1900 ml of Muller Hinton agar is shown in the Table 7. The antibiotic and its dilution in each plate was labeled and kept at 4°C in the refrigerator until further use.

From the pure cultures of bacteria grown overnight on MacConkey agar, a suspension matching 0.5 McFarland standard (1.5×10^8 CFU/ml) was made in nutrient broth. It was further diluted 1 in 10 in sterile saline to give a final concentration of 10^7 CFU/ml. From this diluted suspension, two microliters were transferred to each plate with antibiotic of known dilution. Grids were marked on the reverse of the plates and up to 32 isolates were inoculated per plate. The plates were inoculated starting with the lowest concentration. A Mueller Hinton agar plate without any antibiotic was used as a control. The plates were allowed to sit at room temperature until all the spots dried and then incubated overnight in air at 37°C. Following incubation, the control plate was observed for growth.

The dilution of antibiotic that inhibited visible growth of the test organism was read as the MIC value. *E. coli* ATCC 25922 was used for quality control.

Interpretative susceptibility criteria by MIC estimation (in µg/ml) as per 2010 CLSI guidelines is shown in Table 8 [146].

Table 7: Volume and concentration of antibiotic solutions for 1900 ml Mueller Hinton agar

Stock concentration	solution	Volume of stock solution	Desired concentration
10, 000 µg/ml		24.32 µl	128 µg/ml
10, 000 µg/ml		12.16 µl	64 µg/ml
10, 000 µg/ml		6.08 µl	32 µg/ml
10, 000 µg/ml		3.04 µl	16 µg/ml
1, 000 µg/ml		15.2 µl	8 µg/ml
1, 000 µg/ml		7.6 µl	4 µg/ml
1, 000 µg/ml		3.8 µl	2 µg/ml
1, 000 µg/ml		1.9 µl	1 µg/ml
100 µg/ml		9.5 µl	0.5 µg/ml

Table 8: MIC interpretive criteria of the antibiotics tested by agar dilution method

Antibiotic	Susceptible	Intermediate	Resistance
Cefotaxime (CTX)	≤1	2	≥4
Ceftriaxone (CTR)	≤1	2	≥4
Ceftazidime (CAZ)	≤4	8	≥16
Cefoxitin (CN)	≤8	16	≥32

Transfer of plasmid by mating (conjugation) experiment:

Isolates phenotypically identified as ESBL producers were tested for transferability of the plasmid by conjugation (mating) experiment [260]. Test strains and the recipient *E. coli* J53 AzR⁺ strain were grown overnight separately in Luria Bertani broth at 37°C. The recipient strain is susceptible to cephalosporins but is resistant to 200 µg/ml of sodium azide. Cultures of test strain and recipient strains were mixed in a separate tube at 1:10 ratio and incubated at 37°C overnight. A volume of 50 µl of the mixture was placed as spots on Mueller Hinton agar containing cefotaxime (2 µg/ml) and sodium azide (200 µg/ml) and incubated at 37°C for up to 48 hours. Growth on this medium was interpreted as successful conjugation and such colonies were confirmed for ESBL production by CLSI-PCT. Inability of the original cultures of test strains and the recipient strain to grow on this medium served as internal control.

Statistical analysis:

Categorical data was analyzed by Chi-square test whereas z test for proportion was used to determine the relationship between groups. $P \leq 0.05$ was considered to be statistically significant. Statistical calculations were performed using software tools available online at <http://www.socscistatistics.com/tests/Default.aspx>

Sources of reagents:

All dehydrated culture media, antibiotic disks, sodium azide and ATCC strains were procured from Hi-Media Laboratories, Mumbai. The 0.5 McFarland standard tube was procured from Span Diagnostics, Surat. Pure laboratory grade antibiotic powders and dimethyl sulfoxide were procured from Sigma-Aldrich, Bangalore. *E. coli* J53 AzR⁺ strain was kindly provided by Dr. George Jacoby, Lahey Institute, MA, USA.

*Materials and
Methods-
Genotypic*

Selection of the culture samples:

ESBL producing isolates were preserved in 20% glycerol trypticase soy broth and stored at -20°C. From this collection, a representative sample of 200 isolates each of *E. coli* and *K. pneumoniae* were taken for further genotypic studies. Forty isolates of *E. coli* and *K. pneumoniae* from each of the five centers were taken using the systematic sampling method [261]. For this purpose, the number of ESBL producers detected from each of the centers was divided by 40 and quotient was found out. The isolates from each center were placed in a row and isolates were picked up on the basis of quotient obtained. Thus, in case of *E. coli* isolates, every fourth sample from Bellary, every third sample from Davangere and Mangalore and every second sample from Dharwad and Kolar were selected. In case of *K. pneumoniae* isolates, every second sample from each of the centers were selected. These isolates were sub-cultured on Mueller Hinton agar containing cefotaxime (2 µg/ml) and assessed for viability and purity.

Extraction of plasmid DNA

Bacterial plasmid was extracted using PureSol Plasmid Extraction Kit (Merck GeNei, Bangalore) as per manufacturer's instructions. For this purpose, the isolates were grown overnight in Luria Bertani broth with cefotaxime (2 µg/ml) at 37°C. In a centrifuge vial, 1.5 ml of this culture suspension was taken and centrifuged at 9000 RPM for one minute and the supernatant was discarded. The pellet was resuspended in 200 µl of Solution A and 10 µl of lysozyme. The tubes were placed in a dry bath at 95°C for one minute and immediately cooled on crushed ice for five minutes. The tubes were then centrifuged at 13,000 RPM for 10 minutes and 500 µl of the supernatant was transferred to another vial. To this, 500 µl of isopropanol was added, mixed well and incubated at room temperature for 5 minutes. The vials were then centrifuged for 10

minutes at 13,000 RPM and the supernatant was discarded. The resulting pellet washed with 150 µl cold 70% ethanol and centrifuged again at 13,000 RPM for 5 minutes. The supernatant was discarded and the pellet was air-dried. The pellet was resuspended in 25 µl of solution B with 2 µl of RNase A and held for five minutes. The tubes were then stored at -20°C until further study.

Polymerase chain reaction (PCR)

*bla*TEM and *bla*SHV genes were detected by uniplex PCR in two steps; first step involved screening by amplifying a part of these genes and the second step involved amplification of the complete *bla* gene. Apart from the *bla*TEM and *bla*SHV genes, all plasmid DNA samples were also tested for CTX-M group 1 gene. Samples that were positive for CTX-M group -1 gene, were tested again for *bla*CTX-M-15 gene. Samples that were negative for *bla*TEM, *bla*SHV and CTX-M group 1 genes were further tested for CTX-M groups -2, -8, -9 and -25 genes. Primer pairs, annealing temperatures and product sizes of all PCR reactions are provided in Table 9.

Primers:

Primer pairs for the detection of TEM, SHV (for screening), CTX-M groups (-1,-2,-8,-9,-25), CTX-M-15, SHV (for amplification and DNA sequencing) genes were used as described in previous publications [178, 262-264]. The primer pair for amplification and sequencing DNA of TEM gene was designed in this study using software 'Primer3: WWW primer tool' available online at http://biotools.umassmed.edu/bioapps/primer3_www.cgi. Primers were ordered from Integrated DNA Technologies (USA) through Proteogen Biosciences, Bangalore. They were reconstituted in sterile double distilled water and aliquots of 10 µM/L were made and held at -20°C.

Table 9: Primer pairs, annealing temperatures and product sizes of all PCR reactions

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	Temp	Product size	Ref.
TEM (screening)	CATTTCCGTGTCGCCCTTATTC	CGTTCATCCATAGTTGCCTGAC	58°C	800 bp	[262]
SHV (screening)	AGCCGCTTGAGCAAATTAAAC	ATCCCGCAGATAAATCACCAC	58°C	713 bp	[262]
CTX-M GROUP-1	AAAAATCACTGCGCCAGTTC	AGCTTATTCATCGCCACGTT	55°C	445 bp	[178]
CTX-M GROUP-2	CGACGCTACCCCTGCTATT	CCAGCGTCAGATTTTTCAGG	55°C	552 bp	[178]
CTX-M GROUP-8	TCGCGTTAAGCGGATGATGC	AACCCACGATGTGGGTAGC	55°C	666 bp	[178]
CTX-M GROUP-9	CAAAGAGAGTGCAACGGATG	ATTGGAAAGCGTTCATCACC	55°C	205 bp	[178]
CTX-M GROUP-25	GCACGATGACATTCGGG	AACCCACGATGTGGGTAGC	55°C	327 bp	[178]
CTX-M-15	CAGAATAAGGAATCCCATGGTT	GGCGATAAACAACGGAAT	53°C	938 bp	[263]
TEM (sequencing)	ATGAGTATTCAACATTTYCGTGTCG	ATGCTTAATCAGTGAGGCACCTA	54°C	855 bp	This study
SHV (sequencing)	GGGTTATTCTTATTTGTCGC	TTAGCGTTGCCAGTGCTC	51°C	928 bp	[264]

Master mix for screening TEM and SHV genes were prepared as follows:

Component	final concentration	Component volume
5x green buffer	1x	10 µl
25 mM MgCl ₂	1.75 mM	3.5 µl
10mM each dNTP mix	0.3 mM	1.5 µl
10 µM Forward primer	0.12 µM	0.6 µl
10 µM Reverse primer	0.12 µM	0.6 µl
Taq polymerase	1.5 U	0.3 µl
DNA template		2 µl
Water was added to make up to 50 µl		

Master mix for screening CTX-M (1,2,8,9,25) groups and sequencing CTX-M-15, SHV, TEM genes were prepared as follows:

Component	final concentration	Component volume
5x green buffer	1x	10 µl
25 mM MgCl ₂	1.5 mM	3 µl
10 mM each dNTP mix	0.2 mM	1 µl
Forward primer	0.1 µM	0.5 µl
Reverse primer	0.1 µM	0.5 µl
Taq polymerase	1.25 U	0.25 µl
DNA template		2 µl
Water was added to make up to 50 µl		

PCR cycling conditions:

- Initial denaturation at 94°C for 60 seconds
- 35 cycles of- denaturation at 94°C for 40 seconds, annealing (at temperatures as shown in Table 9) for 40 seconds, and elongation at 72°C for 60 seconds
- Final elongation at 72°C for seven minutes

QB-96 Satellite Thermocycler (Quanta Biotech, UK) was used to run PCR cycles; PCR run conditions were programmed using the supplied software.

Post PCR validation

Post PCR validation was performed by gel electrophoresis as per manufacturer's recommendation. Agarose gel (1.5%) with ethidium bromide (0.5 µg/ml) having 32 wells was prepared. A volume of 10 µl of the PCR product was loaded into each well; 10 µl of 100 bp DNA ladder was also loaded into the last well. The tray was filled with 1x TBE buffer and subjected to electrophoresis at 110 v, 90 amps for 30 minutes. Bands were visualized using GelDoc system and photographed using the supplied imaging software.

Controls:

Plasmid extract of isolates known to possess *bla* gene were used as positive controls for each of the three gene types tested. Purified plasmid DNA positive for *bla*TEM, *bla*SHV and *bla*CTX-M genes were kindly provided by Dr. Hans-Jürg Monstein (Sweden). CTX-M group genes for controls were kindly provided by Dr. Branka Bedenic (Croatia). Isolates harbouring *bla*TEM, *bla*SHV and *bla*CTX-M-15 genes were kindly provided by Dr. Ananad Manoharan (Christian Medical College, Vellore). *K. pneumoniae* ATCC 700603 also served as a positive control for *bla*SHV gene.

Consumables:

PCR buffer, dNTP mix and Taq polymerase were obtained from Promega (USA) supplied via Proteogen Biosciences, Bangalore and were held at -20°C. PCR vials, microcentrifuge vials, barrier filter tips, DNase free water, agarose, TBE buffer, 100bp DNA ladder, gel loading buffer were purchased from Merck Genei, Bangalore. They were used and stored as recommended by the manufacturer.

DNA sequencing:

The amplified PCR products were purified using PCR Clean-Up Kit and AMPureE Gel DNA Extraction Spin kit (Amnion Biosciences, Bangalore). After purification, the DNA was sequenced by Sanger Dideoxy Chain Termination Technique using ABI3130XL Genetic Analyzer and read with software BDT version 3.1. Both purification and sequencing were performed on commercial basis by Amnion Biosciences, Bangalore. The results were obtained in FASTA format.

Bioinformatics and analysis:

Consensus sequences were obtained from both forward and reverse sequences using the software tool CAP3 Sequence Assembly Program available online at <http://doua.prabi.fr/software/cap3>.

Aligning DNA sequences:

The consensus sequences were aligned with known nucleotide sequences of TEM-1 (GenBank Accession number: J01749), SHV-1 (GenBank Accession number: AF148850), CTX-M-15 (GenBank Accession number: AY044436) using the NCBI's BLAST tool available online at <http://blast.ncbi.nlm.nih.gov/BlastAlign.cgi>.

Phylogenetic tree:

The phylogenetic tree was developed using Mega 6.0 software using the maximum likelihood and maximum parsimony criterion [351]. The scale bar of 0.05 represents 5% amino-acid difference.

Translation into amino acid sequences:

The nucleotide sequences were converted into amino acid sequences using the “DNA to Protein Translation” software tool available online at

<http://bio.lundberg.gu.se/edu/translat.html> and EMBOSS Transeq tool available online at http://www.ebi.ac.uk/Tools/st/emboss_transeq/.

Identification of TEM, SHV and CTX-M-15 types by protein BLAST:

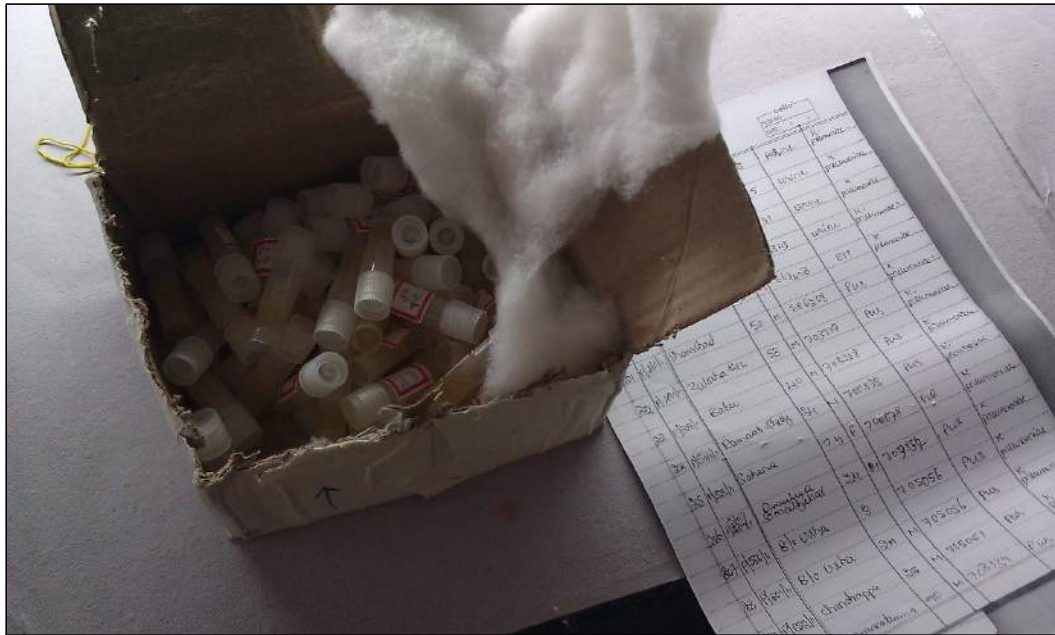
Translated amino acid sequences of TEM and SHV genes were matched against published sequences maintained at “THE LACTAMASE ENGINEERING DATABASE” by the University of Stuttgart, Germany (available online at <http://www.laced.uni-stuttgart.de/>) and the table maintained by Dr. George Jacoby and Dr. Karen Bush (available online at <http://www.lahey.org/studies/>). Similarly, translated amino sequences of CTX-M-15 gene were identified using the NCBI’s Standard Protein BLAST available online at <http://blast.ncbi.nlm.nih.gov/BlastAlign.cgi>.

Detection of amino acid substitutions:

Amino acid substitutions were identified by comparing the amino acid sequences with known amino acid sequences using the MULTALIN software tool available online at http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_multalin.html. Amino acid substitutions that did not match with the known substitutions published in the literature were noted down. Such sequences were sent to Dr. George Jacoby (Lahey Institute, Massachusetts, USA) for identification.

Colour Plates

Bacterial cultures received from various centers



***E. coli* colonies on MacConkey agar**



***K. pneumoniae* colonies on MacConkey agar**



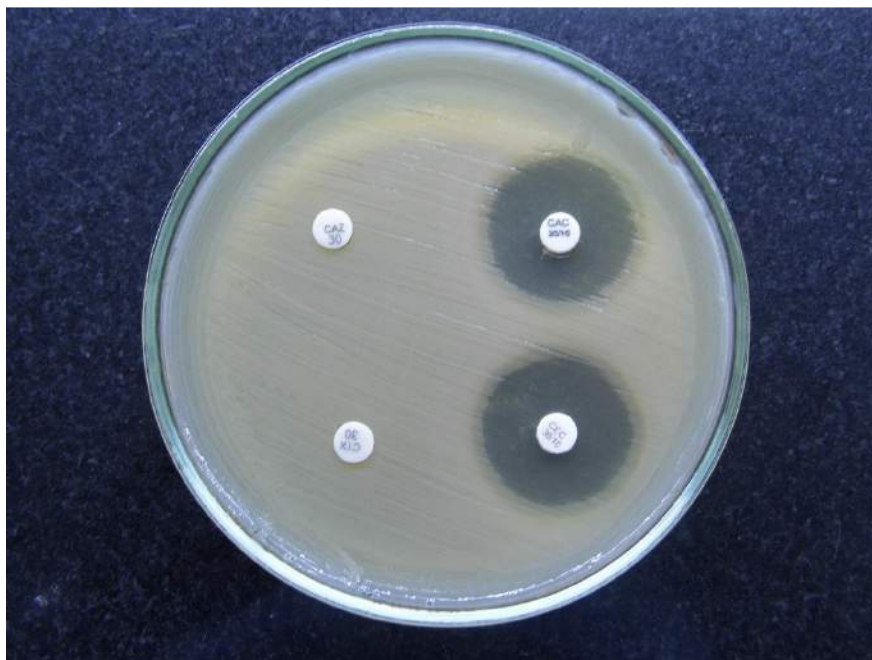
Screening for ESBL production in *E. coli* isolate



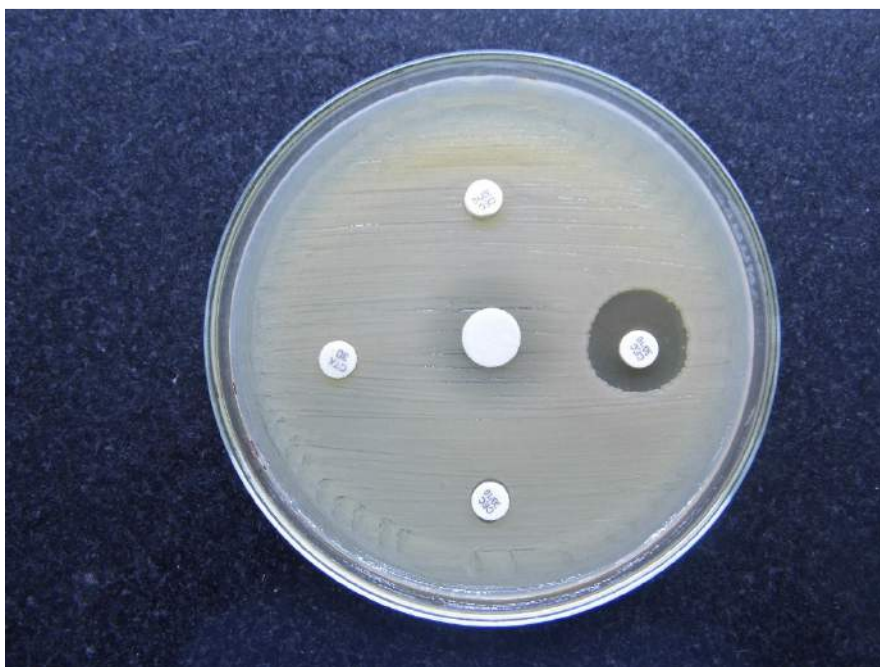
Screening for ESBL production in *K. pneumoniae* isolate



ESBL detection by Phenotypic Confirmatory Test (CLSI-PCT)



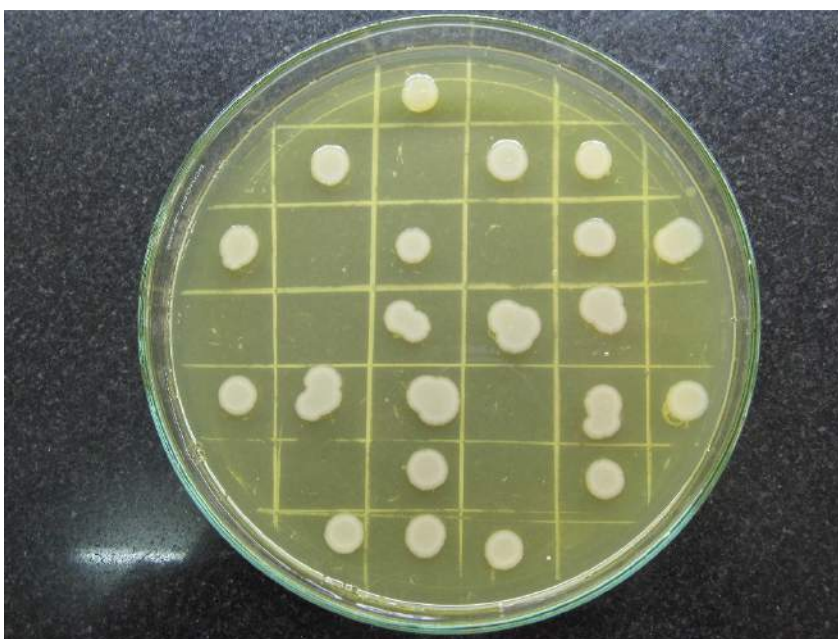
Detection of ESBL production in isolates also producing AmpC beta-lactamase



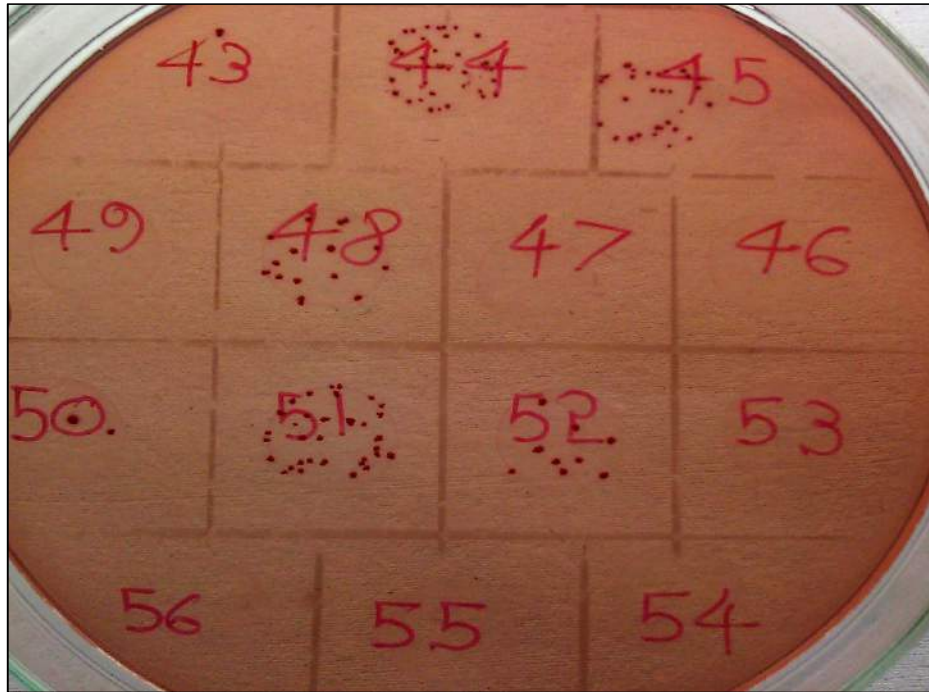
**Antibiotic susceptibility testing to beta-lactam and beta-lactam+beta-lactamase
inhibitors**



Estimation of MIC values by agar dilution method



Plasmid transfer by mating (conjugation) experiment



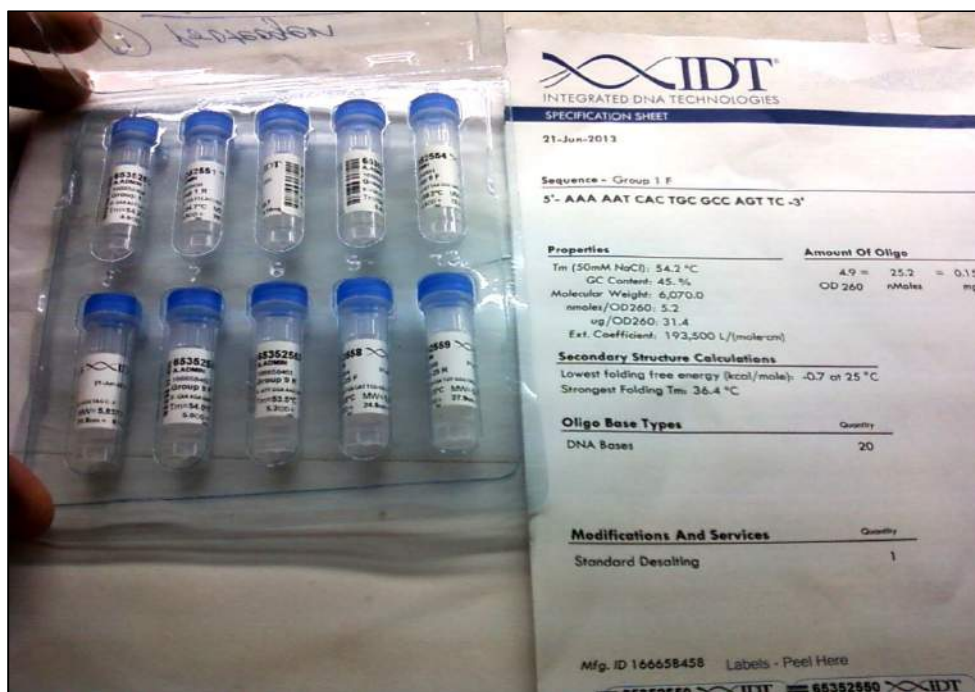
E. coli ATCC 25922 and *K. pneumoniae* ATCC 700603 standard strains



Plasmid extraction kit (Merck-GeNei, Bangalore)



Primers for PCR, (IDT)



Taq polymerase, dNTPs and buffer for PCR (Promega, USA)



QB-96 Satellite Thermocycler (Quanta Biotech, UK)



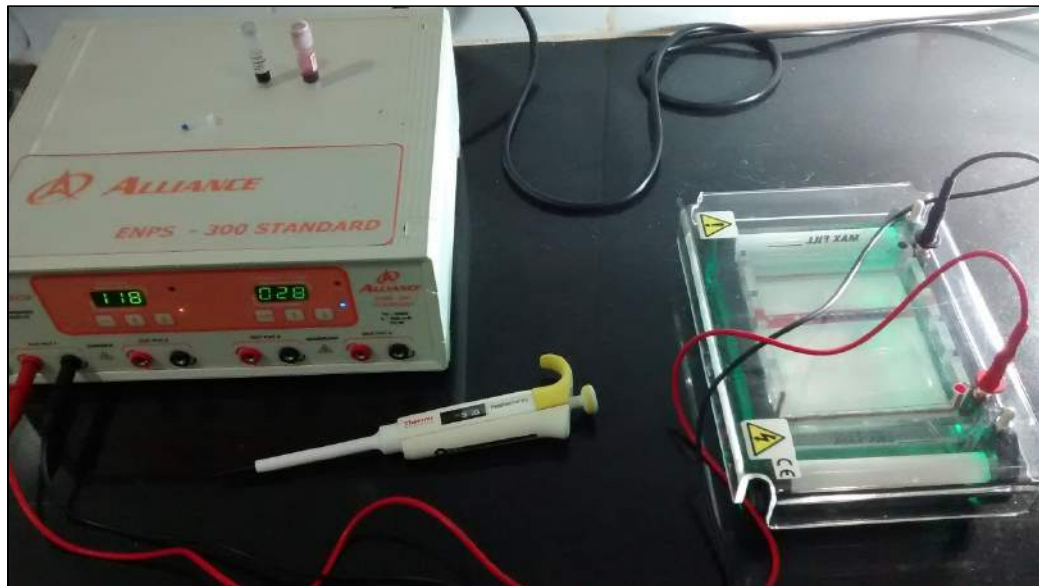
Programmed PCR cycles



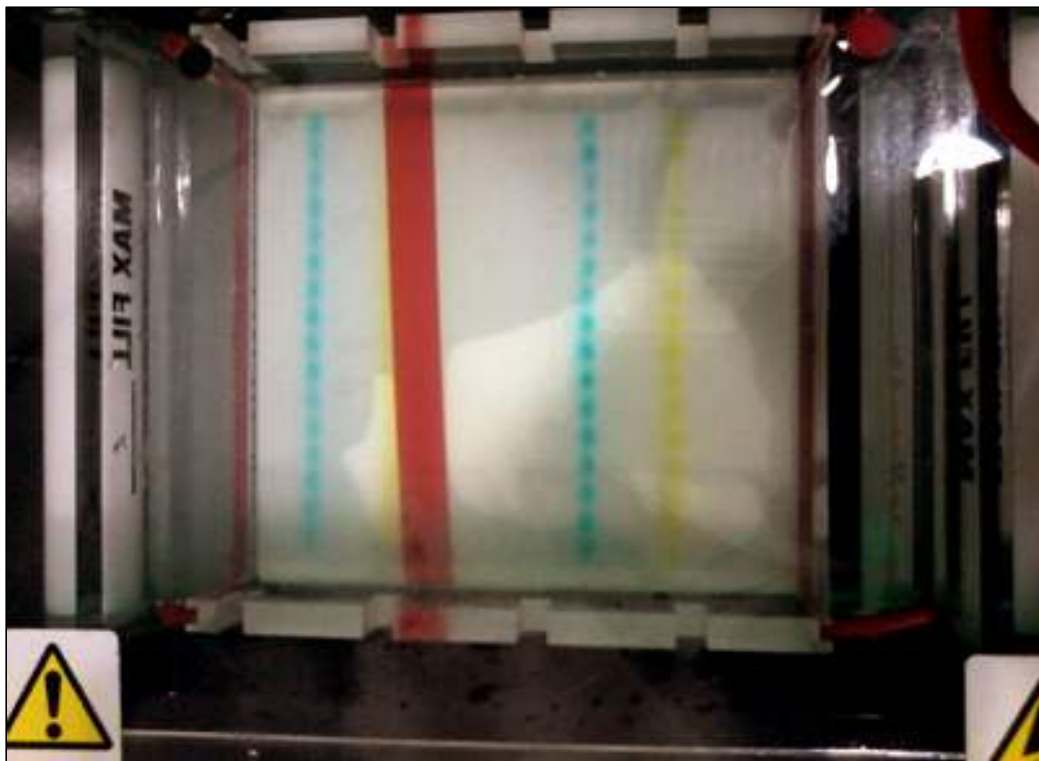
Agarose, buffer, ethidium bromide, gel loading dye (Merck-GeNei, Bangalore)



Electrophoresis unit



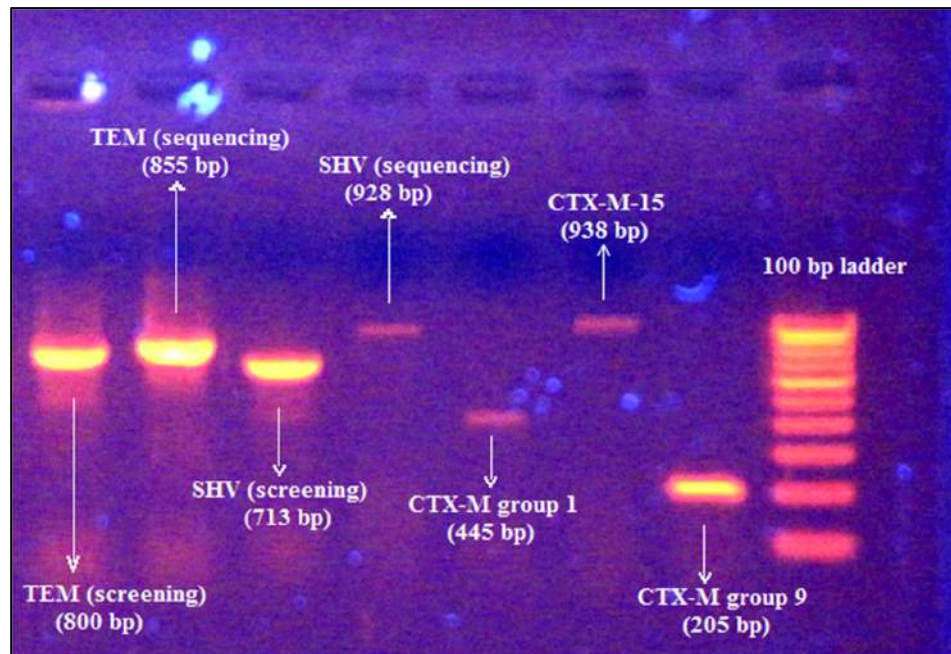
Electrophoresis under run



GelDoc system with camera



Bands of all *bla* genes detected by PCR after staining



Results

Clinical samples

One thousand isolates each of *E. coli* and *K. pneumoniae* isolates were cultured from various clinical samples. The distribution of the various samples and the organisms isolated from them is displayed in the Table 10. Urine (42.9%), pus (29.8%) and sputum (14.5%) were the common samples submitted for culture. Isolates of *E. coli* were obtained predominantly from urine (61.4%) samples, whereas *K. pneumoniae* isolates were obtained mostly from pus (35.3%) and urine (24.3%) samples.

Table 10: *E. coli* and *K. pneumoniae* from various clinical samples included in this study

Clinical sample	Number of samples n=2000 (%)	<i>E. coli</i> n=1000 (%)	<i>K. pneumoniae</i> n= 1000 (%)
Urine	857 (42.9)	614 (61.4)	243 (24.3)
Pus	595 (29.8)	242 (24.2)	353 (35.3)
Sputum	290 (14.5)	61 (6.1)	229 (22.9)
Blood	92 (4.6)	35 (3.5)	57 (5.7)
ET tube	67 (3.4)	3 (0.3)	64 (6.4)
Throat swab	20 (1)	4 (0.4)	16 (1.6)
Ascitic fluid	19 (1)	13 (1.3)	6 (0.6)
Vaginal swab	14 (0.7)	9 (0.9)	5 (0.5)
Pleural fluid	13 (0.7)	5 (0.5)	8 (0.8)
Suction tip	12 (0.6)	2 (0.2)	10 (1)
Rectal swab	8 (0.4)	7 (0.7)	1 (0.1)
Cervical swab	6 (0.3)	3 (0.3)	3 (0.3)
Gastric lavage	3 (0.15)	-	3 (0.3)
CSF	2 (0.1)	2 (0.2)	-
Bronchoalveolar lavage	2 (0.1)	-	2 (0.2)

Among the patients, from whom the clinical samples were collected, 57.1% were male and 42.9% were female. The age of the patients ranged from <1 year to 99 years. The mean age of patients was 38.8 years and the median age was 38 years. The median age was 43 years among men and 30 years among women. The age group of the patients from whom these clinical samples were obtained is displayed in Table 11. Among these patients included in this study, 63.3% of males and 69% of females were 21-60 years old.

Table 11: Age group of the patients from whom the samples were obtained.

Age group (in years)	No. of patients n (%)	Male n (%)	Female n (%)
0-20	373 (18.6)	194 (17)	179 (20.9)
21-40	748 (37.4)	348 (30.5)	400 (46.6)
41-60	567 (28.4)	376 (32.9)	191 (22.3)
61-80	278 (13.9)	197 (17.2)	81 (9.5)
81-100	34 (1.7)	28 (2.4)	6 (0.7)
	2000	1143 (57.1)	857 (42.9)

Screening test for ESBL production:

In the screening test, which involved detection of resistance to one or more of the four cephalosporin antibiotics (ceftazidime, cefotaxime, ceftriaxone and aztreonam), 1,276 (63.8%) isolates were found to be resistant. Resistance to cefotaxime was observed in 1,274 (99.8%), aztreonam in 1,273 (99.8%), aztreonam in 1,273 (99.8%), ceftazidime in 1,271 (99.6%) and ceftriaxone in 1,268 (99.4%) isolates.

Of the 1,000 *E. coli* isolates, 740 (74%) were found to be resistant to one or more of the screening agents. Among them 738 (99.7%) were resistant to all the four screening agents. Of the 1,000 *K. pneumoniae* isolates, 536 (53.6%) were found to be resistant to one or more of the screening agents. Among them 529 (98.7%) were resistant to all the four screening agents.

Confirmation of ESBL production by CLSI-PCT:

Of the 1,276 isolates that were positive in the screening test, ESBL production was confirmed by PCT in 1,076 (84.3%) isolates. Of the 740 *E. coli* isolates that were positive in the screening test, 614 (83%) were phenotypically confirmed as ESBL producers. Similarly, of the 536 *K. pneumoniae* isolates, 462 (86.2%) were identified as ESBL producers. Thus, of the 1000 isolates each studied, 614 (61.4%) *E. coli* and 462 (46.2%) *K. pneumoniae* isolates were phenotypically identified as ESBL producers. ESBL detection among the isolates using the two combinations of disks is displayed in Table 12.

Table 12: Isolates detected as ESBL producers by CLSI-PCT method

Isolates	Both combinations	CTX-CEC disks		CAZ-CAC disks	
		CTX-CEC only	CTX-CEC total	CAZ-CAC only	CAZ-CAC total
EC	585	24	609	5	590
KP	382	79	461	1	383
Total	967 (89.9%)	103 (9.6%)	1070 (99.4%)	6 (0.5%)	973 (90.7%)

CAZ: ceftazidime, CAC: ceftazidime+clavulanic acid, CTX: cefotaxime, CEC: cefotaxime+clavulanic acid

ESBLs were detected in 1070 (99.4%) isolates by using CTX-CEC disks and in 973 (90.7%) isolates by using CAZ-CAC disks. Combinations of both CTX-CEC and

CAZ-CAC disks were able to detect ESBL production in 967 (89.9%) of isolates tested. While 103 (9.6%) isolates were detected by CTX-CEC disks only, 6 (0.5%) isolates were detected by CAZ-CAC disks only.

The distribution of ESBL producing *E. coli* and *K. pneumoniae* from various centers is as shown in Table 13 and Figure 20. The prevalence of ESBL producing *E. coli* (ESBL-EC) varied from 47 to 83.5% and that of ESBL producing *K. pneumoniae* (ESBL-KP) varied from 38.5 to 49% across the five centers.

Table 13: Results of screening and confirmatory test among 1000 isolates each of *E. coli* and *K. pneumoniae* across the five centers

Location	Screening test positive n(%)		ESBL positive n(%)	
	EC	KP	EC	KP
Mangalore	165 (82.5)	101 (50.5)	127 (63.5)	96 (48)
Dharwad	124 (62)	102 (51)	99 (49.5)	77 (38.5)
Bellary	175 (87.5)	103 (51.5)	167 (83.5)	98 (49)
Davangere	171 (85.5)	110 (55)	127 (63.5)	95 (47.5)
Kolar	105 (52.5)	120 (60)	94 (47)	96 (48)
Total	740 (74)	536 (53.6)	614 (61.4)	462 (46.2)

EC= *E. coli*, KP= *K. pneumoniae*

The isolation of ESBL-EC and ESBL-KP from various clinical samples is displayed in Table 14. ESBL producers were isolated from urine (47%), pus (26.9%), sputum (12.6%), blood (5.8%), ET tube (3.2%), ascitic fluid (0.9%) and from 38 (3.5%) other samples. ESBL-EC were predominantly isolated from urine samples (64%), whereas ESBL-KP were isolated predominantly from pus samples (33.8%) and urine (24.5%).

Table 14: Distribution of ESBL producers according to the clinical samples

Clinical sample	Total	<i>E. coli</i> n =614 n (%)	<i>K. pneumoniae</i> n =462 n (%)
Urine	506	393 (64)	113 (24.5)
Pus	290	134 (21.8)	156 (33.8)
Sputum	136	41 (6.7)	95 (20.6)
Blood	62	18 (2.9)	44 (9.5)
ET tube	34	3 (0.5)	31 (6.7)
Ascitic fluid	10	7 (1.1)	3 (0.6)
Others	38	18 (2.9)	20 (4.3)

Co-production of ESBL and AmpC beta-lactamase:

By using amino-phenylboronic acid disk method, 50 (5%) *E. coli* and 2 (0.2%) *K. pneumoniae* isolates were identified as co-producers of both ESBL and AmpC beta-lactamase. The proportion of ESBL and AmpC beta-lactamases among the tested isolates is displayed in Figure 21. Isolates of *E. coli* producing both ESBL and AmpC beta-lactamase ranged from 0.1- 21% across the centers. In contrast to this, *K. pneumoniae* isolates co-producing both the enzymes were detected only in 2 (0.2%) isolates. By the same method, 35 (3.5%) *E. coli* and 9 (0.9%) *K. pneumoniae* isolates were identified as only AmpC beta-lactamase producers. In 41 (4.1%) *E. coli* and 63 (6.3%) *K. pneumoniae* isolates, neither ESBL nor AmpC beta-lactamases were detected. The distribution of beta-lactamases among *E. coli* and *K. pneumoniae* isolates according to the region is displayed in Table 15 and 16.

Table 15: Distribution of beta-lactamases among *E. coli* isolates across five centers

Location	Positive in screening test	ESBL only	ESBL and AmpC	AmpC only	Unknown mechanism
MNG	165	127	11	5	22
DWD	124	99	14	6	5
BEL	175	167	1	4	3
DVG	171	127	21	12	11
KOL	105	94	3	8	0
Total	740	614	50	35	41

DVG: Davangere, DWD: Dharwad, KOL: Kolar, BEL: Bellary, MNG: Mangalore

Table 16: Distribution of beta-lactamases among *K. pneumoniae* isolates across five centers

Location	Positive in screening test	ESBL only	ESBL and AmpC	AmpC only	Unknown mechanism
MNG	101	96	0	2	3
DWD	102	77	0	2	23
BEL	103	98	0	0	5
DVG	110	95	2	2	11
KOL	120	96	0	3	21
Total	536	462	2	9	63

DVG: Davangere, DWD: Dharwad, KOL: Kolar, BEL: Bellary, MNG: Mangalore

Figure 20: Distribution of ESBL producing *E. coli* and *K. pneumoniae* across the centers

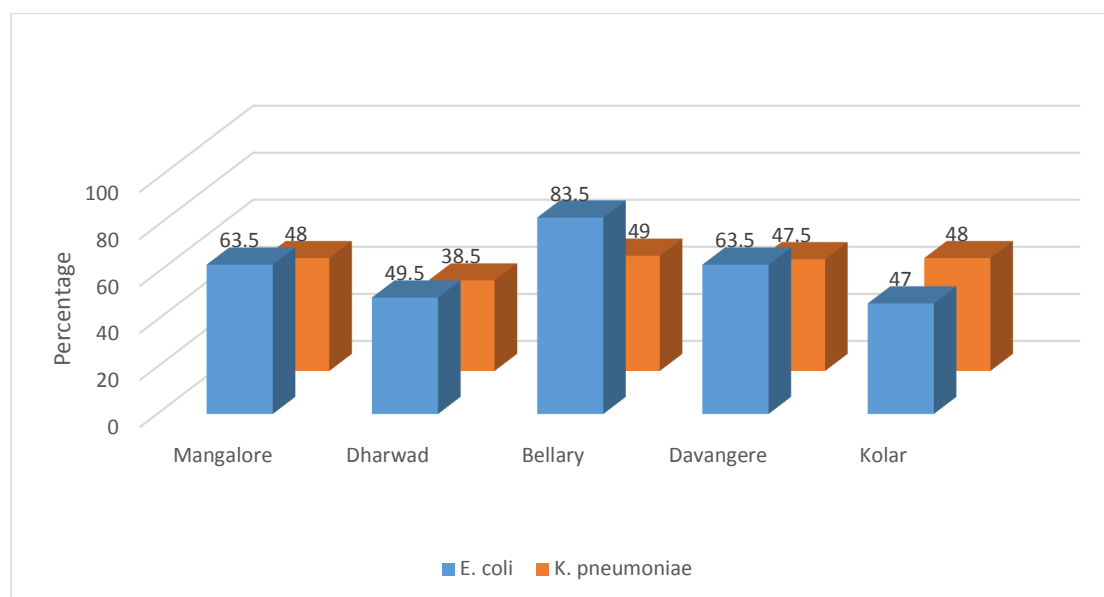
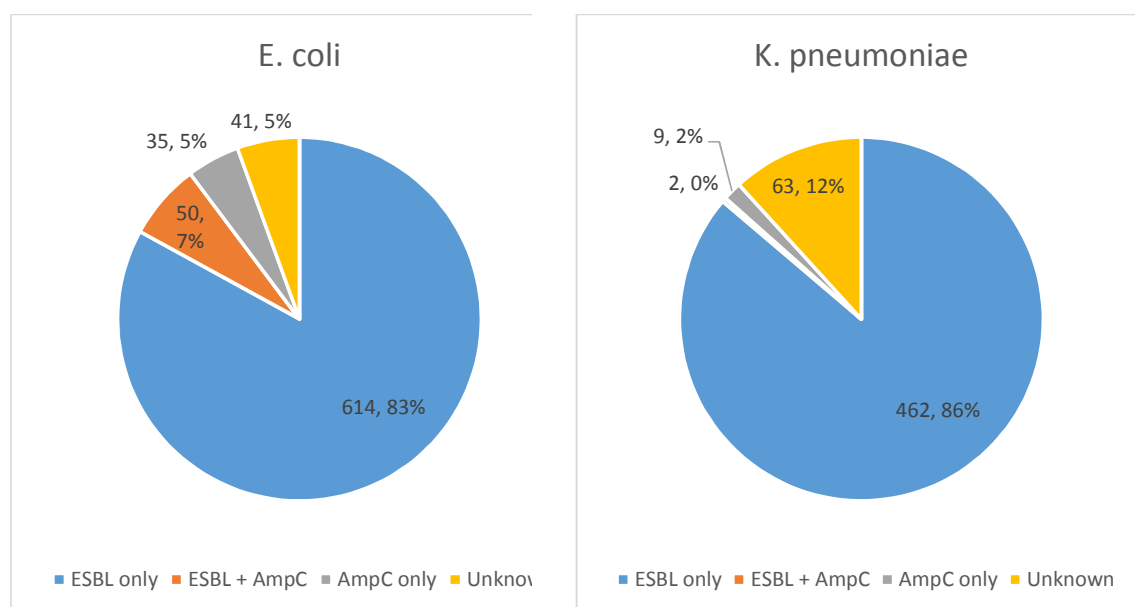


Figure 21: Beta-lactamases detected among 200 isolates of *E. coli* and *K. pneumoniae* each



Antibiotic susceptibility testing results:

All the ESBL producing isolates were resistant to aztreonam, ceftazidime (CAZ), ceftriaxone (CTR) and cefotaxime (CTX) by disk diffusion test. Similarly, agar dilution method also detected decreased susceptibility of the ESBL producers to cefotaxime and ceftriaxone (MIC levels of ≥ 2 $\mu\text{g/ml}$) and ceftazidime (≥ 8 $\mu\text{g/ml}$). Only seven isolates, which included four *E. coli* and three *K. pneumoniae* were susceptible to aztreonam. The MIC values of CTX, CTR and CAZ among both ESBL-EC and ESBL-KP are as displayed in Table 17:

Table 17: MIC values of CAZ, CTX and CTR among ESBL producers

	MIC range ($\mu\text{g/ml}$)		MIC ₅₀ ($\mu\text{g/ml}$)		MIC ₉₀ ($\mu\text{g/ml}$)	
	ESBL-EC	ESBL-KP	ESBL-EC	ESBL-KP	ESBL-EC	ESBL-KP
CAZ	8 to >128	8 to >128	64	128	>128	>128
CTX	4 to >128	4 to >128	>128	>128	>128	>128
CTR	8 to >128	4 to >128	>128	>128	>128	>128

CAZ: Ceftazidime, CTX: Cefotaxime, CTR: Ceftriaxone

The antibiotic susceptibility of ESBL producers to beta-lactam and beta-lactam and beta-lactamase inhibitor combinations is displayed in Table 18 and Figure 22. The resistance to CAZ was overcome in 443 (72.1%) *E. coli* and 177 (25.3%) *K. pneumoniae* isolates when the ceftazidime was combined with clavulanic acid (CAC). This difference between *E. coli* and *K. pneumoniae* isolates was found to be statistically significant ($P < 0.001$). The resistance to CTX was overcome in 82 (13.4%) *E. coli* and 83 (18%) *K. pneumoniae* isolates when it was combined with clavulanic acid (CEC), but this difference was not found to be significant ($P = 0.03752$).

Among the ESBL producers, 23 (3.7%) *E. coli* and 61 (13.2%) *K. pneumoniae* isolates were susceptible to cefepime. The difference in susceptibility to cefepime between *E. coli* and *K. pneumoniae* was statistically significant ($P < 0.001$). Among the cefepime-resistant ESBL producers, 422/547 (77.1%) *E. coli* and 191/369 (51.8%) *K. pneumoniae* isolates became susceptible to cefepime when it was combined with clavulanic acid. Similarly, 499/547 (91.2%) *E. coli* and 262/369 (71%) *K. pneumoniae* isolates became susceptible to cefepime when it was combined with tazobactam. Difference between *E. coli* and *K. pneumoniae* in susceptibility to the combination of cefepime with clavulanic acid or tazobactam was found to be statistically significant ($P < 0.001$). Such a significant difference was also noted with the combination of cefixime and clavulanic acid.

Cefoxitin resistance was noted among 377 (61.4%) ESBL-EC and 238 (51.5%) ESBL-KP; the difference in susceptibility to cefoxitin between *E. coli* and *K. pneumoniae* was found to be significant ($P = 0.0012$). Among the ESBL producers, 606 (98.7%) *E. coli* and 419 (90.7%) *K. pneumoniae* were susceptible to imipenem. The difference between *E. coli* and *K. pneumoniae* in susceptibility to imipenem was also found to be statistically significant ($P < 0.001$).

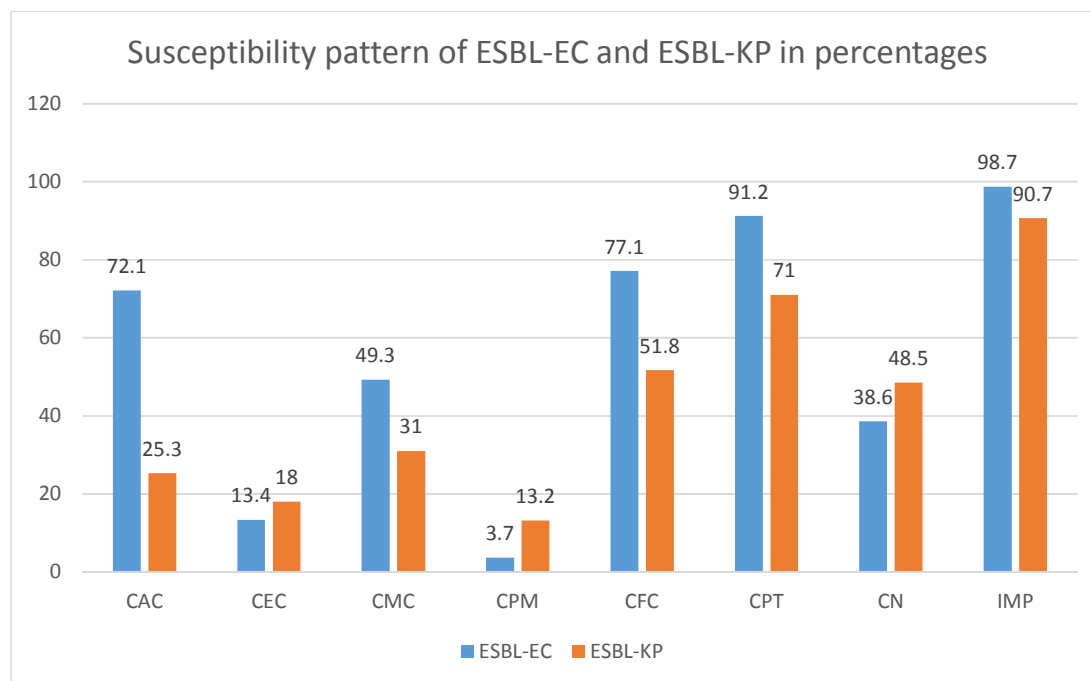
Plasmid transfer by conjugation:

Plasmid mediated resistance transfer by mating experiment was successfully demonstrated in 362 (59%) isolates of ESBL-EC and 249 (53.9%) isolates of ESBL-KP.

Table 18: Antibiotic susceptibility pattern of ESBL producers

Antibiotic	<i>E. coli</i>	<i>K. pneumoniae</i>	P value
MIC $\geq$ 128 μ g/ml to cefotaxime	486/614 (79.1%)	343/462 (74.2%)	0.05744
MIC $\geq$ 128 μ g/ml to ceftriaxone	493/614 (80.3%)	382/462 (82.7%)	0.31732
MIC $\geq$ 128 μ g/ml to ceftazidime	228/614 (37.1%)	253/462 (54.8%)	<0.001
Ceftazidime + clavulanic acid	443/614 (72.1%)	177/462 (25.3%)	<0.001
Cefotaxime + clavulanic acid	82/614 (13.4%)	83/462 (18%)	0.03752
Cefixime + clavulanic acid	303/614 (49.3%)	143/462 (31%)	<0.001
Cefepime	23/614 (3.7%)	61/462 (13.2%)	<0.001
Cefepime + clavulanic acid	422/547 (77.1%)	191/369 (51.8%)	<0.001
Cefepime + tazobactam	499/547 (91.2%)	262/369 (71%)	<0.001
Imipenem	606/614 (98.7%)	419/462 (90.7%)	<0.001
Cefoxitin	237/614 (38.6%)	224/462 (48.5%)	0.0012

Figure 22. Antibiotic susceptibility pattern of ESBL-EC and ESBL-KP



CAC: Ceftazidime+clavulanic acid, CEC: cefotaxime+clavulanic acid, CMC: cefixime+clavulanic acid, CPM: cefepime, CFC: cefepime+clavulanic acid, CPT: cefepime+tazobactam, CN: ceftazidime, IMP: imipenem

Detection of *bla* genes by PCR:

Attempts to detect one or more *bla* genes by PCR in 400 isolates phenotypically identified as ESBL producers was successful in 391 (97.8%) isolates. This included 196 (98%) isolates of *E. coli* and 195 (97.5%) isolates of *K. pneumoniae*.

Detection of *bla* genes in *E. coli*:

Among the 200 *E. coli* isolates, 186 (94.9%) harboured *bla*_{CTX-M} gene, 23 (11.7%) *bla*_{SHV} gene and 61 (31.1%) *bla*_{TEM} gene. Detection of *bla* gene types among the *E. coli* isolates across the centers is displayed in Table 19.

Table 19: Detection of *bla* gene types among 40 *E. coli* isolates from each center

Center	<i>bla</i> TEM n (%)	<i>bla</i> SHV n (%)	<i>bla</i> CTX-M n (%)	No genes
MNG	8 (20)	4 (10)	37 (92.5)	1
DWD	11 (27.5)	1 (2.5)	38 (95)	-
BEL	22 (55)	10 (25)	39 (97.5)	-
DVG	19 (47.5)	7 (17.5)	34 (85)	2
KOL	1 (2.5)	1 (2.5)	38 (95)	1
Total (n=200)	61 (30.5)	23 (11.5)	186 (93)	4 (2%)

The prevalence of *bla*CTX-M among the *E. coli* isolates tested across the centers varied between 85 and 97.5%. The prevalence of *bla*SHV varied between 2.5 and 25% across the center, whereas the prevalence of *bla*TEM varied between 2.5 and 55% across the centers. Among these isolates, single *bla* gene could be detected in 135 (67.5%), two genes in 48 (24%) and three genes in 13 (6.5%) isolates. To be more specific, *bla*CTX-M was the only gene found in 127 (63.5%) of the *E. coli* isolates tested. Only four (2%) *E. coli* isolates harboured either *bla*TEM or *bla*SHV genes alone. Two different *bla* genes occurred in three combinations among 48 isolates. Of these, *bla*CTX-M in combination with *bla*TEM gene was found in 42 (21%) isolates. Distribution of *bla* genes and their combinations in these isolates is displayed in Table 20 and Figure 23.

Detection of *bla* genes in *K. pneumoniae*:

Among the 200 *K. pneumoniae* isolates tested, 60 (82%) harboured *bla*CTX-M gene, 177 (90.8%) *bla*SHV gene and 118 (60.5%) *bla*TEM gene. The prevalence of *bla*CTX-M genes across the centers in the population of 200 isolates varied from 52.5

to 100%, *blaSHV* genes from 85 to 95%, and *blaTEM* genes from 45 to 72.5%. Detection of *bla* gene types among the *K. pneumoniae* isolates across the centers is displayed in Table 21. Distribution of *bla* genes and their combinations in these isolates is displayed in Table 22 and Figure 24.

Table 20: Distribution of *bla* genes among 40 *E. coli* isolates each across five centers

Center	TEM only	SHV only	CTX-M only	TEM+ SHV	TEM + CTX-M	SHV+ CTX-M	TEM+ SHV+ CTX-M	None
DVG	3	1	17	1	12	2	3	1
MNG	0	1	28	0	6	1	2	2
DWD	1	0	29	1	9	0	0	0
BEL	0	1	16	0	14	1	8	0
KOL	0	1	37	0	1	0	0	1
Total	4	4	127	2	42	4	13	4

Table 21: Detection of *bla* genes among 40 *K. pneumoniae* isolates each across five centers

Center	<i>blaTEM</i> n (%)	<i>blaSHV</i> n (%)	<i>blaCTX-M</i> n (%)	No genes
MNG	23 (57.5)	34 (85)	33 (82.5)	3
DWD	29 (72.5)	35 (87.5)	40 (100)	0
BEL	18 (45)	38 (95)	21 (52.5)	0
DVG	25 (62.5)	34 (85)	34 (85)	2
KOL	23 (57.5)	36 (90)	32 (80)	0
Total (n=200)	118 (59)	177 (88.5)	160 (80)	5 (2.5)

Table 22: Distribution of *bla* gene types among 40 *K. pneumoniae* isolates each across centers

Center	TEM only	SHV only	CTX-M only	TEM+ SHV	TEM + CTX-M	SHV+ CTX-M	TEM+ SHV+ CTX-M	None
MNG	0	4	1	0	2	9	21	3
DWD	0	0	3	0	2	8	27	0
BEL	1	18	0	0	1	4	16	0
DVG	0	2	2	2	2	9	21	2
KOL	0	8	2	0	2	7	21	0
Total	1	32	8	2	9	37	106	5

Among these isolates, single *bla* gene could be detected in 41 (20.5%), two genes in 48 (24%) and three genes in 106 (53%) isolates. *bla*CTX-M was the only gene found in 8 (4%) of the *K. pneumoniae* isolates tested, whereas *bla*SHV alone was found in 32 (16%) isolates. Among the combination of two *bla* genes, the combination of *bla*SHV and *bla*CTX-M was found in 37 (18.5%) isolates. Individual and various combinations of *bla* genes in these isolates is displayed in Table 23 and Figure 25.

Table 23: Individual and combinations of *bla* genes among ESBL-EC and ESBL-KP

<i>bla</i> type	<i>E. coli</i>	<i>K. pneumoniae</i>
<i>bla</i> CTX-M	127	8
<i>bla</i> SHV	4	32
<i>bla</i> TEM	4	1
<i>bla</i> TEM+ <i>bla</i> CTX-M	42	9
<i>bla</i> TEM+ <i>bla</i> SHV	2	2
<i>bla</i> SHV+ <i>bla</i> CTX-M	4	37
<i>bla</i> TEM+ <i>bla</i> SHV+ <i>bla</i> CTX-M	13	106
Total	196	195

Figure 23: Distribution of *bla* genes among 200 *E. coli* isolates

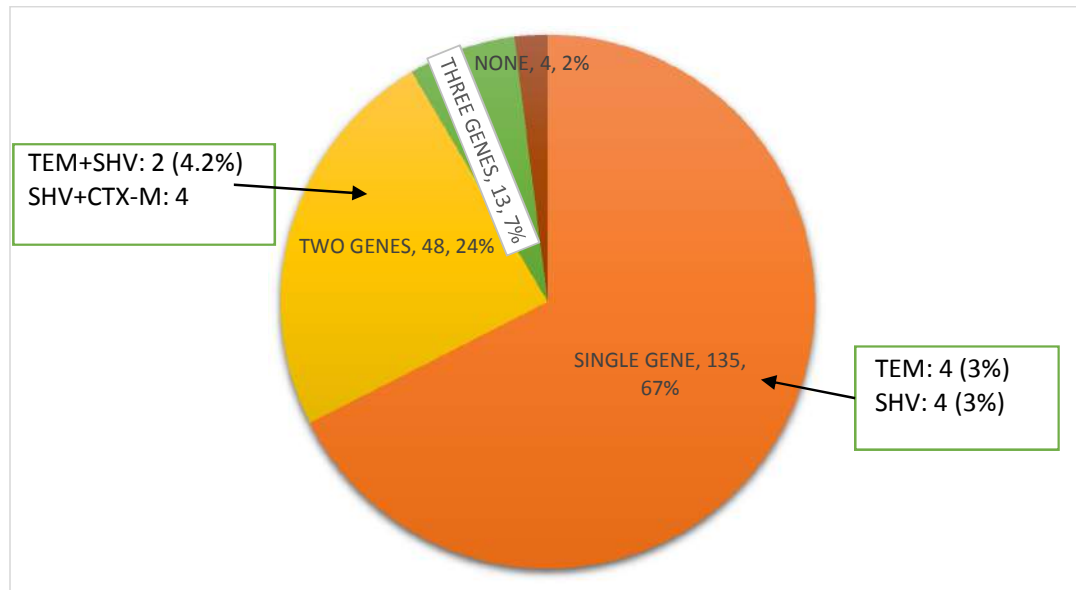


Figure 24: Distribution of *bla* genes among 200 *K. pneumoniae* isolates

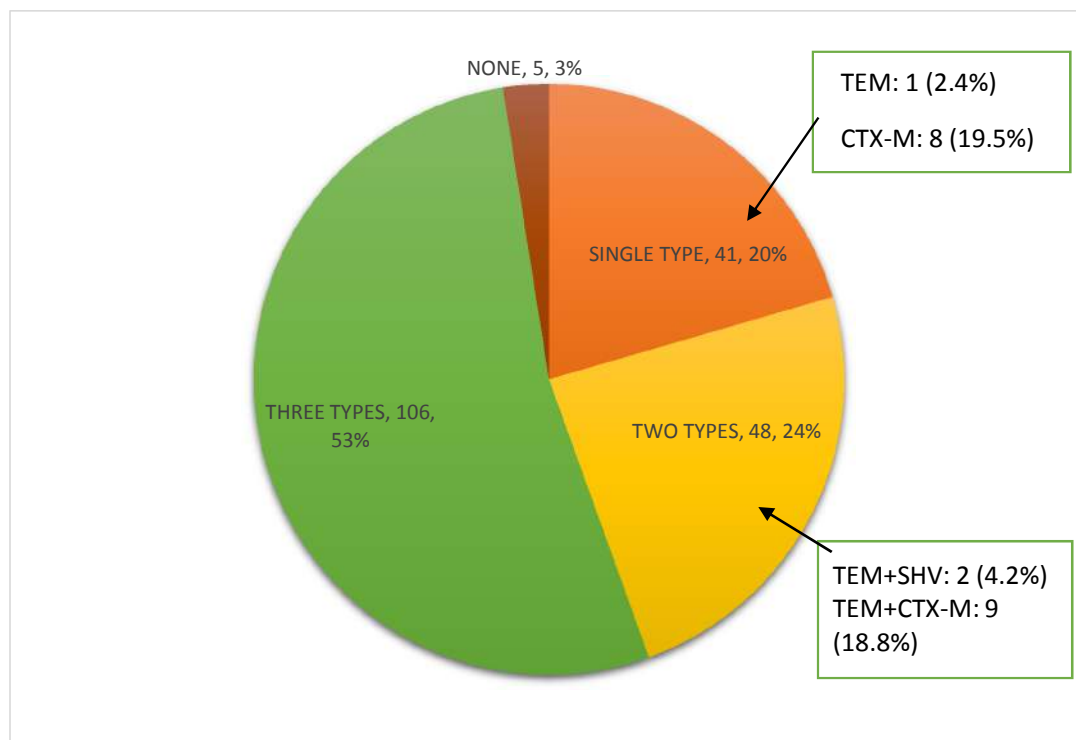
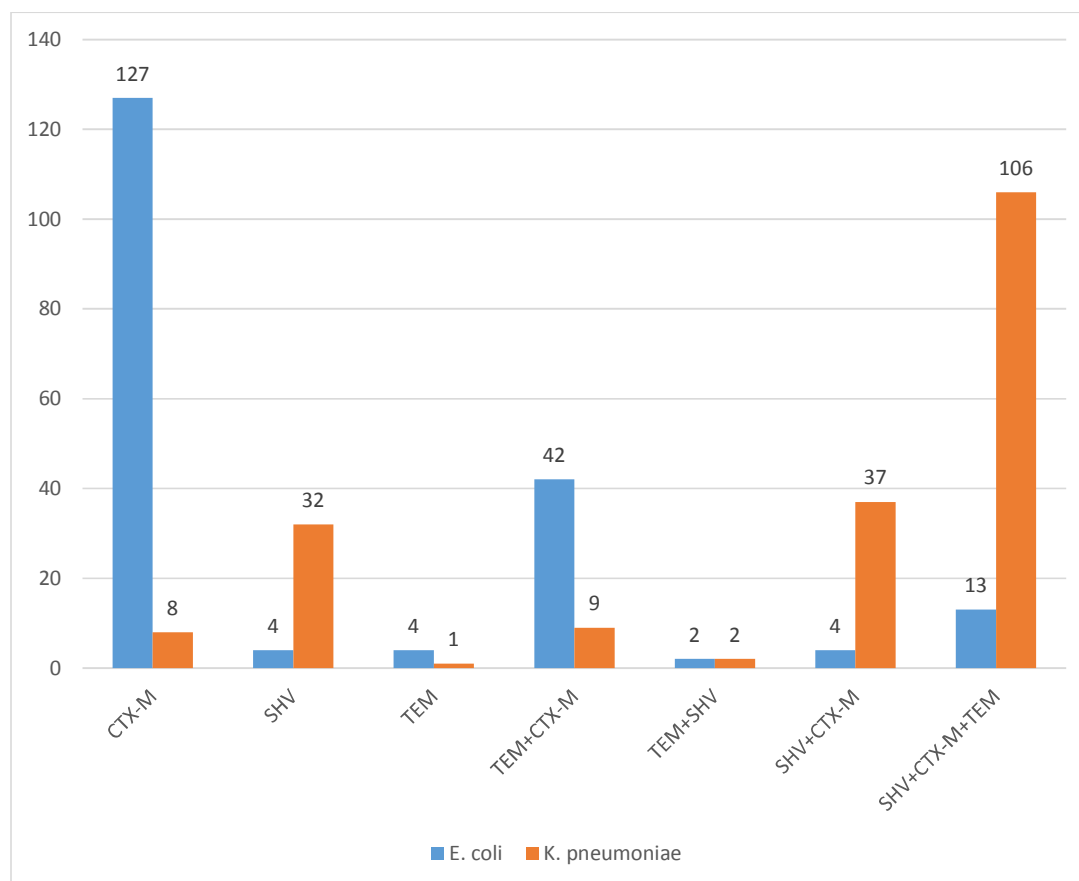


Figure 25: Graphical representation of occurrences of *bla* genes among ESBL-EC and ESBL-KP



Nucleotide substitutions

The *bla* genes were sequenced and the nucleotide sequences were translated into amino acid sequences, which led to the identification of several beta-lactamase types. The nucleotide sequences of all the *bla*TEM-1, *bla*SHV-1, *bla*SHV-11 and *bla*CTX-M-15 genes identified in this study were analyzed by BLAST tool as they were the dominant *bla* types detected in this study. The complete nucleotide sequences of these genes were compared with the reference nucleotide sequences from the accession numbers available at www.lahey.org/studies. While *bla*TEM-1 and *bla*CTX-M-15

were detected among both *E. coli* and *K. pneumoniae*, *blaSHV-1* and *blaSHV-11* were predominantly detected among *K. pneumoniae* isolates.

The number of nucleotide substitutions among both *E. coli* and *K. pneumoniae* in comparison to the reference sequences is shown in Table 24.

Table 24: Nucleotide substitutions encountered in among TEM, SHV and CTX-M-15 genes

<i>bla</i>	No. of genes	Number of nucleotide substitutions						
		n (%)						
		0	1	2	3	4	5	6
<i>bla</i> TEM-1	59	11	4	6	38	-	-	-
EC		(18.6)	(6.8)	(10.2)	(64.4)			
<i>bla</i> TEM-1	116	39	20	4	52	1	-	-
KP		(33.6)	(17.2)	(3.4)	(44.8)	(0.9)		
<i>bla</i> SHV-1	45	12	3	6	13	2	8	1
		(26.7)	(6.7)	(13.3)	(28.9)	(4.4)	(17.8)	(2.2)
<i>bla</i> SHV-11	49	-	11	24	1	4	5	4
			(22.4)	(49)	(2)	(8.2)	(10.2)	(8.2)
<i>bla</i> CTX-M-15	346	339	5	2	-	-	-	-
		(98)	(1.4)	(0.6)				

EC: *E. coli*, KP: *K. pneumoniae*

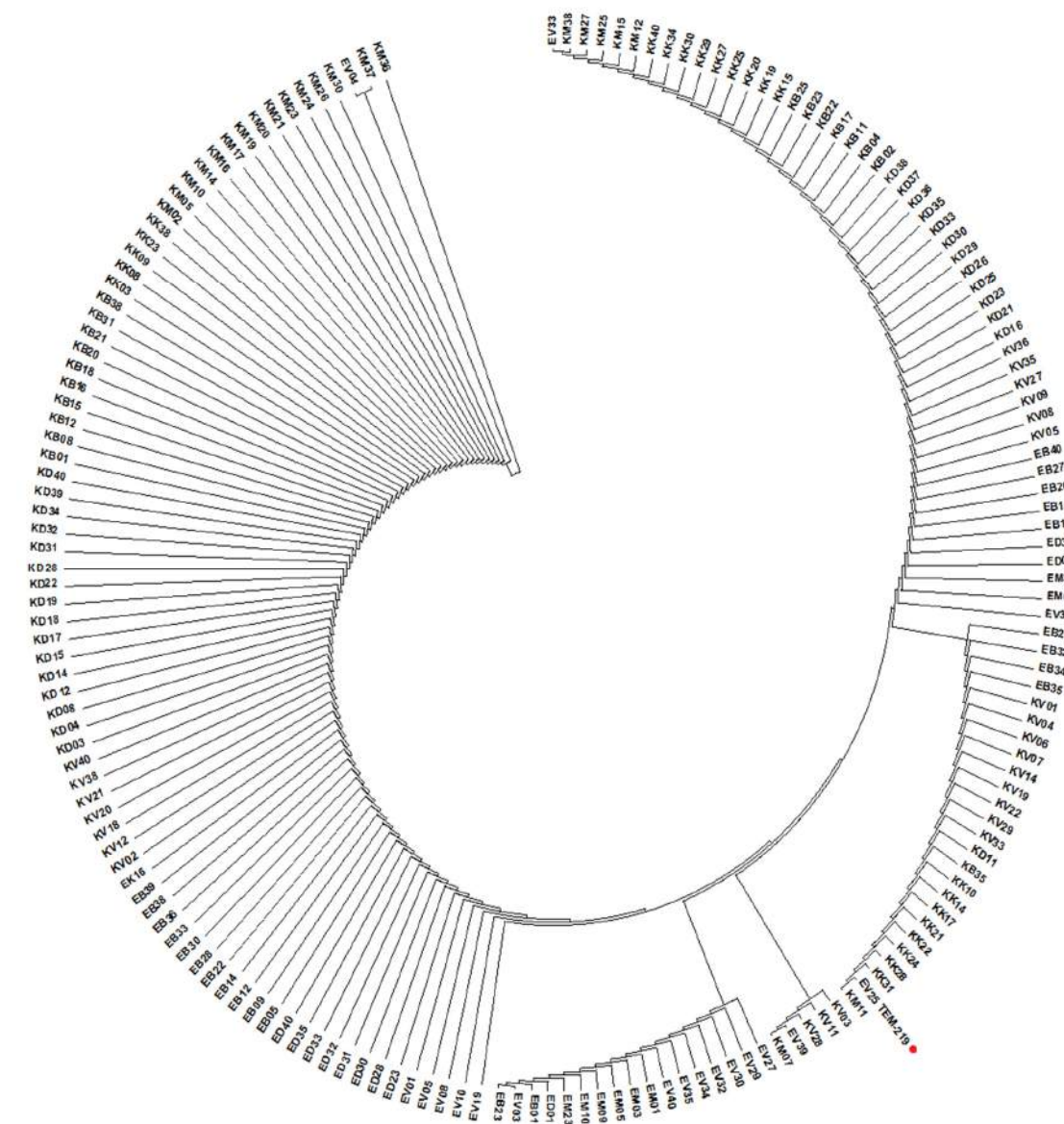
When the nucleotide sequences of all *bla*TEM-1 genes were compared to the sequences of the reference *bla*TEM-1 gene (Accession No.J01749) by BLAST tool, it was found that sequences of 11 (18.6%) *E. coli* and 39 (29.3%) *K. pneumoniae* were identical to the reference sequence. In the remaining 48 *E. coli* and 77 *K. pneumoniae* isolates, the *bla*TEM-1 genes contained up to four nucleotide substitutions. All the detected four nucleotide substitutions were noted in a single *K. pneumoniae* isolate from Kolar.

Of the 45 *blaSHV*-1 genes detected, 12 were identical to the reference nucleotide sequence (Accession No. AF148850). Rest of the 33 genes exhibited 1-6 nucleotide substitutions, with 13 (28.9%) of them bearing three nucleotide substitutions. Compared to the reference nucleotide sequence of *blaSHV*-11 (Accession No. X98101), the nucleotide sequences of *blaSHV*-11 genes detected in this study exhibited 1-6 nucleotide substitutions, with 24 (49%) of them bearing two nucleotide substitutions.

When nucleotide sequences of *blaCTX*-M-15 genes detected in this study were analyzed, 98% of them were found to be identical to the reference sequence (Accession No. AY044436). Among the rest, two genes bore two substitutions and five genes bore single nucleotide substitutions.

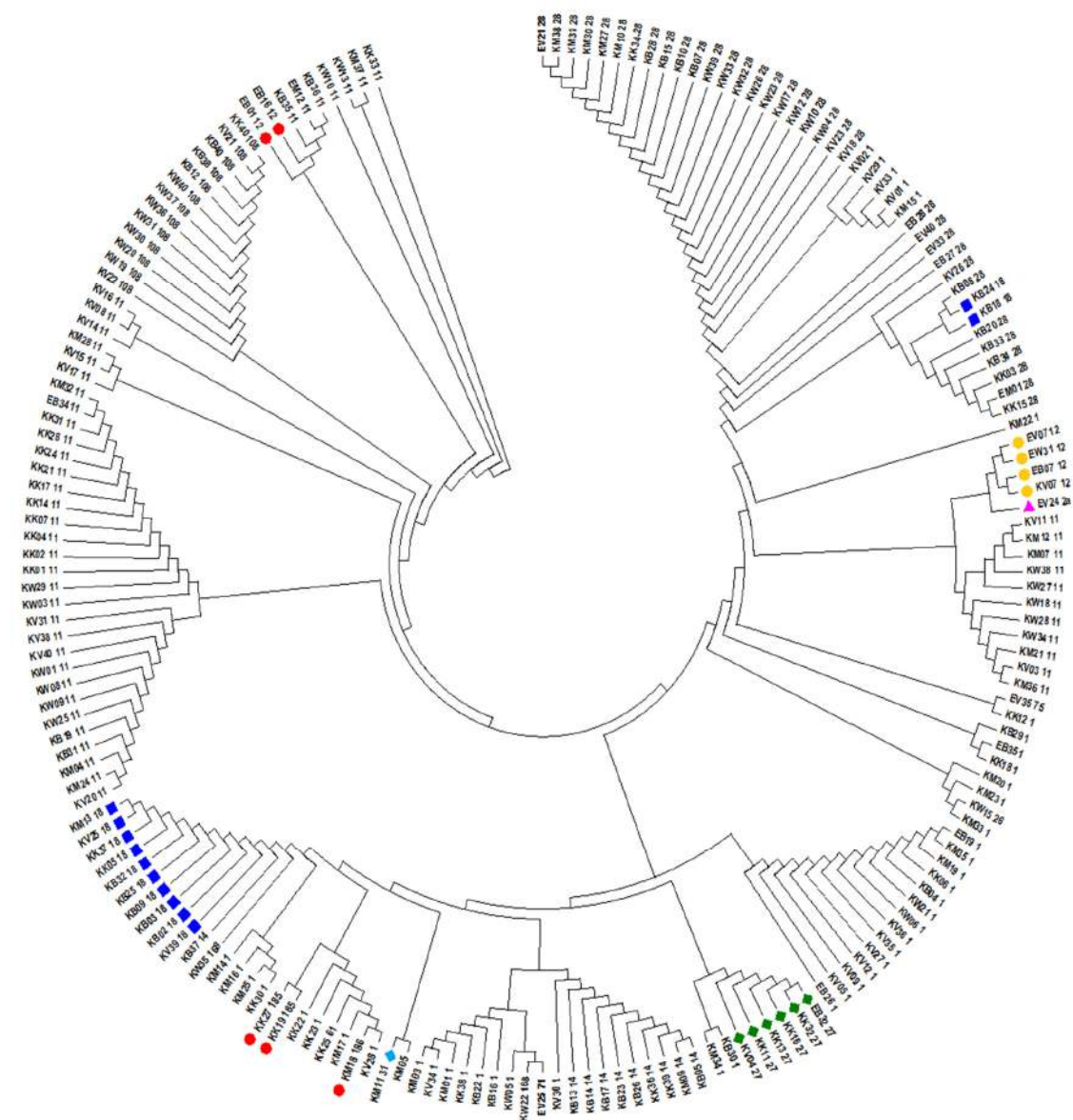
Since none of these nucleotide substitutions resulted in amino-acid substitution, they could be considered as synonymous substitutions. The phylogenetic trees of *blaTEM* and *blaSHV* genes from the nucleotide sequences obtained in this study are displayed in Figure 26 and 27. A phylogenetic tree of *blaCTX*-M-15 genes was not prepared as 98% of the sequences were identical. Nucleotide sequences of the identified *bla* genes and their translated amino acid sequences are provided in Annexure 3.

Figure 26: Phylogenetic tree of all *bla*TEM genes detected in this study



Note: *bla*TEM-219, the novel TEM type discovered in this study is marked by a red dot, whereas the rest are all *bla*TEM-1. Despite coding for the same protein, six clusters are apparent in this phylogenetic tree.

Figure 27: Phylogenetic tree of all the *bla*SHV genes detected in this study

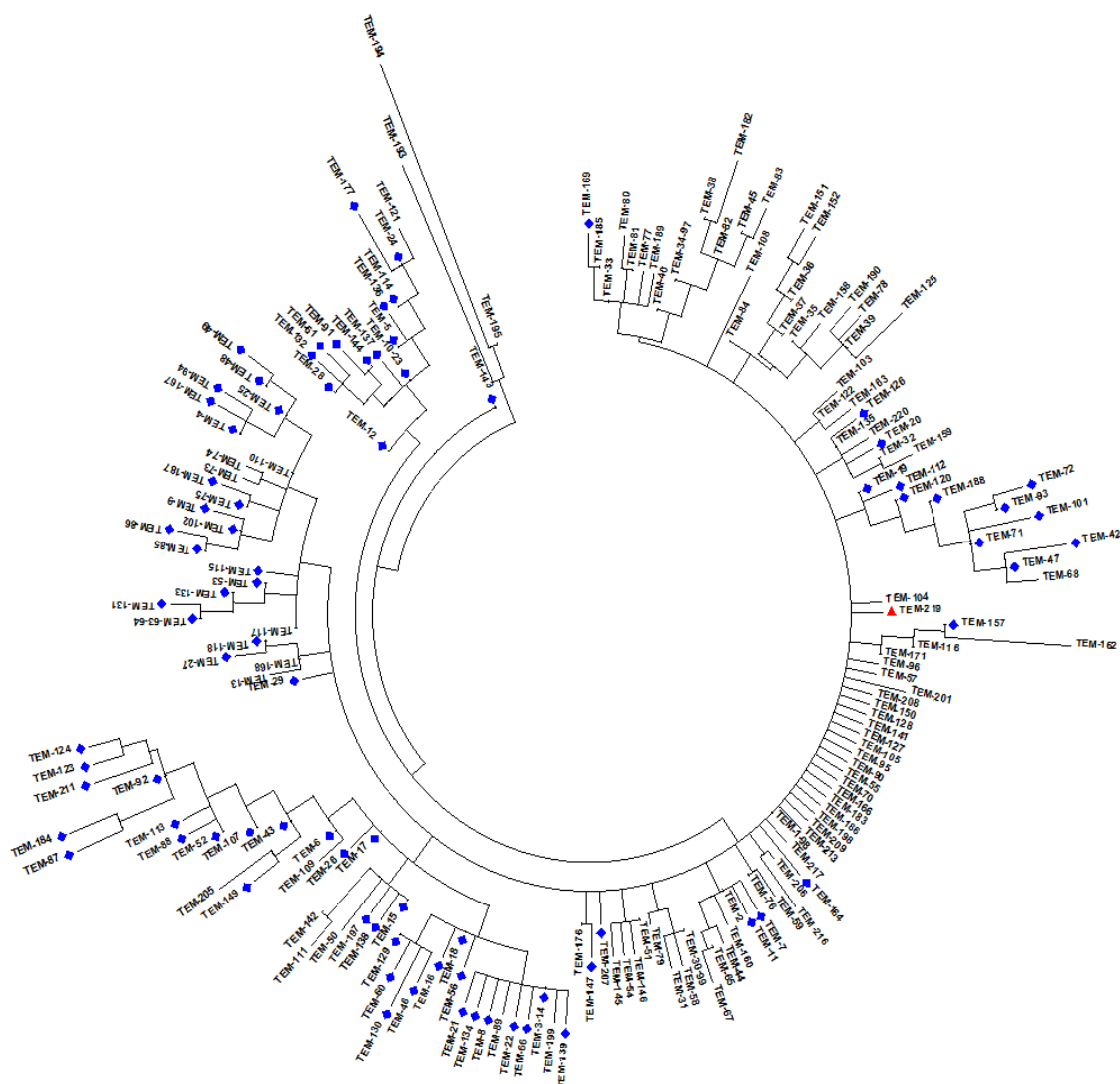


Note: Different ESBL types are marked in cyan, blue, green, pink and orange colours whereas the novel *bla*SHV-185 and *bla*SHV-186 types discovered in this study are marked by red dots. The phylogenetic tree shows several branches as the tree is composed of 17 different *bla*SHV types.

Using the accession numbers available at www.lahey.org/studies/ website, nucleotide sequences of hitherto known *bla*TEM, *bla*SHV genes and *bla*CTX-M genes

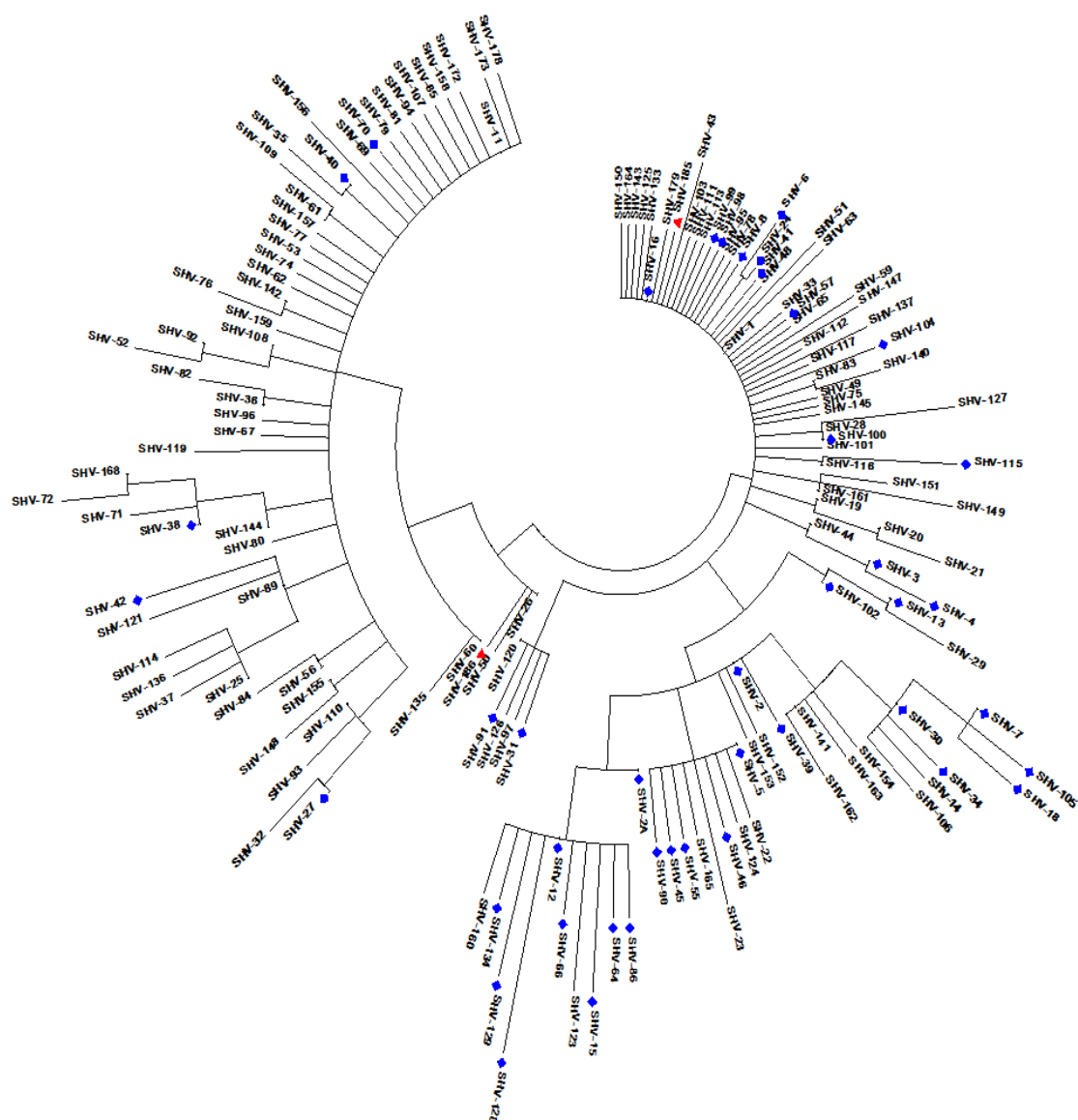
were obtained from GenBank. From the aligned sequences, phylogenetic trees were constructed. Figures 28, 29 and 30 represent the phylogenetic trees of TEM, SHV and CTX-M beta-lactamases, respectively along with the position of newly discovered *bla* types in the present study.

Figure 28: Phylogenetic tree of TEM beta-lactamases



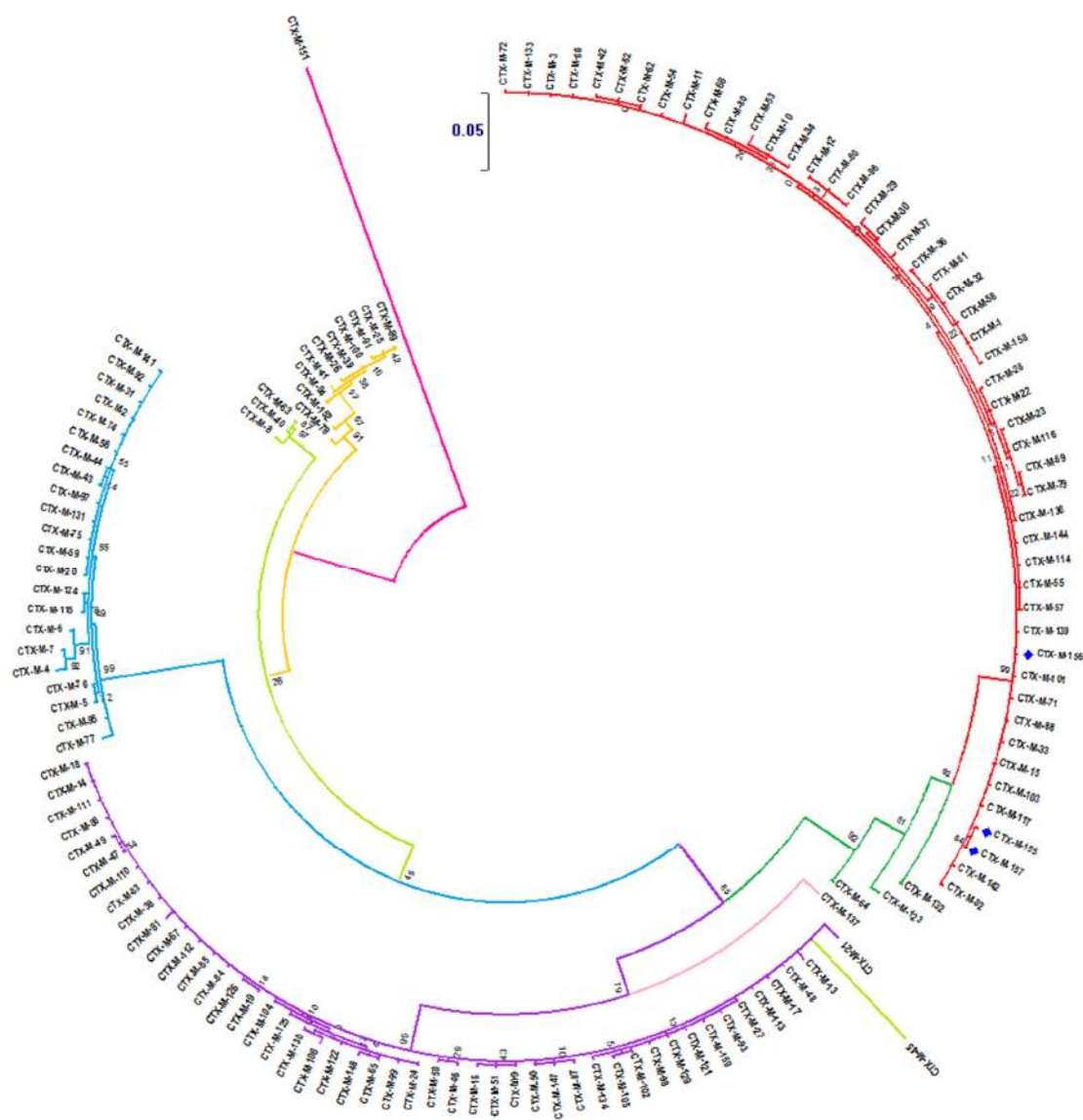
Note: The TEM types with ESBL phenotype characterized until now are marked by blue dots, whereas the novel TEM-219 detected in this study is marked by a red triangle. There appears to several clusters among TEM type beta-lactamases

Figure 29: Phylogenetic tree of SHV beta-lactamases



Note: The SHV types with ESBL phenotype characterized until now are marked by blue dots, whereas the novel SHV-185 and SHV-186 discovered in this study are marked by red triangle. Similar to the TEM type, there appears to be several clusters in among the SHV-type beta-lactamases.

Figure 30: Phylogenetic tree of CTX-M beta-lactamases



Note: The branched of the nine different clusters are displayed in different colours. CTX-M-155, CTX-M-156 and CTX-M-157 discovered in this study are marked by blue dots. From this picture it appears that in addition to the seven clusters described earlier, there are two more new clusters: cluster 151 and cluster 137, which are represented by CTX-M-151 and CTX-M-137, respectively.

Identification of beta-lactamase type by DNA sequencing:

The translated amino-acid sequences from the DNA sequences of the *bla* gene revealed that beta-lactamases could be categorized into three types: ESBLs (belonging

to Bush's 2be subgroup), non-ESBLs (belonging to 2b or 2br subgroup) and the unclassified type. While 2b subgroup comprises of classical beta-lactamases not capable of hydrolyzing oxyimino-cephalosporins, 2br subgroup comprises of inhibitor resistant types.

From the analysis of 400 isolates, a total of 725 *bla* genes were characterized, which included 270 from *E. coli* and 455 from *K. pneumoniae*. Of the 725 *bla* genes, only 372 (51.3%) were confirmed as ESBL types, which included 346 *bla*CTX-M and 26 *bla*SHV genes and the rest were either non-ESBL or of unclassified type.

Thus, from 400 ESBL positive isolates, ESBL genes were detected in only 361 (90.3%) isolates. This included 189 (94.5%) *E. coli* and 172 (86%) *K. pneumoniae* isolates. While all the *bla*CTX-M coded for ESBL, none of the *bla*TEM gene encoded for known ESBL type. Among the non-ESBL *bla*SHV genes characterized, 110 coded for beta-lactamase types of 2b or 2br subgroup and 52 were unclassified. The varieties of *bla* genes identified among the ESBL producers is depicted in Figures 31-34.

Characterization of *bla* types in *E. coli*

Following characterization of *bla* genes in *E. coli* isolates, five ESBL types were identified, which included three SHV types and two CTX-M types. ESBL genes of a single type were found in 185 (92.5%) isolates whereas both CTX-M and SHV ESBL types were found in four (2%) isolates. Among 186 *bla*CTX-M detected, 185 belonged to CTX-M-1 group and one to CTX-M-9 group. All the types in CTX-M-1 group were identified as *bla*CTX-M-15. The lone CTX-M-9 group gene was detected in an *E. coli* isolate from Davangere.

Figure 31: Functional types of the characterized *bla* genes

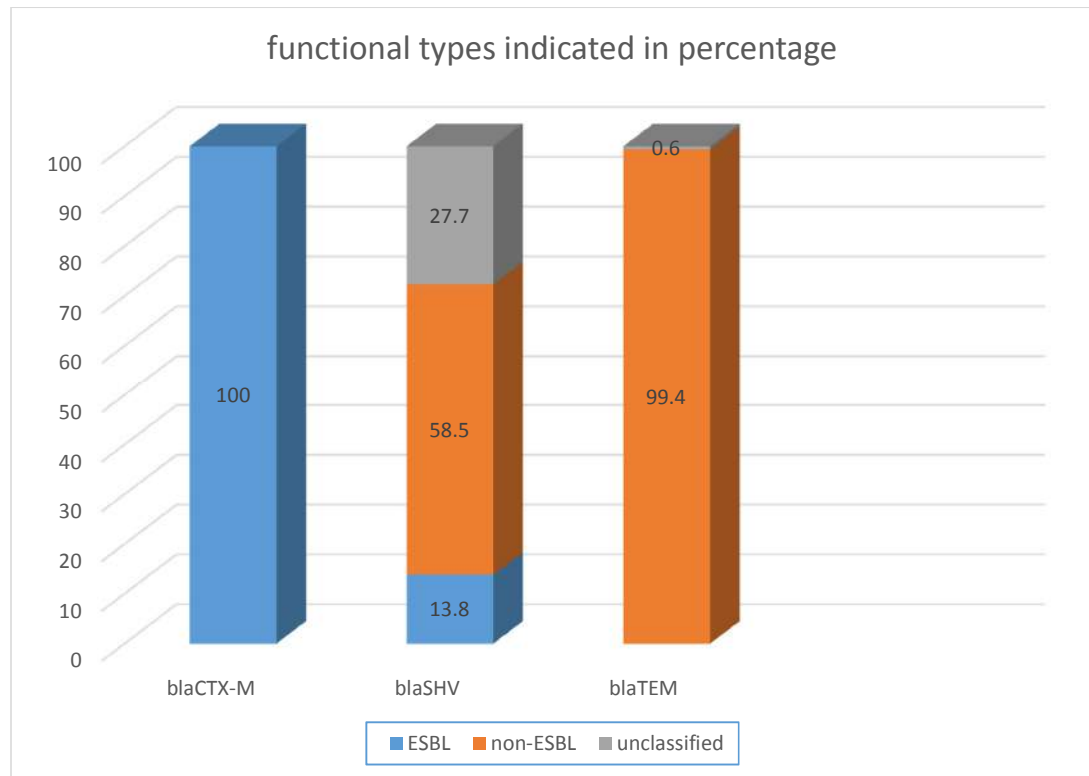


Figure 32: Functional types of *blaSHV* among *E. coli* and *K. pneumoniae*

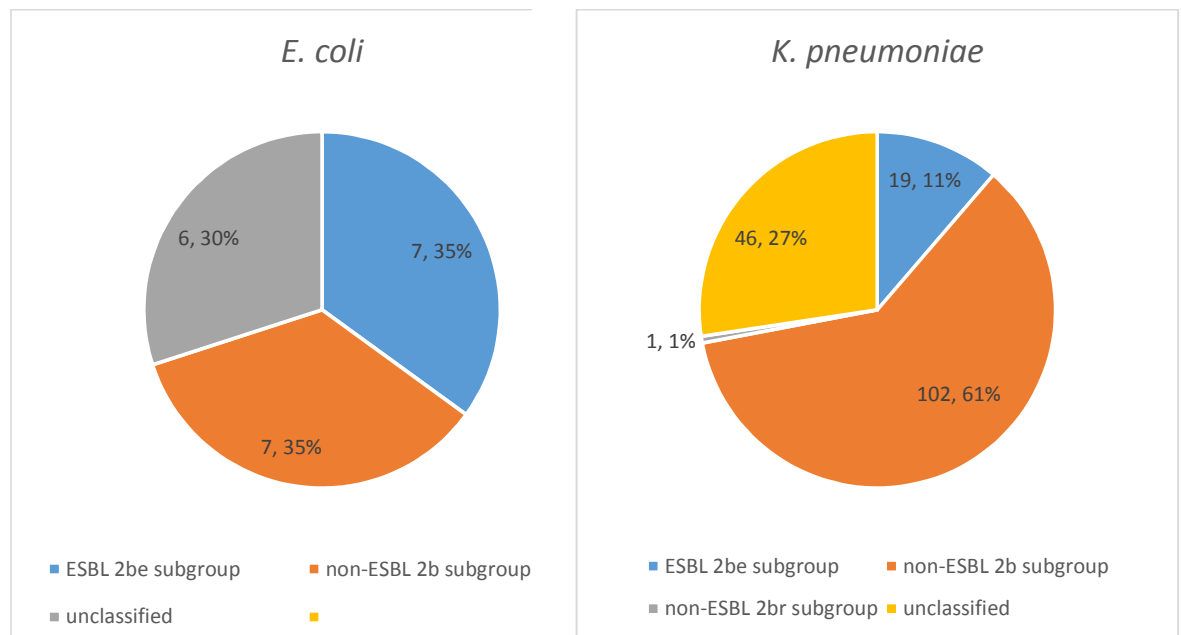


Figure 33: Numbers of bla genes detected among ESBL-EC and ESBL-KP

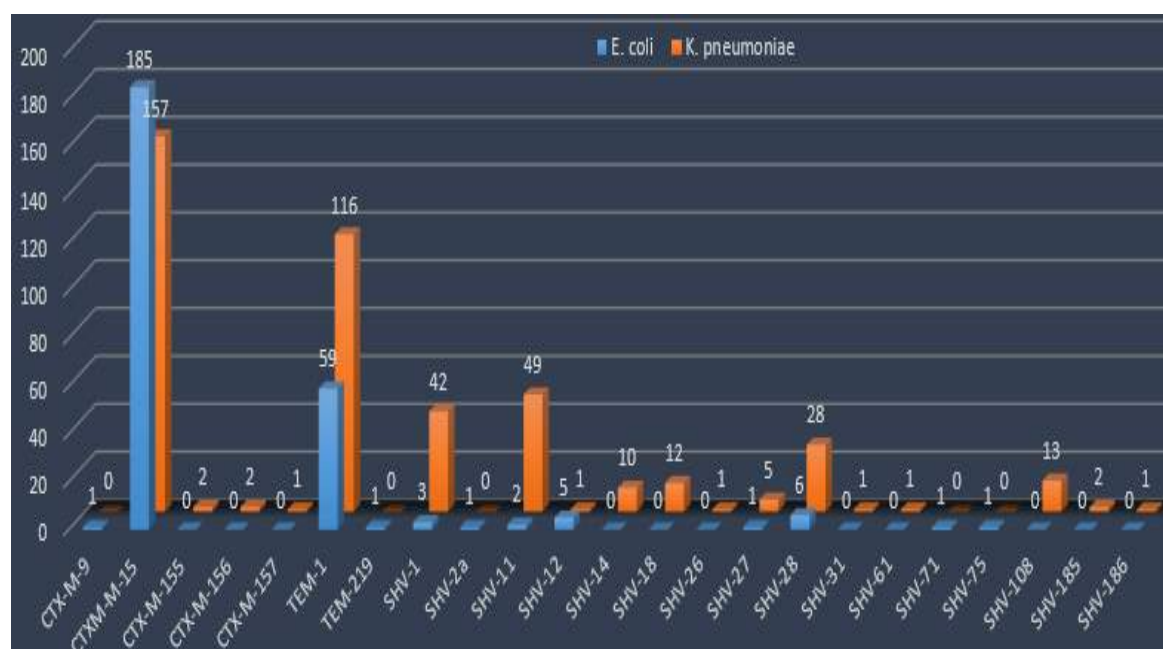
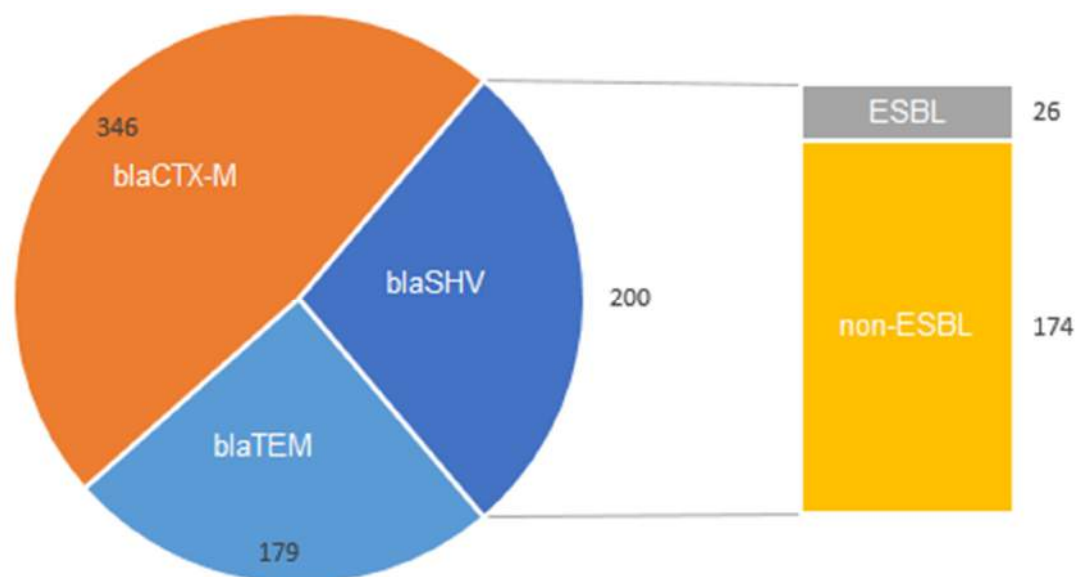


Figure 34: bla types among ESBL producers



Among the *bla*SHV genes, ESBL types detected were *bla*SHV-2a, *bla*SHV-12 and *bla*SHV-27. The non-ESBL 2b subgroup types detected were *bla*SHV-1, *bla*SHV-11, *bla*SHV-71 and *bla*SHV-75. *bla*SHV-28 was the only type whose status is currently unclassified. All the *bla*TEM genes belonged to the non-ESBL *bla*TEM-1 type with an exception. The exception detected was a novel type designated as *bla*TEM-219, which had unique amino acid substitution: Val262Phe (Figure 35). Its current status in Bush's functional grouping is uncertain. Distribution of various *bla* types across the five centers is displayed in Table 25.

Table 25: Distribution of *bla* types in *E. coli* and *K. pneumoniae* isolates across the centers

bla types among E. coli isolates							
		MNG	DWD	BEL	DVG	KOL	
E	CTX-M-9 Group	1					
	CTX-M-15	37	38	39	33	38	
	SHV-2a	1					
	SHV-12	1		3	1		
	SHV-27	1					
NE	TEM-1	8	11	22	17	1	
	SHV-1	3					
	SHV-11	1	1				
	SHV-71	1					
	SHV-75	1					
UC	SHV-28	1	2		3		
	TEM-219	1					

bla types among K. pneumoniae isolates							
		MNG	DWD	BEL	DVG	KOL	
E	CTX-M-15	33	39	21	32	32	
	CTX-M-155	2					
	CTX-M-156	1		1			
	CTX-M-157	1					
	SHV-12	1					
	SHV-18	1	7		2	2	
	SHV-27	1				4	
	SHV-31	1					
NE	SHV-1	15	2	5	13	7	
	SHV-11	9	14	4	11	11	
	SHV-14	1	7		2		
	SHV-26	1					
	SHV-61	1					
	TEM-1	22	29	18	25	22	
UC	SHV-28	5	9	8	3	3	
	SHV-108	7		3	2	1	
	SHV-168	2					
	SHV-185	2					
	SHV-186	1					

E: ESBL, NE: non-ESBL, UC: unclassified

Characterization of *bla* types in *K. pneumoniae*

Following characterization of *bla* genes in *K. pneumoniae* isolates, eight ESBL types were identified, which included four SHV types and four CTX-M types. ESBL genes of a single type were found in 165 (82.5%) isolates whereas both CTX-M and SHV type ESBL genes were found in seven (3.5%) isolates.

All the *bla*CTX-M genes detected among *K. pneumoniae* belonged to CTX-M-1 group. They were identified as *bla*CTX-M-15 with three exceptions. The exceptions were *bla*CTX-M-155, *bla*CTX-M-156 and *bla*CTX-M-157 types, which were novel types detected in this study (Figure 35). CTX-M-155 differed from CTX-M-15 by two substitutions (Glu166Ala and Ala282Gly), CTX-M-156 differed from CTX-M-15 by one substitution (Lys271Asn) and CTX-M-157 differed from CTX-M-15 by one substitution (Glu166Ala).

*bla*SHV genes identified as ESBLs included *bla*SHV-12, *bla*SHV-18, *bla*SHV-27 and *bla*SHV-31 types. Non-ESBL types (2b subgroup) included *bla*SHV-1, *bla*SHV-11, *bla*SHV-14, and *bla*SHV-61. One of the *bla*SHV genes, identified as *bla*SHV-26, was found to be a non-ESBL inhibitor resistant type (IRT) belonging to 2br subgroup. *bla*SHV-28, *bla*SHV-108, *bla*SHV-168, *bla*SHV-185 and *bla*SHV-186 types are currently not assigned to any functional subgroup. Among them, *bla*SHV-185 and *bla*SHV-186 were the novel types discovered in this study (Figure 35). SHV-185 differed from SHV-1 by one novel substitution (Val262Met) whereas SHV-186 differed from SHV-1 by two novel substitutions (Ala187Thr and Gly242Cys). All *bla*TEM genes were identified as non-ESBL *bla*TEM-1 types.

The amino acid substitutions of the novel *bla* types is displayed in Figure 34. The flowchart of identification of *bla* types among *E. coli* and *K. pneumoniae* is depicted in Figure 36 and 37.

The complete nucleotide and translated amino acid sequences of these newly discovered beta-lactamase variants have been submitted to GenBank. Following are the accession numbers: TEM-219 (KM114268), SHV-185 (KM233164), SHV-186 (KM233165), CTX-M-155 (KM211508), CTX-M-156 (KM211509) and CTX-M-157 (KM211510).

Figure 35: Amino-acid substitutions among the novel beta-lactamases

TEM-1 vs TEM-219	
	250 260 270 280 290
TEM_1	RGSRGIIAALGP-DGKPSRIVVITYTTGSQATMDERNRQIAEIGASLIKHW
TEM_219	RGSRGIIAALGP-DGKPSRIVFITYTTGSQATMDERNRQIAEIGASLIKHW
SHV-1 vs SHV-185 vs SHV-186	
	190 200 210 220 230 240
SHV_1	TTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRAGPLIRSVLPAGWFIADKTGAG-E
SHV_185	TTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRAGPLIRSVLPAGWFIADKTGAG-E
SHV_186	TTPASMTATLRKLLTSQRLSARSQRQLLQWMVDDRAGPLIRSVLPAGWFIADKTGAG-E
	250 260 270 280 290
SHV_1	RGARGIVALLGP-NNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHWQR
SHV_185	RGARGIVALLGP-NNKAERIVMIYLRDTPASMAERNQQIAGIGAALIEHWQR
SHV_186	RCARGIVALLGP-NNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHWQR
CTX-M-15 vs CTX-M-155 vs CTX-M-156 vs CTX-M-157	
	130 140 150 160 170 180
CTX_M_15	NGTMSLAELSAAALQYSDNVAMNKLIHVGGPASVTAFARQLGDETFRLDRTTEPTLNTAI
CTX_M_155	NGTMSLAELSAAALQYSDNVAMNKLIHVGGPASVTAFARQLGDETFRLDRTAPT LNTAI
CTX_M_157	NGTMSLAELSAAALQYSDNVAMNKLIHVGGPASVTAFARQLGDETFRLDRTAPT LNTAI
CTX_M_156	NGTMSLAELSAAALQYSDNVAMNKLIHVGGPASVTAFARQLGDETFRLDRTTEPTLNTAI
	250 260 270 280 290
CTX_M_15	DKTGSGGYGTTNDIAVIWPKDRAPLILVITYFTQPQPKAESRRDVLASAAKIVTDGL
CTX_M_155	DKTGSGGYGTTNDIAVIWPKDRAPLILVITYFTQPQPKAESRRDVLASGAKIVTDGL
CTX_M_157	DKTGSGGYGTTNDIAVIWPKDRAPLILVITYFTQPQPKAESRRDVLASAAKIVTDGL
CTX_M_156	DKTGSGGYGTTNDIAVIWPKDRAPLILVITYFTQPQPKAESRRDVLASAAKIVTDGL

The positions of amino-acid substitutions are marked in blue. TEM-1 is compared to TEM-219, SHV-185, -186 compared to SHV-1, CTX-M-155, -156, -157 compared to CTX-M-15 sequences.

Figure 36: Flowchart showing detection of various *bla* gene types in *E. coli*

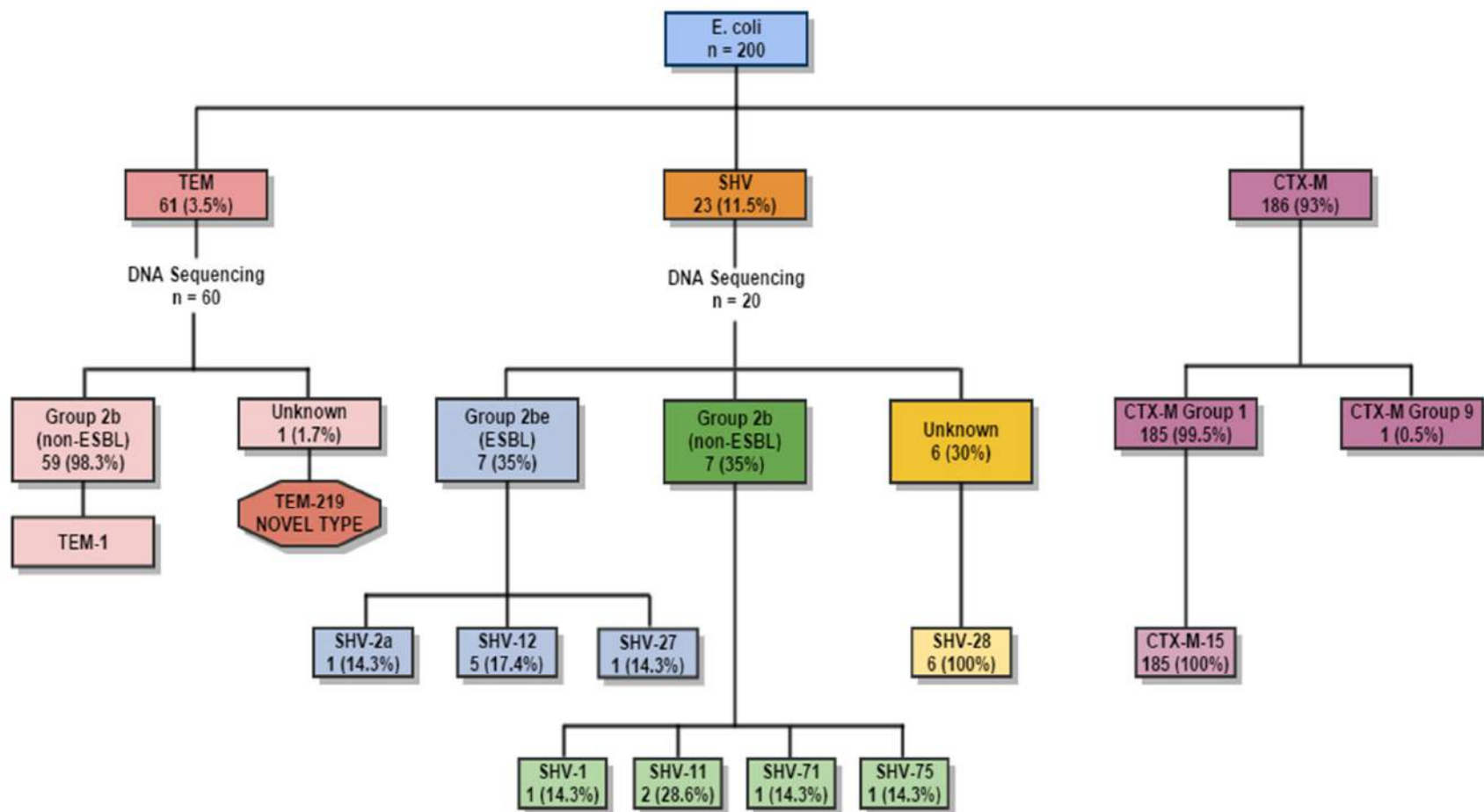
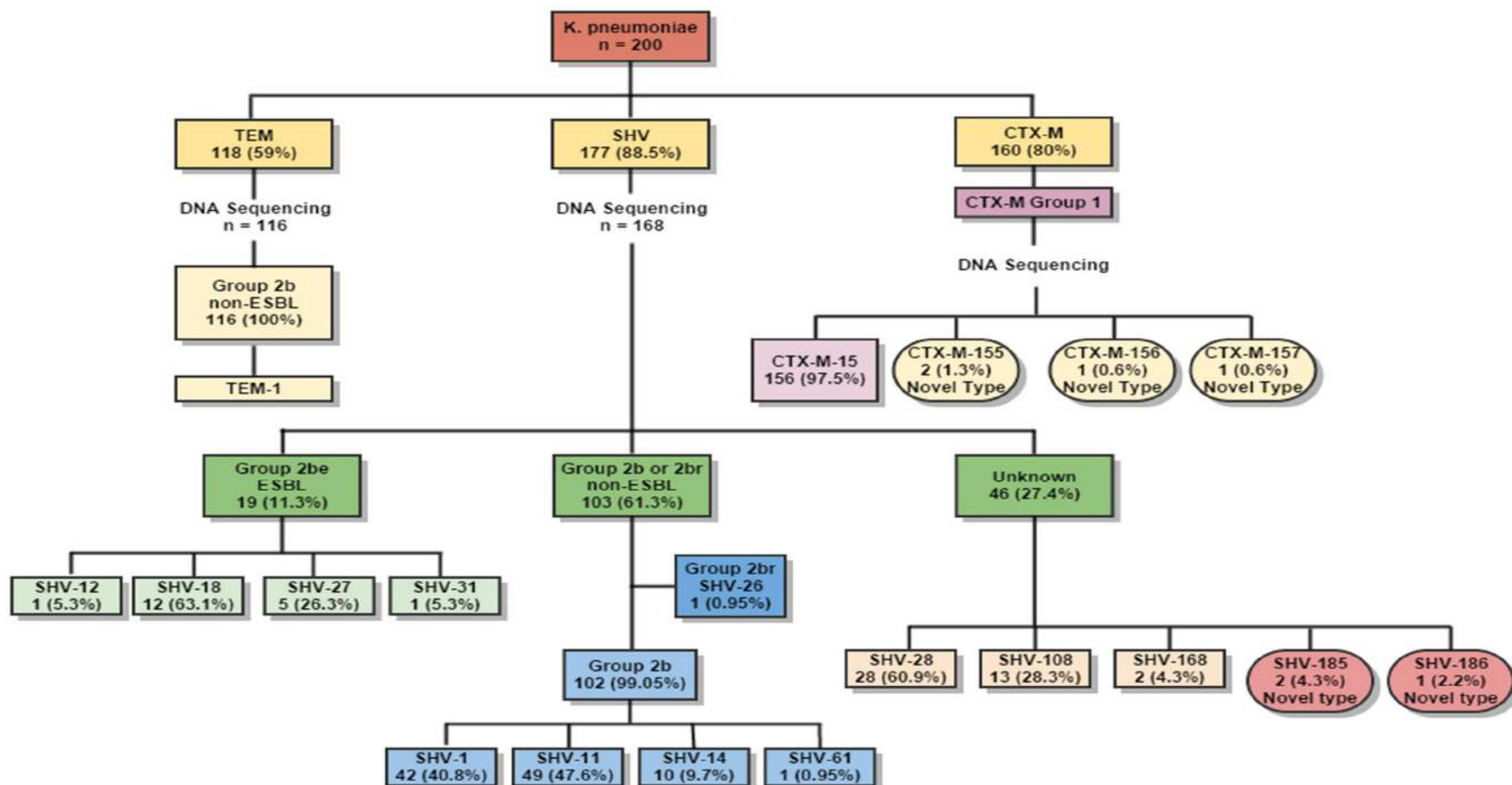


Figure 37: Flowchart showing detection of various *bla* gene types in *K. pneumoniae*



Discussion

In order to understand the diversity and distribution of ESBL types across Karnataka, one thousand clinical isolates each of *E. coli* and *K. pneumoniae* collected from five different centers were studied. These isolates were screened for resistance to four oxyimino-cephalosporin screening agents and tested for ESBL production by phenotypic method. Two hundred isolates each of *E. coli* and *K. pneumoniae* representing the total population of ESBL producers from the five centers were studied by molecular methods to characterize the *bla*TEM, *bla*SHV and *bla*CTX-M genes. Antibiotic susceptibility pattern of ESBL producers were also determined.

Majority of the clinical samples yielding the isolates used in this study came from male patients (57.1%) and most patients (65.8%) from either gender belonged to the age group of 21-60 years. Urine (42.9%), pus (29.8%) and sputum (14.5%) formed the bulk of the samples that yielded cultures of *E. coli* and *K. pneumoniae*. *E. coli* isolates were predominantly isolated from urine samples (61.4%) whereas *K. pneumoniae* isolates were predominantly isolated from pus (35.3%) and urine samples (24.3%). Comparatively more numbers of *K. pneumoniae* isolates were recovered from blood samples than *E. coli* (5.7% vs 3.5%). Isolation of *K. pneumoniae* from bloodstream infections were particularly high in the pediatric age group (48.3%). Expectedly, sputum cultures yielded higher numbers of *K. pneumoniae* (22.9%) than *E. coli* (6.1%) isolates.

In 2009, CLSI introduced two-step procedure for the detection of ESBL producers. In the first step, isolates of *E. coli* and *K. pneumoniae* should be screened for resistance to one or more of third-generation indicator cephalosporin (ceftriaxone, cefotaxime, ceftazidime, cefpodoxime or aztreonam). Since ESBLs vary in their

hydrolysis of these cephalosporins as substrates, resistance to at least one of them was considered as positive in the screening test.

In the present study, 74% of *E. coli* and 53.6% of *K. pneumoniae* were found to be positive for possible production of ESBLs in the screening test. The screening test involved ceftriaxone, cefotaxime, ceftazidime and aztreonam disks but not cefpodoxime. In the pilot study, the cefpodoxime disk did not meet the expected quality standard and therefore could not be included. False positive screening results with cefpodoxime too have been documented, which have been attributed to a variety of mechanisms including hyperproduction of TEM-1 beta-lactamase, production of OXA-30 beta-lactamase and elevated chromosomal AmpC beta-lactamase production [265]. The EUCAST guidelines mentions that cefpodoxime is less specific than the combination of cefotaxime (or ceftriaxone) and ceftazidime for screening ESBLs.

Nonetheless, each the four cephalosporins were able to detect resistance in >99% of isolates. Despite the miniscule difference in resistances noted in this study, it is worthy to note that eight strains would have been missed if ceftriaxone disk alone were to be used. In contrast to this finding, Nogueira *et al* reported highest sensitivity with the ceftriaxone disk [266]. In a deviation from the choices of screening agents, a study from Imphal in India reported cefepime to be the best screening agent [132]. However, CLSI guidelines does not include cefepime disk for screening.

There are some disadvantages associated with the screening test. The two-step detection process adds to the increased cost and delay in reporting results. It is impractical for laboratories that receive large number of samples or in situations where patients are suffering from severe infections. Although the sensitivity of screening test is high, its specificity is lower because a positive test does not necessarily indicate

ESBL production alone. Production of other beta-lactamases can also give a positive screening test [267, 268]. Rice LB *et al* described a *K. pneumoniae* isolate that exhibited ceftazidime MIC of 32 µg/ml, but was positive only for chromosomally encoded *bla*SHV-1. This strain hyperproduced SHV-1 due to mutation in the promoter region and at the same time exhibited decreased production of an OMP [269]. These evidences reveal the role of multiple mechanisms in the resistance to cephalosporins. In this study, false positive results in the screening test was observed in 148 (13.8%) isolates.

In the second step recommended by CLSI, isolates that are positive in the screening test are confirmed for ESBL production by clavulanic acid based test. Although ESBLs are inhibited by clavulanic acid, sulbactam and tazobactam, most phenotypic methods are based on clavulanic acid. In this study, both ceftazidime, ceftazidime+clavulanic acid (CAZ/CAC) and cefotaxime, cefotaxime+clavulanic acid (CTX/CEC) disks were used as per CLSI protocols. CTX/CEC disks were able to detect ESBL production in 99.4% of isolates that were positive in the screening test. CAZ/CAC disks were able to detect ESBLs in only 90.4% of isolates, suggesting that CTX/CEC disks were more sensitive in detecting ESBLs. However, if CTX/CEC disks alone were to be used, six ESBL producers would have been missed. Similarly, 103 ESBL producers would have been missed if only CAZ/CAC disks were to be used.

CLSI recommends that both cefotaxime and ceftazidime, alone and in combination with clavulanic acid must be used for detection of ESBLs. The reason for such recommendation is due to the fact that ESBLs vary in their ability to hydrolyze third generation cephalosporins as substrates. Cefotaximase type ESBLs would be missed if only ceftazidime were to be used and ceftazidimases would be missed if only cefotaxime were to be used. Most CTX-M type ESBLs except those hydrolyze ceftazidime as well, would be missed if only ceftazidime were to be used. Yet, several

studies from India have used either of them in their disk diffusion assays [270-275]. Such studies are likely to have underreported the detection of ESBLs. Thus, the CLSI guidelines on using both disk combinations is imperative.

Of the 1,276 isolates that were positive in the screening test in this study, 1076 (84.3%) isolates could be phenotypically confirmed as ESBL producers by the CLSI-PCT method. Detection of ESBL production among the isolates positive in the screening test has been shown to range from 78% to 91.1% in the other studies [276-278].

Out of the 740 *E. coli* isolates that were initially positive in the screening test, ESBL production was detected only in 614 (83%) isolates. Similarly, out of 536 *K. pneumoniae* isolates, 462 (86.2%) were identified as ESBL producers. Thus, from the total population of bacteria included in this study, ESBLs were detected in 614 (61.4%) *E. coli* and 462 (46.2%) *K. pneumoniae* isolates.

The data on the prevalence of ESBL producers as gleaned from different studies reported from the Karnataka state reviewed between 2007 and 2014 is presented in Table 26. Among *E. coli*, the prevalence of ESBL production has been shown to vary from 25.4% to 88.5%, with both highest and lowest being reported from Manipal center, albeit at different time period. Among *K. pneumoniae*, the prevalence ranged from 9.6% (Bangalore) to 96% (Gulbarga-Raichur). In majority of the studies, the prevalence of ESBLs among both *E. coli* and *K. pneumoniae* across Karnataka has been reported to be above 60%.

From the literature published during 2013-14 across India, the detection rates of ESBL-EC and ESBL-KP is shown to vary from 18 to 90% and 9 to 80%, respectively. The data on studies cited in this period is displayed in Table 27. Even the studies cited

here vary in several parameters and might not reflect the true prevalence of ESBL producers. The wide variation in the findings are probably the result of variations in the methodology adopted in those studies rather than the actual prevalence itself.

Table 26: Prevalence of ESBL-EC and ESBL-KP across Karnataka (2007-14 data)

Center	Year	ESBL-EC	ESBL-KP	Reference
Manipal	2007	88.5%	86%	[279]
Davangere	2008	62.9%	62.2%	[280]
Gulbarga- Raichur	2008	-	96%	[151]
Gulbarga- Raichur	2009	-	20%	[281]
Manipal	2009	25.4%	27.4%	[282]
Belgaum	2010	-	74%	[283]
Bijapur	2010	31.7%	46.4%	[284]
Mangalore	2010	60%	75%	[285]
Bangalore	2011	42.2-81	9.6-67.1%	[286, 287]
Hubli	2011	62.2%	71.9%	[288]
Shimoga	2011	86.7%	9.7%	[289]
Bangalore	2013	40%	63.1%	[140]
Raichur	2013	47.5%	-	[290]
Tumkur	2013	66.7%	32.2%	[291]
Chitradurga	2014	73%	51.3%	[139]

From the northern part of India, data were available from Amritsar, Delhi, Varanasi, Baddi (HP), Noida and Nainital. Studies from these centers revealed that the detection of both ESBL-EC and ESBL-KP were least from Nainital and most from Baddi. The detection rates of ESBL-EC ranged from 24% (Nainital) to 90% (Baddi),

whereas the detection of ESBL-KP ranged from 9% (Nainital) to 80% (Baddi, HP) in these centers. Data from the eastern part were available from Aizawl, Guwahati, Imphal and Kolkata. In these centers, detection of ESBL-EC ranged from 12.3% (Aizawl) to 73.6% (Guwahati) whereas the detection of ESBL-KP ranged from 26.2% (Imphal) to 93% (Kolkata). From the central part of India, data were available from Bhopal and Indore, where the detection of ESBL-EC ranged from 41.6% (Bhopal) to 50.1% (Indore) and ESBL-KP ranged from 26% (Indore) to 48.3% (Bhopal). From the western part of India, data were available from Jaipur, Pune, Ahmedabad and Baroda. In these centers, the detection of ESBL-EC ranged from 20% (Baroda) to 62.5% (Ahmedabad) whereas the detection of ESBL-KP ranged from 15.6% (Jaipur) to 67% (Baroda). From the southern part of India, data were available from Secunderabad, Vellore, Raichur, Bangalore, Tumkur and Chitradurga. The detection of ESBL-EC in these centers ranged from 18.4% (Secunderabad) to 73% (Chitradurga) whereas the detection of ESBL-KP ranged from 32.2% (Tumkur) to 63.1% (Bangalore).

Despite the variations noted in the detection rates of ESBL producers across the country, the prevalence rates are indeed high. In the Asia-Pacific surveillance study of 2011, India ranked second in terms of high prevalence [127]. In some other studies, the ESBL detection rates have reported to be 100% among the bacterial population studied [Annexure 3]. These data imply that if strict infection control measures and judicious usage of antibiotics are not implemented now, the prevalence rates of ESBL producers are likely to increase further. ESBL detection rates are known to vary across the studies conducted in the same geographical area (Table 26 and 27). Many of these variations are probably due to the variations in the study design. Epidemiological typing of the isolates can nullify this assumption. Due to wide variations in the methodologies followed, study population, sample sizes and period of

studies across India, it is improbable to derive a meaningful picture. Therefore, the importance of uniform nation-wide surveillance program is felt.

Table 27: Prevalence of ESBL-EC and ESBL-KP across India (2013-14 data)

Region	Center	ESBL-EC	ESBL-KP	Reference
Northern	Amritsar, Punjab	46-56%	15-52%	[292, 293]
	Baddi, HP	89%	80%	[129]
	Delhi	61-75%	60-74%	[294, 295]
	Nainital, Uttarakhand	24%	9%	[128]
	Noida, Uttar Pradesh	55%	-	[296]
	Varanasi, Uttar Pradesh	75%	-	[148]
Eastern	Aizawl, Mizoram	12%	-	[130]
	Guwahati, Assam	73%	67%	[297]
	Imphal, Manipur	34%	26%	[132]
	Kolkata, West Bengal	57-89%	50-93%	[131, 298]
Central	Bhopal, Madhya Pradesh	50%	48%	[133]
	Indore, Madhya Pradesh	41%	26%	[134]
Western	Ahmedabad, Gujarat	62%	65%	[136]
	Baroda, Gujarat	20%	67%	[135]
	Jaipur, Rajasthan	30-57%	15-67%	[137, 299]
	Pune, Maharashtra	44%	32%	[300]
Southern	Bangalore, Karnataka	40%	63%	[140]
	Chitradurga, Karnataka	73%	51%	[139]
	Raichur, Karnataka	47%	-	[290]
	Secunderabad	18%	33%	[301]
	Vellore, Tamil Nadu	60%	-	[302]

In the present study, variations were noted across the centers despite maintaining uniform study methodology. The prevalence of ESBL-EC was noticed to be highest from Bellary center (83.5%) followed by Mangalore and Davangere (63.5% each) and lesser from Dharwad (49.5%) and Kolar (47%) centers. Differences in the prevalence of ESBL-EC across Karnataka was significant ($P < 0.001$); on the other hand, the prevalence of ESBL-KP across Karnataka was not significant ($P = 0.2$). The reason for these variations are not clear, but might reflect the possibility of different strains becoming endemic in those centers. This may also be related to the different levels of exposure to antibiotics among the local strains.

Despite being effective in the detection of ESBL production, CLSI-PCT method suffers from both false positive and false negative results. Clavulanate based tests can give false positive results in strains of *K. oxytoca* hyperproducing K1 beta-lactamase [303]. Other causes of false positive results include hyper-production of non-ESBL TEM or SHV beta-lactamases [304, 305]. Miro E *et al.* reported *E. coli* isolates exhibiting MIC of ceftazidime up to 32 µg/ml but susceptible to cefotaxime and aztreonam. These isolates even exhibited a synergy between amoxycylav and ceftazidime suggesting the presence of an ESBL. However, genotypic study revealed hyperproduction of SHV-1 beta-lactamase in those isolates [306]. False negative results are often observed in isolates that also produce high levels of AmpC beta-lactamase, OXA-1 and KPC enzymes [211]. These enzymes are able to mask the clavulanic acid based inhibition of ESBL as they are not inhibited by it. AmpC beta-lactamases are chromosomally encoded in *E. coli* but is conspicuously absent among *K. pneumoniae*. However, plasmid mediated AmpC can exist in both *E. coli* and *K. pneumoniae* [307]. Low level expression is often not a problem but derepressed mutants that produce excessive AmpC enzymes can readily mask clavulanic acid based tests. Several

approaches have been devised to detect ESBL production in isolates that also produce AmpC beta-lactamases. These include inhibition of AmpC beta-lactamases by oxacillin, amino-phenylboronic acid or using a cephalosporin (e.g., cefepime) that is unaffected by it [305].

By using amino-phenylboronic acid disk method, only 4% of isolates in this study were deemed to produce both ESBLs and AmpC beta-lactamase enzymes. Although co-producers were mainly identified by amino-phenylboronic acid method in this study, all the co-producers were also found to be positive by cefepime/cefepime+clavulanic acid disk method. Although on the lesser side, co-production of ESBL and AmpC beta-lactamase was found to be comparatively higher among *E. coli* (5%) than *K. pneumoniae* (0.2%) isolates. In contrast to the present study, several other studies from India have reported the higher prevalence of ESBL and AmpC beta-lactamases co-producing *E. coli* or *K. pneumoniae* isolates, which ranged from 22-92% [308, 309, 286, 300, 310]. In these studies, some detected only inducible AmpC and few other detected constitutive AmpC enzymes. Varying methodologies such as cefepime/cefepime+clavulanic acid, E-test, AmpC disk method, amino-phenylboronic acid disk method, ceftazidime/ceftazidime+amino-phenylboronic acid disk method, and AmpC E test etc. were used in these studies. The prevalence of AmpC beta-lactamase producing *E. coli* (3.5%) and *K. pneumoniae* (0.9%) isolates alone too were low among both in this study. In a study from Gulbarga in Karnataka, AmpC beta-lactamases were detected among 3.3% of *E. coli* and 2.2% of *K. pneumoniae* isolates tested [311].

The variation in the prevalence of co-producers across the studies may partly be because of variation in the techniques used and the bacterial population under study. Co-producers were not pursued further in this study. It is also of interest that among

4.1% *E. coli* and 6.3% *K. pneumoniae* isolates, neither ESBL nor AmpC beta-lactamases were detected in this study. It is possible that these isolates expressed other beta-lactamase types such as KPC, OXA, or MBL, or had porin deficiencies.

In the present study, all ESBL producers were uniformly resistant to ceftriaxone, ceftazidime, cefotaxime and aztreonam, rendering them inappropriate for treatment. None of the ESBL producers tested in this study exhibited susceptibility MIC breakpoint of ≤ 1 $\mu\text{g/ml}$ to cefotaxime and ceftriaxone and ≤ 4 $\mu\text{g/ml}$ to ceftazidime. While ESBL-EC and ESBL-KP were similarly resistant to cefotaxime and ceftriaxone at MIC level of ≥ 128 $\mu\text{g/ml}$ in this study, significantly more number of ESBL-KP were resistant to ceftazidime than ESBL-EC at the same concentration.

In the present study MIC₉₀ of cefotaxime, ceftriaxone and ceftazidime amongst all ESBL producers were $>128\mu\text{g/ml}$ indicating high level resistance. MIC₅₀ of cefotaxime and ceftriaxone too were $>128\mu\text{g/ml}$ but that of ceftazidime was slightly lower, being $64\mu\text{g/ml}$ in case of ESBL-EC. Similarly, in few other studies higher MIC levels of cefotaxime and ceftriaxone than ceftazidime were reported [107, 312, 313]. In contrast to these findings, a Greek study reported slightly higher MIC values of ceftazidime than cefotaxime [314] among ESBL producers. Even the type of CTX-M ESBL can influence the MIC values. In a Korean study, the MIC₉₀ of cefotaxime among CTX-M-14 and CTX-M-15 ESBL producers were >128 $\mu\text{g/ml}$ but differed with respect to ceftazidime [118]. While the MIC₉₀ of ceftazidime was 32 $\mu\text{g/ml}$ in CTX-M-14 producers, it was 256 $\mu\text{g/ml}$ in CTX-M-15 ESBL producers. This difference is obviously due to the fact that CTX-M-15 can hydrolyze ceftazidime better than CTX-M-14.

Among the non-Indian studies, the susceptibility to ceftazidime among ESBL-EC has been shown to vary from 7 to 77%, whereas it ranged from 9% to 16.7% among ESBL-KP. Susceptibility pattern of ESBL producers to cefotaxime, ceftazidime, cefepime and ceftazidime across various studies is displayed in Table 28. Data from these studies too suggest that ceftazidime susceptibility is considerably higher among *E. coli* than *K. pneumoniae*. This could perhaps also be due to the inability of the bulkier ceftazidime molecule to pass through the narrow porins of *K. pneumoniae*.

Table 28. Susceptibility pattern of ESBL-EC and ESBL-KP in various studies

Ceftazidime		Cefotaxime		Cefepime		Cefoxitin		Center	Ref.
EC	KP	EC	KP	EC	KP	EC	KP		
24.2	27.5	0	0	7.8	7.8	76.6	82.4	Vietnam	[315]
13.6		8.6		28.4		35.2		Greece	[314]
51.4	-	0	-	8.3	-	-	-	China	[114]
-	9.1	-	0	-	-	-	64	USA	[107]
77	-	0	-	4	-	95	-	Spain	[313]
35	13.5	18.3	18.9	76.7	64.9	70	64.9	S. Arabia	[316]
7	-	0	-	32.7	-	38	-	Japan	[116]
27	-	8	-	23	-	90	-	France	[317]
-	16.7	-	16.7	-	40	-	40	Egypt	[318]
36	19	0	0	45	24	73	56	Brazil	[319]
35.5	-	31.1	-	35.5	-	31.1	-	Noida	[296]
50	70	25	30	-	-	94	70	Delhi	[294]
-	2.5	-	10.1	-	57	-	100	Pune	[320]

Note: all numbers indicate percentages; EC: *E. coli*; KP: *K. pneumoniae*

OmpK37 porin, which is believed to be present in most clinical isolates of *K. pneumoniae*, has a narrow pore thereby contributing to increased resistance to beta-lactam antibiotics [321]. With respect to cefotaxime, the susceptibility among ESBL-EC ranged from 8% to 18.3% and from 16.7% to 18.9% among ESBL-KP, suggesting that both these species do not differ much in their susceptibility to cefotaxime.

In studies from India, susceptibility of ESBL-EC to ceftazidime ranged from 35.5% to 50% and that among ESBL-KP ranged from 2.5% to 70%. Susceptibility of ESBL-EC to cefotaxime ranged from 25% to 31.1% and that among ESBL-KP ranged from 10.1% to 30% (Table 28). In few other studies, none of the ESBL producers were found to be susceptible to cefotaxime or ceftazidime [322]. These observations suggests that ESBL producers exhibit significantly high level of resistance towards cefotaxime and ceftriaxone than to ceftazidime, which may be because of presence of CTX-M-15 enzymes that hydrolyze cefotaxime and ceftriaxone better than ceftazidime. In one *E. coli* isolate that produced CTX-M-9 group ESBL in this study, the MIC of cefotaxime and ceftriaxone was 16 times more than that of ceftazidime indicating that it was predominantly a cefotaximase type ESBL.

In the present study, cefepime fared poorly against ESBL producers, as only 3.7% of ESBL-EC and 13.2% of ESBL-KP were susceptible to it. Significantly more numbers of ESBL-KP were resistant to cefepime than ESBL-EC in this study. In various studies outside India, susceptibility of ESBL-EC to cefepime ranged from 4% to 76.7% whereas among ESBL-KP, it ranged from 7.8% to 64.9% (Table 28). In studies from India, the susceptibility to cefepime among ESBL-EC ranged from 13.5% to 35.5%, whereas it ranged from 20.8% to 57% among ESBL-KP [296, 299, 320]. Few other studies have also reported complete resistance to cefepime among ESBL producers [322, 323]. Susceptibility to cefepime also depends on the type of ESBL

produced by the bacterium. While TEM-5, -12, -15, -19, and -26 are susceptible, TEM-3, -4, -6, -7, -9 are resistant to it [324]. Cefepime resistance has been documented in isolates producing SHV-2, -3, -4, -5 type ESBLs [325]. All isolates that produced only SHV type ESBLs in this study were fully susceptible to cefepime. However, MIC values of cefepime would have been a better marker of susceptibility. Resistance in the other isolates is probably because of CTX-M-15 enzymes, which are capable of hydrolyzing cefepime [64]. In fact, high MIC levels are thought to be predictors of CTX-M type ESBLs. It has been suggested that cefepime might be used for treatment of infections by ESBL producers depending on the ESBL type and MIC value of cefepime [326, 327]. Cefepime has been used in various studies to treat infections by ESBL producers but the outcomes have been mixed [327]. Despite its utility, it has been cautioned that cefepime must be avoided in intensive care units as it has the propensity to select multi-drug resistant organisms [328].

Since ESBLs are susceptible to inhibition by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam, a good approach is to combine them with oxyimino-cephalosporins. While these inhibitors inactivate the ESBLs, the combined antibiotic is free to act on the bacteria. Despite the possibility of large numbers of beta-lactam+beta-lactamase inhibitor combinations, only a few are available for clinical use. The popular ones include ampicillin+sulbactam, amoxicillin+clavulanic acid, ticarcillin+clavulanic acid, cefoperazone+sulbactam, and piperacillin+tazobactam [17]. Few other combinations such as cefixime+clavulanic acid, cefpodoxime+clavulanic acid, cefadroxil+clavulanic acid, ampicillin+clavulanic acid, cefuroxime+clavulanic acid, ceftriaxone+sulbactam, ceftriaxone+tazobactam, cefepime+tazobactam, ceftriaxone+tazobactam etc. are also available selectively in some countries for clinical use. However, the susceptibility breakpoints of only amoxicillin-clavulanic acid,

ampicillin-sulbactam, piperacillin+tazobactam and ticarcillin+clavulanate are available for testing Enterobacteriaceae members in the in the CLSI guidelines.

In this study, the resistance to cefepime was reversed more efficiently when it was combined with tazobactam than clavulanic acid ($P < 0.001$). The combination of clavulanic acid with cefepime was recommended by Livermore *et al* since this combination is useful against isolates that produce both ESBL and AmpC beta-lactamase producers [224]. However, this combination is not yet commercially available. Susceptibility breakpoints for cefepime in combination with beta-lactamase inhibitors is not available in CLSI guidelines. Studies that have tested combination of cefepime with beta-lactamase inhibitors have adopted the breakpoints of cefepime. Cefepime+tazobactam combination has been evaluated in a few studies. In a study from Jaipur, the combination of cefepime+tazobactam was shown to be active against 100% of ESBL-EC and ESBL-KP [137]. In a study from Delhi, the susceptibility of ESBL-EC and ESBL-KP was reported to be 86% and 41.5%, respectively [329]. A clinical study in Chennai has showed that this combination can be safely used in the treatment of bacteremia by gram negative bacteria [229]. This combination appears better than cefepime+clavulanic acid as tazobactam is a better inhibitor of CTX-M type ESBLs. Further clinical trials are warranted if cefepime+tazobactam is to be used as a carbapenem-sparing antibiotic.

In this study, 27.9% of ESBL-EC and 74.7% of ESBL-KP were resistant to the combination of ceftazidime + clavulanic acid. Likewise, 86.6% of ESBL-EC and 82% of ESBL-KP were resistant to the combination of cefotaxime+clavulanic acid. Although both ESBL-EC and ESBL-KP displayed almost similar resistance to cefotaxime+clavulanic acid, but significantly more number of ESBL-KP were resistant to ceftazidime+clavulanic than ESBL-EC.

Even though ESBLs are inhibited by clavulanic acid, not all ESBL producers are susceptible to the combination of cephalosporin and clavulanic acid. This could be due to hyperproduction of non-ESBL TEM or SHV beta-lactamases, which are also expressed by many ESBL producers. In this study 30.7% of ESBL-EC and 90.1% of ESBL-KP also produced non-ESBL TEM/SHV beta-lactamases.

Susceptibility to these two combinations have not been explored in many studies as these are not available for clinical use. Beta-lactam-beta-lactamase inhibitor combinations such as ticarcillin+clavulanic acid, cefoperazone+sulbactam and piperacillin+tazobactam have been found useful in the treatment of infections by ESBL producers. A review of several studies from India and abroad revealed that the susceptibility of ESBL producers to piperacillin+tazobactam ranged 90-100% or exhibited good inhibitory activity in many cases [225-227, 275, 330-335]. These antibiotic combinations were not tested in this study.

Despite not being hydrolyzed by ESBLs, ceftioxin resistance was found in 46% of isolates in this study. Only 38.6% of ESBL-EC and 48.5% of ESBL-KP were found susceptible to ceftioxin. Since the resistant isolates did not produce AmpC beta-lactamases, the resistance was probably due to porin mutations that disallowed the entry of ceftioxin into their periplasmic spaces or active efflux [336]. The susceptibility of ESBL-EC and ESBL-KP among non-Indian studies have been shown to range from 31.1% to 95% and 40% to 82.4%, respectively (Table 28). Among the Indian studies, susceptibility to ceftioxin has been shown to range from 31.1% to 94% and 70% to 100% among ESBL-EC and ESBL-KP, respectively (Table 28). In contrast, a study from Pondicherry reported resistance in 100% of ESBL-KP isolates [323]. Clinical studies on the efficacy of ceftioxin are few and more studies are warranted in this direction.

Since ESBLs do not confer resistance to carbapenems, carbapenems have become the antibiotics of choice in the treatment of serious infections by beta-lactamase producing gram negative bacteria. In this study, 98.7% of ESBL-EC and 90.7% of ESBL-KP were susceptible to Imipenem. Similarly, high susceptibility to imipenem has been shown across several studies. Clinical failures with carbapenem have been reported to occur during the course of treatment, which have been attributed to selection of porin mutants and co-production of AmpC beta-lactamase or KPC enzymes [241, 242].

Thus, for most antibiotics tested here, significantly more numbers of ESBL-KP were resistant to the beta-lactam antibiotics tested than ESBL-EC, which may be associated with tendency of *K. pneumoniae* to produce multiple beta-lactamases. When the antibiotic susceptibility results were extrapolated to the genotypically identified ESBL producers, both *E. coli* and *K. pneumoniae* exhibited similar level of resistance ($P = 0.238$) to cefepime. The reason for this discrepancy is not clear. Cefepime was found to be effective against SHV type ESBL producers.

Oxyimino-cephalosporins such as ceftazidime, cefotaxime and ceftriaxone had been the mainstay of treatment of infections caused by Enterobacteriaceae members since their introduction. Their utility in treatment of severe infections has drastically fallen in the recent times due to increase in ESBL mediated resistance [337]. Cephalosporins may be used in the treatment as long as their MIC clearly fall in the susceptible category. Hence, it is vital that diagnostic laboratories determine the MICs of cephalosporin antibiotics among ESBL producers. It is believed that inappropriate and rampant usage of these antibiotics has led to emergence and selection of ESBL producers [338, 339]. Restriction in usage of these antibiotics have been shown to be associated with reduction in incidence of ESBL production [340, 341]. These studies

have demonstrated that implementation of effective antibiotic policies can help to reduce the burden of ESBL producers.

Plasmids encoding *bla* genes transfer themselves to other strains or species. Horizontal transfer has been thought to be responsible for the widespread dissemination of ESBL producers both in the hospital and the community. Mating experiment using a standard recipient strain is often used to confirm the location of *bla* gene on a plasmid. In this study, presence of ESBL genes on plasmids was demonstrated successfully by mating experiment in 56.8% isolates. *E. coli* isolates were found to readily transfer the plasmids than *K. pneumoniae*, although this observation was statistically not significant. Wang J *et al* reported successful conjugation only in 28.1% of *E. coli* isolates [342]. In another study, Barguigua *et al* reported successful conjugation in only 50% of *K. pneumoniae* isolates [343]. Mating experiments on *bla*TEM or *bla*SHV encoding plasmids have revealed transfer frequencies in the range of 10^{-2} to 10^{-9} [81]. A study conducted at Mumbai, which evaluated horizontal transfer rates of ESBL carrying plasmids by conjugation reported transfer of resistance under laboratory and environmental conditions at a frequency of $3-4 \times 10^{-5}$, which is high [344]. The present study did not evaluate the frequency of conjugation among ESBL producers. Despite that, the promiscuity of the isolates in transferring the plasmid containing *bla* gene does not seem to be very high in this study.

When the nucleotide sequences of *bla*TEM-1, *bla*SHV-1, *bla*SHV-11 and *bla*CTX-M-15 were compared with the reference sequences, 1-6 substitutions were found. Only those nucleotide substitutions that lead to amino acid substitutions in the final protein are significant as they result in new beta-lactamase type. Lack of nucleotide substitution in the *bla*TEM-1 gene was significantly more among *K. pneumoniae* (33.6%) than *E. coli* (18.6%) isolates ($p = 0.03846$), whereas occurrence

of three nucleotide substitution was significantly more among *E. coli* (64.4%) than *K. pneumoniae* (44.8%) isolates ($p=0.01428$). No such difference was found with respect to other *bla* genes studied. Since there was no difference in the translated amino acid sequences of TEM-1, SHV-1, SHV-11 and CTX-M-15, it can be safely considered that the synonymous nucleotide substitutions were the result of silent mutations.

Although some ESBL genes are found encoded on the chromosome, most are located on plasmids [84, 348]. Mobile genetic elements such as transposons or insertion sequences are known to carry chromosomal gene into plasmids [57, 346]. In this study, plasmids extracted from the bacteria served as templates for PCR. Of the 400 isolates, only 361 (90.3 %) isolates, including 189 (94.5%) *E. coli* and 172 (86%) *K. pneumoniae* could be confirmed as ESBL producers by the detection of ESBL gene. Among those isolates in which no ESBL genes were detected, nine possessed *bla*TEM or *bla*SHV gene but their genes could not be sequenced. A positive phenotypic test could also be possible due to hyperproduction of classical beta-lactamases. Absence of ESBL gene could also be possible due to missing out on the detection of ESBL gene or the loss of encoding plasmid during storage. A total of 24 different *bla* gene types were identified, of which only 10 (41.7%) were identified as ESBL types. While all the *bla*CTX-M genes were of ESBL types, only five *bla*SHV but none of *bla*TEM were confirmed as ESBL types. These observations reiterate the importance of sequencing the *bla*TEM and *bla*SHV genes. Among the studies from India very few had undertaken DNA sequencing for confirmation of ESBL genes, hence this study gains importance.

All over the world, CTX-M ESBLs have replaced the TEM and SHV type ESBLs and this phenomenon has been described as the “CTX-M β -lactamase pandemic” [81]. In the Indian subcontinent, CTX-M-1 group, which includes the CTX-M-15 type, is endemic. As reported worldwide, CTX-M type ESBLs predominated

among both *E. coli* (93%) and *K. pneumoniae* (80%) isolates in this study. With one exception, all the *bla*CTX-M genes were identified as *bla*CTX-M-15 belonging to the CTX-M-1 group.

In addition, this study reports three new variants of CTX-M-15, designated as CTX-M-155, CTX-M -156 and CTX-M -157. Amino acid substitutions, such as Val103Asp, Asn104Asp, Asn106Lys, and Pro107Ser, which are located in the 103VNYN106 loop, are considered important in cefotaxime hydrolysis. While substitution Ser237Ala, Ser130Gly, and Arg276Asn are important in cefotaxime hydrolysis, Arg164His, Pro167Ser and Asp240Gly substitutions are important in ceftazidime hydrolysis [347]. None of the substitutions noted among the novel CTX-M types discovered in this study seems to have occurred at significant locations. The antibiotic resistance of isolates producing these novel CTX-M types were similar to those of CTX-M-15 producers suggesting that the substitutions did not affect the antibiotic resistance profile.

Prevalence of CTX-M ESBL producing *E. coli* across India range from 4.5% (Aligarh) to 100% (Delhi). Among *K. pneumoniae*, CTX-M producers range from 36% (Pondicherry) to 100% (Ajmer) [Annexure 3]. Despite these variations, the average prevalence of CTX-M producers in both *E. coli* and *K. pneumoniae* is generally between 65-70%. Among the studies published from India, only a few identified the CTX-M-15 types and the rest are unknown, but are likely to belong to CTX-M-1 group. Though CTX-M group 1 genes are most commonly encountered in India, rarely CTX-M-8 and CTX-M-9 groups have been also reported. A study from Delhi reported detection of a CTX-M-8 group gene in a single isolate obtained from the stool of a neonate [348]. The *bla*CTX-M gene belonging to CTX-M-9 group detected in an *E. coli* isolate in this study could not be sequenced. There are at least three documented

reports on detection of CTX-M-9 from India. These include isolates of *K. pneumoniae* and *E. coli* isolated from the stool sample of neonates at a hospital in Kolkata, *E. coli* isolates from Baddi and Varanasi [129, 148, 349]. Apart from these, there are isolated reports of other CTX-M types such as CTX-M-1, CTX-M-3, CTX-M-28 and CTX-M-72 [144, 148, 149]. Current knowledge suggests that CTX-M-15 is the predominant ESBL type among Indian isolates of *E. coli* and *K. pneumoniae*. As more studies undertake molecular characterization of *bla* genes, we might come across other CTX-M types too.

SHV type ESBLs are derived from the chromosomal SHV beta-lactamases belonging to the 2b subgroup following one or several mutations in the active site of the enzyme. *blaSHV-1* and *blaSHV-11* are the common chromosomal *bla* genes among isolates of *K. pneumoniae* but can also exist in plasmids. Of the 17 different SHV types detected in this study, only five (29.4%) types coded for ESBL phenotypes. These included SHV-2a, SHV-12, SHV-18, SHV-27 and SHV-31. While SHV-12 was common among *E. coli*, SHV-18 was common among *K. pneumoniae* isolates in this study. SHV-12 and SHV-27 were detected among both *E. coli* and *K. pneumoniae* isolates.

Non-ESBL SHV type beta-lactamases were detected in 35% of *E. coli* and 61% of *K. pneumoniae* isolates in this study. Among them SHV-1 and SHV-11 were common among both *E. coli* and *K. pneumoniae*, although they were more frequently detected among *K. pneumoniae* isolates. Production of these enzymes are thought to help the bacteria hydrolyze aminopenicillins and early cephalosporins, which are hydrolyzed less efficiently by the ESBLs. Hammond *et al* have shown by in-vitro experiments that *K. pneumoniae* isolates harbouring non-ESBL *blaSHV* on plasmids can mutate under exposure to antibiotics and evolve into ESBL encoding phenotype

[346]. This observation suggests that presence of non-ESBL *bla* types may serve as reservoir for development into ESBL types.

One *K. pneumoniae* isolate in this study also produced SHV-26, a non-ESBL enzyme with inhibitor resistant phenotype. This isolate, which also produced TEM-1 and CTX-M-15 ESBL, exhibited MIC of ceftazidime and cefotaxime at 32 µg/ml but ceftriaxone MC of >128 µg/ml. The inhibitor resistant property of SHV-26 does not seem to have been expressed in this isolate as it gave a positive phenotypic test for ESBL production.

In this study, 30% of SHV types detected among *E. coli* and 27% among *K. pneumoniae* isolates, are currently unclassified. Among them, SHV-28 was the most common, which was detected among both *E. coli* and *K. pneumoniae* isolates. Another unclassified variant, SHV-108 was exclusively seen in *K. pneumoniae* isolates. This study reports SHV-26, SHV-61, SHV-71, SHV-75 and SHV-168 for the first time in India and more importantly, SHV-185 and SHV-186 as the novel SHV types discovered in this study.

Amino acid substitutions at positions 146, 156, 169, 179, 205, 238 or 240 are responsible for ESBL phenotype in SHV enzymes [55]. The Val262Met substitution in SHV-185 and Ala187Thr and Gly242Cys substitutions in SHV-186 seems to have occurred at locations, which do not seem to affect hydrolytic properties of these enzymes. At the same time, the isolates that produced these novel SHV types also produced CTX-M-15 ESBL, hence the hydrolytic property of these new SHV variants could not be ascertained.

Although several studies have detected *bla*SHV gene by PCR in India, reports on SHV-type ESBLs are scanty, as most of these studies did not sequence the entire *bla*

gene. Among the published reports, SHV-12 appears to be the most common ESBL type. One study from Baddi, reported detection of SHV-2, SHV-4 and SHV-5 ESBLs. Among the non-ESBL SHV types, SHV-1, SHV-11, and SHV-28 have been reported in the literature [Annexure 3].

In this study, 179 *bla*TEM genes were detected among the ESBL producers. Sequencing revealed that all except one belonged to the non-ESBL *bla*TEM-1 type. The lone exception, named as TEM-219 was a novel discovery in this study and had Val262Phe amino acid substitution. ESBL phenotype among TEM enzymes is associated with amino acid substitutions at positions 104, 164, 238, or 240. The substitution at position 262 in TEM-219 seems to have no bearing on its hydrolytic property. The *E. coli* isolate, which produced it also produced SHV-71 and CTX-M-15 enzymes, hence the hydrolytic property of TEM-219 could not be ascertained.

*bla*TEM genes have been detected in many studies across India but not many had characterized them by DNA sequencing, hence the actual prevalence of TEM ESBLs is unknown. Among the few studies that characterized *bla*TEM genes, the ESBL types included TEM-3, TEM-10, TEM-20, TEM-52, TEM-136, TEM-149 and TEM-169, but they were all isolated reports. Among the non-ESBLs, TEM-1 was the most commonly identified TEM type and rest rare ones included TEM-33, TEM-50, TEM-116, and TEM-190 [Annexure 3].

Before the emergence of CTX-M ESBLs, most ESBLs belonged to the TEM or SHV types. In a study conducted during 1996-97 that involved seven countries, SHV type ESBLs (67.1%) were the most common and TEM-type ESBLs were detected in only 16.4% of isolates [350]. That was the period when CTX-M ESBLs were making their appearances across the world. In that study, CTX-M ESBLs were detected from

Australia, Belgium, Turkey, and South Africa for the first time. Nonetheless, they were discovered in 23.3% of isolates in all countries except USA. Much has changed since then. At present CTX-M is the predominant ESBL type all over the world. Observations from several national and international studies suggest that perhaps these bacteria are slowly giving up TEM and SHV type ESBLs in favor of more potent CTX-M ESBLs. At the same time, the bacteria are retaining classical beta-lactamases, which may serve two purposes; 1) to hydrolyze aminopencillins and early cephalosporins, against which CTX-M ESBLs are not highly efficient and 2) by overproducing these enzymes, the bacteria can overcome the inhibitory effects of beta-lactamase inhibitors.

Some variations were observed in types and distributions of *bla* genes among the isolates obtained from the five centers. Among *E. coli* isolates, least *bla*TEM types were noticed from Kolar (2.5%) and most from Bellary (55%) center. Similarly least numbers of *bla*SHV genes were noted in isolates from Dharwad and Kolar (2.5% each) centers but most were from Bellary (25%) center. Among *K. pneumoniae* isolates, least *bla*TEM types were noted from Bellary (45%) and most from Dharwad (72.5%) center. With respect to *bla*SHV genes in *K. pneumoniae*, the distribution across the centers were fairly uniform but most SHV type ESBLs were found in isolates from Bellary. While the distribution of *K. pneumoniae* with *bla*CTX-M gene were seen fairly uniform (~85%) across Davangere, Mangalore, Kolar and Dharwad centers, it was significantly less ($p < 0.0001$) from Bellary center (45%). Compared to the other centers, isolates from Bellary center presents a unique picture in three ways; a) *bla*TEM genes were found to be highest among *E. coli* but least among *K. pneumoniae* isolates, b) *bla*SHV genes were highest among both *E. coli* and *K. pneumoniae* isolates and c) *K. pneumoniae* possessed least numbers of *bla*CTX-M genes. These variations perhaps reflect prevalence of limited number of plasmid types in bacteria or endemicity of

certain strains, which are restricted to certain geographical area. Only typing methods such as plasmid typing or MLST can clarify this assumption.

Certain differences in the types and prevalence of *bla* genes among *E. coli* and *K. pneumoniae* were also noted in this study. These differences are summarized in the Figure 38. Among *E. coli* isolates *bla*CTX-M (93%) was the predominant gene, whereas *bla*SHV (88.5%) predominated among *K. pneumoniae* isolates. Similar observation was made in studies from Vellore, Amritsar, Jaipur and Tumkur where *bla*CTX-M was the predominant gene type among both *E. coli* and *K. pneumoniae*. An exception to this observation was noted in the study from Guwahati among *K. pneumoniae* isolates where *bla*TEM genes predominated over *bla*CTX-M genes. The comparative data from these centers is displayed in Figure 39. Although *bla*CTX-M was detected among both *E. coli* and *K. pneumoniae* isolates in large numbers in this study, it was significantly higher among *E. coli* isolates ($P = 0.00014$). Similar observations were made in studies from Guwahati, Tumkur, Vellore, Amritsar and Delhi. In contrast, a slightly higher prevalence of CTX-M ESBLs were noted among *K. pneumoniae* than *E. coli* isolates from Jaipur.

In this study, detection of *bla*TEM and *bla*SHV genes were significantly higher among *K. pneumoniae* than *E. coli* ($P < 0.001$). Similar observations were reported in studies from Ahmedabad, Guwahati, Jaipur, Delhi, Amritsar and Vellore. In contrast, the prevalence of *bla*TEM genes were higher among *E. coli* isolates in the study from Tumkur.

While possession of single *bla* gene type was significantly more among *E. coli*, occurrence of multiple *bla* gene types was significantly more among *K. pneumoniae* ($P < 0.001$) in this study. Similar observations were reported in studies from Vellore,

Amritsar, Jaipur, Guwahati, and Ahmedabad. In contrast, detection of single *bla* gene types was reported to be higher among *K. pneumoniae* than *E. coli* in a study from Tumkur. In *E. coli* isolates from Vellore and Jaipur, presence of two *bla* types predominated over single *bla* type. In this study, equal numbers (48 each) of *E. coli* and *K. pneumoniae* possessed a combination of two *bla* genes. While the combination of *bla*CTX-M and *bla*TEM was predominant in *E. coli*, the combination of *bla*CTX-M and *bla*SHV was predominant in *K. pneumoniae* isolates.

Unlike *E. coli* (6.5%), more numbers of *K. pneumoniae* (53%) isolates harboured all the three genes (*bla*TEM, *bla*SHV and *bla*CTX-M) in this study. Besides the present study, studies from Vellore and Jaipur reported predominance of three *bla* types over two types in *K. pneumoniae* isolates. On the contrary, the study from Guwahati reported prevalence of two *bla* types over three *bla* types among *K. pneumoniae* isolates. Presence of multiple *bla* genes is advantageous to *K. pneumoniae* as it confers resistance to multiple class of beta-lactam antibiotics and thereby provide survival advantage.

This study establishes that considerable genetic diversity and variations in the distribution of ESBL producers and their *bla* genes exist in the clinical isolates of *E. coli* and *K. pneumoniae* across Karnataka. Such studies have to be carried on a continuous basis to detect the emergence of newer resistant strains in the region. Regional and national-level surveillance programs conducted on a periodic basis are vital in understanding and documenting the evolution and spread of beta-lactamase mediated resistant bacteria. Genotypic variations among the bacteria in the population may also influence the choice of strategy for their control and outcome.

Figure 38: Some differences found between *E. coli* and *K. pneumoniae* isolates in this study

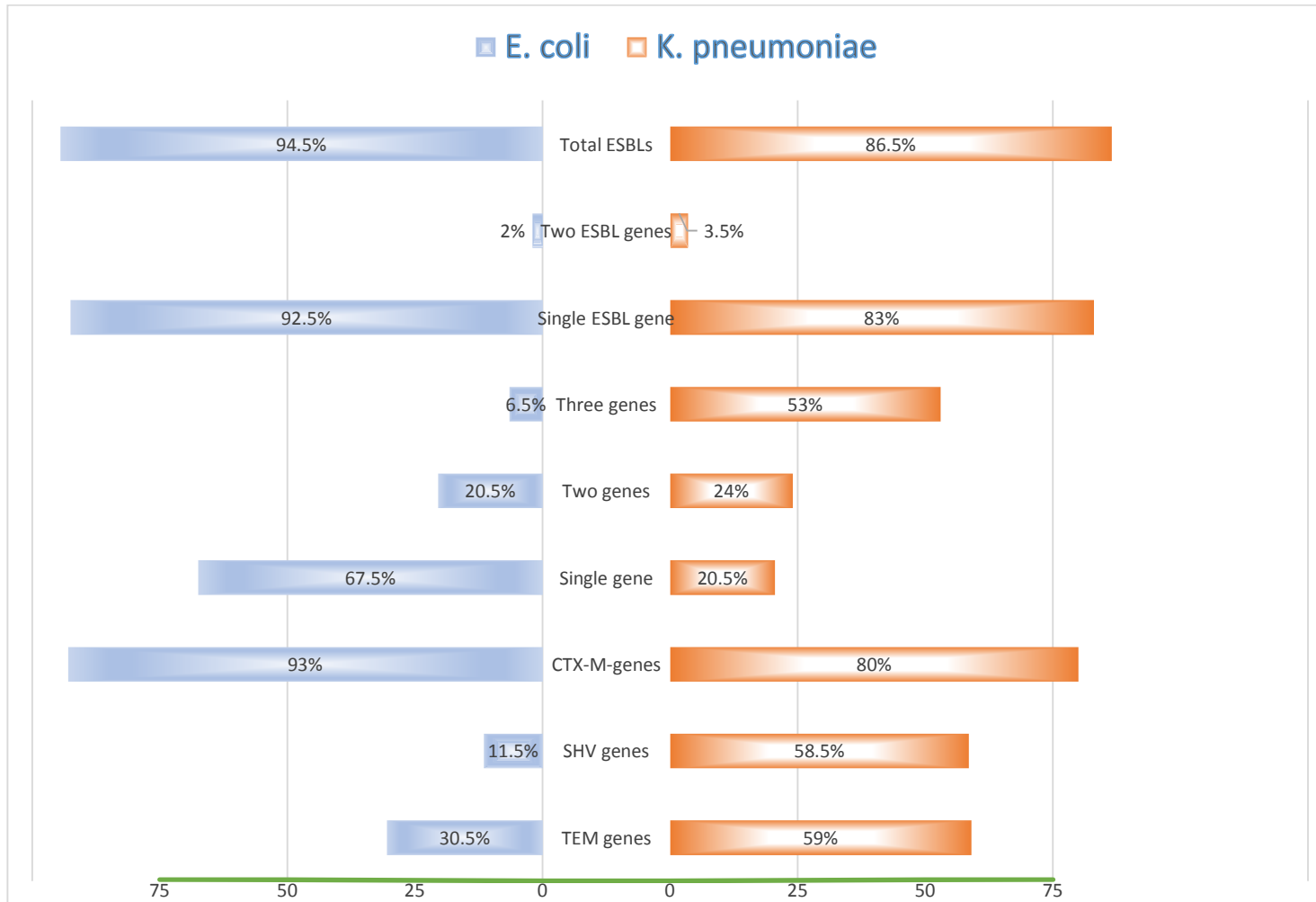
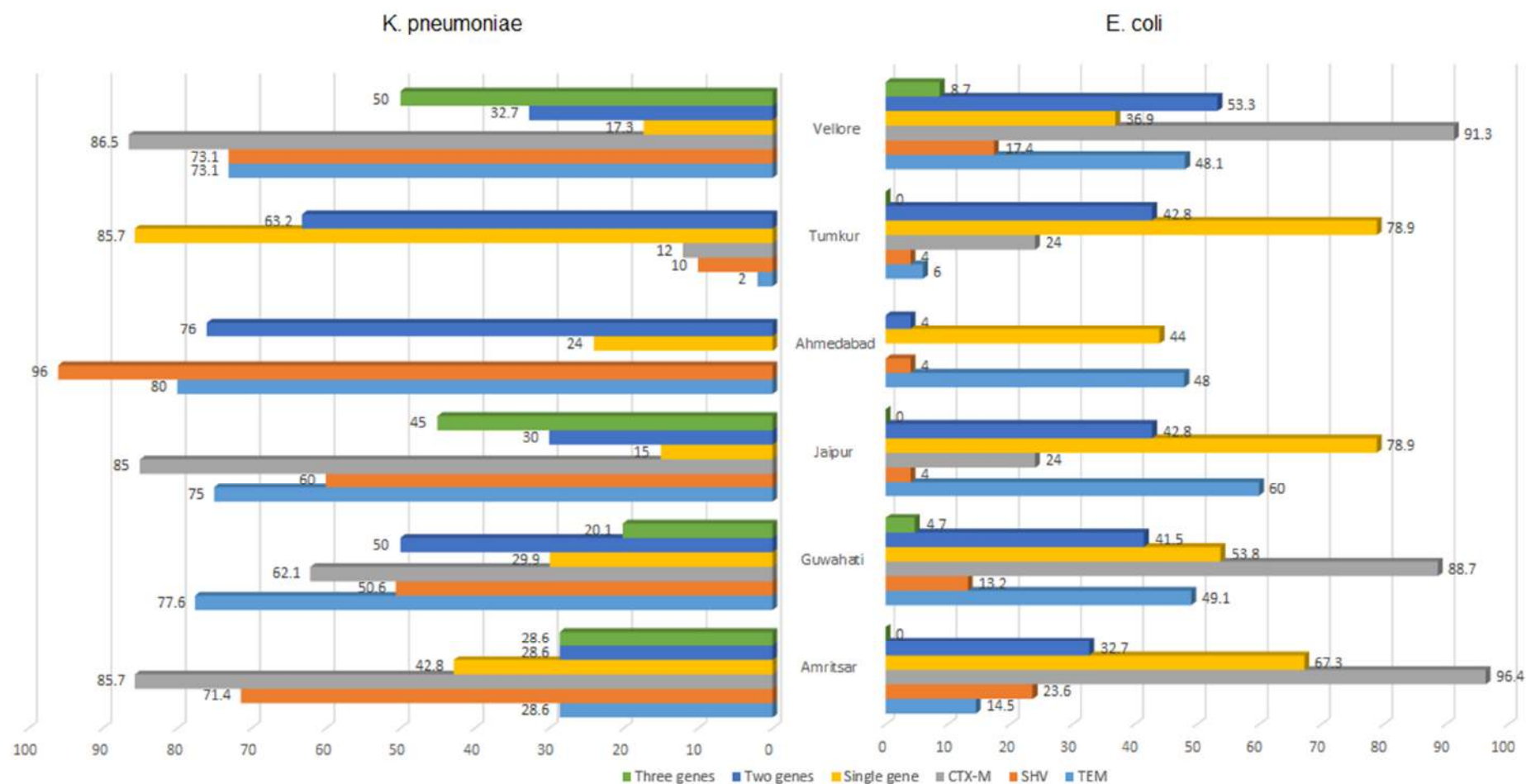


Figure 39: Comparative data of the three *bla* types and their combinations from various studies across India



Summary

One thousand isolates each of *E. coli* and *K. pneumoniae* cultured from various clinical samples obtained from five centers across Karnataka (Mangalore, Dharwad, Bellary, Davangere and Kolar) were studied for ESBL production. ESBLs in these isolates were detected by two-step method as recommended by the CLSI: screening for resistance to four cephalosporin antibiotics and disk potentiation using cefotaxime and ceftazidime alone and with clavulanic acid. By phenotypic methods, 61.4% of *E. coli* and 46.2% of *K. pneumoniae* were identified as ESBL producers. The positive result in the screening test was also due to co-production of AmpC beta-lactamases in 5% of *E. coli* and 0.2% of *K. pneumoniae*. In 4.1% of *E. coli* and 6.3% of *K. pneumoniae*, neither ESBL nor AmpC beta-lactamase enzymes were detected suggesting that some other mechanism of resistance could be at play in those isolates.

It was noted that while the prevalence of ESBL producing *E. coli* isolates varied across the five centers, the distribution of *K. pneumoniae* was fairly uniform. When the ESBL producers were subjected to antibiotic susceptibility testing, it was observed that all of them were resistant to third generation cephalosporins such as cefotaxime, ceftriaxone and ceftazidime. By estimating MIC values, high-level resistance was observed among the ESBL producers to cefotaxime and ceftriaxone than to ceftazidime. This is a feature of CTX-M-15 ESBLs. At the same time, resistance to ceftazidime was pronounced among *K. pneumoniae* isolates than *E. coli*. Resistance to cefepime among ESBL producing *E. coli* (96.3%) and *K. pneumoniae* (86.8%) was found to be high. Cefepime+tazobactam was inhibitory to 91.2% of ESBL producing *E. coli* and 71% of ESBL producing *K. pneumoniae* suggesting that this could be explored as a carbapenem-sparing alternative. Cefoxitin resistance was noted in 61.4% of ESBL-EC and 51.5% of *K. pneumoniae*. Since these isolates tested negative for AmpC beta-

lactamases, porin mutations or efflux pump mediated resistance could be the reason for cefoxitin resistance. More than 90% of ESBL producers were susceptible to imipenem suggesting that these drugs continue to be effective against ESBL producers.

By PCR technique, *bla*TEM, *bla*SHV and *bla*CTX-M genes were detected among a representative sample of 200 isolates each of ESBL producing *E. coli* and *K. pneumoniae*. *bla*CTX-M was the dominant *bla* type among both *E. coli* and *K. pneumoniae* although it was higher among *E. coli*. The other two genes, *bla*TEM and *bla*SHV were detected in higher numbers among *K. pneumoniae* than *E. coli* isolates. While single *bla* type was common among *E. coli* isolates, multiple *bla* types were common among *K. pneumoniae* isolates. ESBLs were identified using the translated amino acid sequences.

Of the 725 *bla* genes characterized in the study, only 372 (51.3%) were confirmed as ESBL types and the rest were either non-ESBLs or unclassified types. By molecular methods, only 189 (94.5%) *E. coli* and 172 (86%) *K. pneumoniae* isolates studied were confirmed as ESBL producers. While most of the CTX-M ESBLs were identified as CTX-M-15, one isolate was positive of CTX-M-9 group gene. In addition, CTX-M-155, CTX-M-156 and CTX-M-157 were the novel CTX-M types discovered in this study.

Of the 17 different SHV types identified in this study, only five (SHV-2a, SHV-12, SHV-18, SHV-27 and SHV-31) were ESBLs. Among the SHV types, 58.5% were identified as the non-ESBL types. In addition, SHV-185 and SHV-186 were the novel SHV types discovered in this study. All the *bla*TEM types with one exception detected in this study were identified as non-ESBL TEM-1 type. TEM-219, the lone exception, was a novel TEM type discovered in this study. This study clearly highlights the

difference in prevalence and diversity of ESBL among the isolates across the five centers. At the same it the study emphasizes the importance of sequencing the entire *bla*SHV and *bla*TEM gene for accurate identification of ESBL types.

Conclusions

Of the 1,000 clinical isolates each of *E. coli* and *K. pneumoniae* tested in this study, ESBLs were detected among 61.4% of *E. coli* and 46.2% of *K. pneumoniae* across the five centers of Karnataka.

The prevalence of ESBL producing *E. coli* was significantly higher than ESBL producing *K. pneumoniae* isolates.

Co-production of ESBL and AmpC beta-lactamases in 5% of *E. coli* and 0.2% of *K. pneumoniae*. In 4.1% of *E. coli* and 6.3% of *K. pneumoniae*, neither ESBL nor AmpC beta-lactamase enzymes were detected.

The prevalence of ESBL producing *E. coli* varied in distribution across the five centers of Karnataka but the distribution of ESBL producing *K. pneumoniae* was fairly uniform.

All ESBL producers were resistant to ceftazidime, cefotaxime and ceftriaxone. High level resistance at MIC levels of >128ug/ml was seen towards cefotaxime and ceftriaxone than ceftazidime. Resistance to ceftazidime was significantly higher among ESBL producing *K. pneumoniae* than ESBL producing *E. coli*.

Resistance to ceftazidime and cefepime alone was high among both the ESBL producing *E. coli* and *K. pneumoniae*. Cefepime+tazobactam exhibited good in-vitro inhibitory activity against ESBL producers. Further clinical trials are required to use cefepime+tazobactam as carbapenem sparing agent. Negligible resistance to imipenem among ESBL producers suggests that carbapenems are still useful therapeutic agents.

Following PCR technique, 725 *bla* genes, comprising of 346 *bla*CTX-M, 200 *bla*SHV and 179 *bla*TEM genes were identified. A total of 24 different *bla* gene types were identified, of which only 10 (41.7%) were identified as ESBL types.

Of the 400 isolates tested, only 361 (90.25%) isolates were confirmed as ESBL producers by the detection of ESBL gene. By genotypic methods, only 189 (94.5%) *E. coli* and 172 (86%) *K. pneumoniae* isolates could be confirmed as ESBL producers.

ESBL producing *K. pneumoniae* harboured higher numbers of *bla*TEM and *bla*SHV than *E. coli*. Multiple *bla* types were common among *K. pneumoniae* whereas single *bla* types were common among *E. coli*.

While all the *bla*CTX-M genes were of ESBL types, only five *bla*SHV but none of *bla*TEM types were confirmed as ESBL types. CTX-M was the dominant ESBL type among both *E. coli* and *K. pneumoniae*, although it was higher among *E. coli*.

Five new *bla* types were detected; CTX-M-155, CTX-M-156, CTX-M-157, SHV-185, SHV-186, and TEM-219. Nucleotide and amino acid sequences of these novel enzymes were submitted to GenBank and accession numbers were obtained.

Regular multi-centric regional and national-level surveillance studies are important to track the evolution and spread of beta-lactamases. Detection of beta-lactamases and evaluation of genetic environment of the clinical isolates would be useful in epidemiological studies and to provide biochemical and molecular basis for bacteriological resistance.

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Annexures

LIST OF ANNEXURES:

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ANNEXURE 1:

Global prevalence of ESBL-EC and ESBL-KP based on international surveillance studies

Duration	Study	Region	ESBL-EC	ESBL-KP	Reference
1994	Individual	Europe	-	22.8%	Livermore DM [1]
		France	-	24%	
		Italy	-	17%	
		Spain	-	1%	
		Portugal	-	49%	
		Turkey	-	59%	
		The Netherlands	-	16%	
		Belgium	-	31%	
		Germany	-	9%	
1997-98	SENTRY	Canada	17.1%	5.3%	Villegas MV [2]
		USA	16.7%	8.5%	
		Europe	20.9%	5.1%	
		Latin America	20.1%	8.8%	
1997-98	SENTRY	Europe	1.3%	18.4%	Nijssen S [3]
1997	SENTRY	Canada	4.2%	4.9%	Winokur PL [4]
		USA	3.3%	7.6%	
		Latin America	8.5%	45.4%	
		Europe	5.3%	22.6%	
1997-99	MYSTIC	Europe	8.5-12.7%		Goossens H [5]
		Belgium	4.9%		
		Germany	0.9-3%		

		Italy	9.6-39.8%		
		Poland	18.5-31.5%		
		Russia	23.9-34.4%		
		Sweden	3.6-4%		
		Turkey	23.4%		
		UK	5.4-22.7%		
1998-99	SENTRY	Australia	0.2%	5.9%	Bell JM [6]
		South Africa	5.1%	36.1%	
		Hong Kong	14.1%	13.8%	
		Mainland China	24.5%	30.7%	
		Philippines	9.4%	31.5%	
		Singapore	5.1%	41.5%	
		Taiwan	5.8%	12.8%	
1997-2000	MYSTIC	Eastern Europe (Russia, Poland ,Turkey)	39-47%		Goossens H [7]
		Germany	1-5%		
		UK	7- 22%		
		Italy	9-15%		
1997-2000	SENTRY	Latin America	7.7%	46.2%	Sader HS [8]
		Argentina	8.7%	45.9%	
		Brazil	6.7%	49.6%	
		Chile	7.6%	46.2%	
		Colombia	7%	39.1%	
		Mexico	11.3%	47.9%	
		Venezuela	2.6%	47.9%	
1997-2000	SENTRY	USA	-	6-7%	Jones RN [9]

1997-2000	MYSTIC	Europe	14.4%	32.8%	Jones RN [10]
1998-2002	SENTRY	Asia-Pacific	5.7%	16.9%	Hirakata Y [11]
		Australia	0.5%	3.7%	
		South Africa	1.5%	28.2%	
		China	24.5%	30.7%	
		Hong Kong	14.3%	10.7%	
		Japan	2.4%	10%	
		Taiwan	5.3%	12.6%	
		Singapore	11.3%	35.6%	
1997-2002	MYSTIC	UK	7.4%		Masterton RG [12]
2002	SMART	Europe	2%	8%	Chow JW [13]
		USA	3%	6%	
		Latin America	12%	23%	
		Asia Pacific	13%	17%	
2002-2004	SMART	Worldwide	8.9%	-	Bochicchio GV [14]
		USA	3%	-	
		Europe	5.3%	-	
		Middle East/Africa	11.1%	-	
		Latin America	11.2%	-	
		Asia/Pacific	16.6%	-	
2002-2007	SMART	Asia-Pacific	21%	21%	Ko WC [15]
2003	SMART	USA	3%	7%	Paterson DL [16]
		Europe	5%	11%	
		Latin America	10%	14%	
		Middle East	13%	20%	

		Asia Pacific	17%	18%	
2004	SMART	USA	2.8%	5.3%	Rossi F [17]
		Europe	6.4%	8.8%	
		Latin America	12%	27.6%	
		Middle East	10%	27.4%	
		Asia Pacific	19.6%	22.9%	
2004	SMART	Asia Pacific	19.6%	-	Hsueh PR [18]
		Australia	5.1%	-	
		New Zealand	0	-	
		China	37.8%	-	
		South Korea	21.2%	-	
		Taiwan	21.9%	-	
		Philippines	1.9%	-	
1997-2004	MYSTIC	Europe	10.8%	13.6%	Goossens H [19]
		USA	1.4%	4.4%	
2005	SMART	Worldwide	13%	15%	Baquero F [20]
		USA	2%	8%	
		Europe	7%	13%	
		Latin America	16%	27%	
		Middle East/Africa	11%	32%	
		Asia-Pacific	26%	17%	
2005-2007	SMART	Worldwide	18%	26.2%	Hawser SP [21]
		Asia-Pacific	34.9%	29.7%	
		Latin America	21.6%	41.5%	
		Middle East/Africa	12.1%	37.6%	

		Europe	8%	16.2%	
		North America	4.8%	9.5%	
2007	SMART	Asia Pacific	42.2%	35.8%	Hawser SP [22]
		Australia	7.7%	0%	
		New Zealand	3.2%	7.1%	
		China	55%	27.5%	
		Hong Kong	17.8%	0%	
		India	79%	69.4%	
		South Korea	22.7%	32.4%	
		Philippines	17%	40%	
		Singapore	33.3%	37.5%	
		Taiwan	12.7%	18.3%	
		Thailand	50.8%	45.5%	
		Vietnam	34.4%	39.1%	
2008	SMART	Latin America	26.8%	37.7%	Winokur PL [23]
2008	SMART	Asia Pacific	34%	22.3%	Hsueh PR [24]
		China	59.1%	34.4%	
		Hong Kong	22%	18.2%	
		India	61.2%	46.8%	
		Malaysia	2.9%	8.3%	
		New Zealand	2.2%	11.1%	
		Philippines	12.2%	35.7%	
		Singapore	12.3%	15.2%	
		South Korea	30.4%	25%	
		Taiwan	5.2%	4.5%	

		Thailand	52.9%	23.1%	
2008	SMART	Europe	11.6%	19.7%	Hawser S [25]
2008	SMART	Latin America	26.8%	37.7%	Villegas MV [26]
2008-2009	SMART	Europe	11%	-	Hawser SP [27]
		France	6.5%	-	
		Germany	11.3%	-	
		Greece	18.9%	-	
		Italy	14.2%	-	
		Portugal	12.8%	-	
		Spain	8%	-	
		Turkey	65%	-	
		UK	40%	-	
2008-2009	SMART	Worldwide	-	22.4%	Hawser SP [28]
		Asia	-	25.6%	
		Europe	-	19.7%	
		North America	-	10%	
		Latin America	-	34.6%	
		South Pacific	-	19.5%	
		Middle East	-	22.6%	
		Africa	-	32.2%	
2008-2009	SMART	Latin America	23.6%	-	Hawser SP [29]
		Argentina	6.3%	-	
		Brazil	13.5%	-	
		Chile	29.5%	-	
		Colombia	8.3%	-	

		Ecuador	35.6%	-	
		Guatemala	30.1	-	
		Mexico	41.4%	-	
		Panama	4.6%	-	
		Venezuela	29.5%	-	
2008-2011	SMART	Worldwide	16.6%	20.1%	Lob SH [30]
2009	SMART	Asia-Pacific	36.1%	25.3%	Chen YH [31]
		Australia	2%	13.3%	
		New Zealand	2.9%	7.8%	
		China	65.4%	31.9%	
		Hong Kong	29.5%	12%	
		India	67.1%	56.8%	
		Malaysia	18%	35.9%	
		Philippines	19.2%	35.5%	
		Singapore	13.5%	18%	
		South Korea	25.7%	8.7%	
		Taiwan	7.5%	7%	
		Thailand	56%	48%	
		Vietnam	42.9%	33.3%	
2008-2010	SENTRY	Argentina	18.1%	60.4%	Gales AC [32]
		Brazil	2.8%	49.9%	
		Chile	23.8%	59.2%	
		Mexico	48.4%	33.3%	
2009-2010	SMART	Worldwide	17.9%	-	Hoban DJ [33]
		Asia-Pacific	27.7%	-	

		Latin America	23.3%	-	
		Europe	18.8%	-	
		Middle East/Africa	16.2%	-	
		North America	7.4%	-	
2009-2010	SMART	Asia-Pacific	28.2%		Lu PL [34]
		Vietnam	60%	54%	
		China	67%	61%	
		Hong Kong	34%	11%	
		Thailand	37%	47%	
		Singapore	26%	33%	
		Malaysia	24%	36%	
		South Korea	20%	20%	
		Taiwan	15%	27%	
		Philippines	16%	33%	
		New Zealand	5%	0	
2009-2010	SMART	USA	8.5%	8.8%	Hoban DJ [35]
		Europe	17.6%	38.9%	
2009-11	SENTRY	USA	9.5-13.7%	10.7-17.2%	Sader HS [36]
		Europe	12.5-16.6%	23.5-29.2%	
2009-12	SENTRY	Europe	19.6%	35.1%	Sader HS [37]
		USA	18.4%	19.5%	
2011	SMART	USA	9.7%	12.7	Hawser SP [38]
2012	SMART	Asia Pacific	28.2%	22.1%	Huang CC [39]
		Australia	6.4%	6.8%	
		New Zealand	3.3%	8%	

		China	55.6%	33.7%	
		Hong Kong	21%	17.8%	
		Thailand	43.1%	40.7%	
		Vietnam	38.6%	40.7%	
		Philippines	10.5%	34.7%	
		Singapore	16.5%	18.6%	
		Malaysia	7.7%	21.4%	
		South Korea	25.9%	24.5%	
		Taiwan	11.5%	12.1%	
2013	RRS	Asia Pacific	48%	47%	Mendes RE [40]
		Australia	12%	15%	
		New Zealand	11%	10%	
		Hong Kong	46%	23%	
		India	78%	64%	
		Indonesia	71%	64%	
		South Korea	37%	40%	
		Malaysia	36%	45%	
		Philippines	47%	55%	
		Singapore	21%	32%	
		Taiwan	91%	75%	
		Thailand	550%	50%	

RRS: Regional Resistance Surveillance; **SMART:** Study for Monitoring Antimicrobial Resistance Trends; **MYSTIC:** Meropenem Yearly Susceptibility Test Information Collection; **SENTRY** Antimicrobial Surveillance Program

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ANNEXURE 2:
Prevalence rates of ESBL producing *E. coli* and *K. pneumoniae* across India

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
Vellore	Multiple specimens	-	93.3%	-	1995	1
Nagpur		-	25.8%	-	1997	2
Chennai	Multiple specimens	-	5.3%	-	2001	3
Chandigarh	Urine	24.7%	38.5%	-	2002	4
Delhi	Multiple specimens	-	80%	-	2002	5
Chennai	Multiple specimens	-	6.7%	-	2002	6
Delhi	Urine	60.7%	78.7%	-	2003	7
Lucknow	Blood	65.3%	87.2%	-	2003	8
Delhi	Pus	72.3%	76.3%	-	2004	9
Delhi	Blood	10.5%	18%	-	2004	10
Haryana	Multiple specimens	16.1%	12.5%	-	2004	11
Aligarh	Multiple specimens	-	30.2%	-	2004	12
Nagpur	Urine	18.5%	25.6%	-	2004	13
Mumbai	Multiple specimens	6.5%	8.5%	-	2004	14
Coimbatore	Urine	41%	40%	-	2004	15
Delhi	Multiple specimens	-	87%	-	2005	16

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
Chennai	Multiple specimens	27.2%	23.6%	-	2005	17
Delhi, Aligarh, Varanasi, Hubli	Multiple specimens	62%	73%	-	2005	18
Delhi	Multiple specimens	-	76.8%	TEM-104	2006	19
Aligarh, Varanasi, Hubli	Multiple specimens	73%	72%	CTX-M-15 (72-73%)	2006	20
Hyderabad	Multiple specimens	63.7%	14%	-	2006	21
Chennai	Multiple specimens	19.2%	21.2%	-	2006	22
Delhi	Blood	100%	52.2%	-	2007	23
Delhi	Multiple specimens	-	97.1%	TEM (20%) SHV (8.4%)	2007	24
Chandigarh	Multiple specimens	40%	69.2%	-	2007	25
Aligarh	Urine	34.4%	27.3%	-	2007	26
Delhi, Mumbai, Indore, Lucknow,	Multiple specimens	92%	96%	CTX-M-15: EC (83%), KP (75%)	2007	27

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
Bangalore, Vellore						
Kuppam	Multiple specimens	53.4%	52.4%	-	2007	28
Manipal	Urine	88.5%	86%	-	2007	29
Pondicherry	Blood	-	97%	-	2007	30
Tiruchirappalli	Urine	42.4%	15.2%	-	2007	31
Delhi	Multiple specimens	72%	38.4%	-	2008	32
Delhi	Urine	29.1%	25.6%	-	2008	33
Chandigarh	Urine	40.2%	51.2%	-	2008	34
Jaipur	Multiple specimens	64.8%	-	-	2008	35
Aligarh	Multiple specimens	14.4%	24.6%	-	2008	36
Lucknow	Blood	-	58%	-	2008	37
Lucknow	Blood	-	49.2%	TEM -75%, SHV- 46.8%	2008	38
Lucknow	Multiple specimens	62.7%	67.6%	-	2008	39
Varanasi	Blood	46.5%	62.7%	-	2008	40
Varanasi	Multiple specimens			CTX-M: EC (80.4%), KP (66.7%) SHV: EC (4.3%), KP (68.3%) TEM: EC (6.8%)	2008	41

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
				OXA-10: EC (0.4%)		
Pune	Multiple specimens	30%	16%	-	2008	42
Mumbai	Exudates, tissue	46.5%	44.4%	-	2008	43
Mumbai	Exudates, tissue	48.4%	51.6%	-	2008	44
Gulbarga, Raichur	Urine	-	96%	CTX-M-15 (94.4%), SHV-12 (1.9%), SHV-28, TEM-1	2008	45
Davangere	Multiple specimens	62.9%	62.2%	-	2008	46
Chennai	Multiple specimens	76.9%		CTX-M: EC (50%), KP (40%) SHV: EC (14%), KP (45%)	2008	47
Chennai	Multiple specimens	44.7%	68.5%	CTX-M (45%), CTX-M-28	2008	48
Chennai	Multiple specimens	15.8%		CTX-M-1 (47.3%)	2008	49
Coimbatore	Multiple specimens	-	-	CTX-M-1 group 82.6%; CTX-M-15 TEM-1 (68%)	2008	50
Coimbatore	Multiple specimens	62.7%	55.9%	-	2008	51
Vellore	Urine, perianal swab	1.6%	-	-	2008	52
Kashmir	Multiple specimens	-	12.5%	-	2009	53
Rohtak	Urine	40%	54.5%	-	2009	54

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
Lucknow	Multiple specimens	63.6%	66.7%	CTX-M: EC (91.8%), KP (66.7%) SHV: EC (29.5%), KP(42.9%), TEM: EC (50.8%), KP (66.7%)	2009	55
Aligarh	Food (veg, non-veg) Urine, pus	5.6% 91.3%		- CTX-M (52.2%), TEM (60.9%), SHV (21.7%)	2009	56
Mumbai	Multiple specimens	51.7%	64.9%	-	2009	57
Gulbarga, Raichur	Feces	-	20%	CTX-M-15 (100%) No TEM or SHV	2009	58
Manipal	Multiple specimens	25.4%	27.4%	-	2009	59
Chennai	Multiple specimens	-	80.9%	CTX-M (75%), TEM (73%), SHV (42%)	2009	60
Delhi	Blood	60%	59%	-	2010	61
Delhi	Blood	41.7%	70.7%	-	2010	62
Chandigarh	Multiple specimens	81.8%	74.5%	-	2010	63
Chandigarh	Multiple specimens	70%	60%	TEM: EC (52%), KP (60%) SHV: EC (48%), KP (72%)	2010	64
Ajmer	Blood Hospital NICU Labour room	-	100% 57% 80%	CTX-M (100%)	2010	65

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
Aligarh	Nose, pharynx, rectum swabs	36.5%	-	CTX-M-15 (5.2%) TEM-1 (2%)	2010	66
Varanasi	Multiple specimens	-	46%	CTX-M (67%), SHV (68%)	2010	67
Mumbai	Multiple specimens	68.3%	61.1%	-	2010	68
Mangalore	Pus, wound swab	60%	75%	-	2010	69
Chennai	Multiple specimens	68%	65.5%	-	2010	70
Salem	Blood	22.2%	7.4%	-	2010	71
Salem	Urine	54.7%	24.1%	-	2010	72
Vellore	Blood	73.5%	72.4%	-	2010	73
Kolkata	Feces	77%		CTX-M-1 group (98.1%), CTX-M-9 group (1.2%)	2011	74
Aligarh	Multiple specimens	61.7%		CTX-M (28.8%): EC (4.5%), KP (39.2%) SHV (13.7%): EC (6.7%), KP (25%) TEM (10.9%): EC (6.7%), KP (17.9%)	2011	75
Aligarh	Multiples specimens	86.5%		CTX-M (82.6%) SHV (59.6%) TEM (42.3%)	2011	76
Jamshedpur	Urine	91.3%	8.7%	-	2011	77

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
Shimoga		86.7%	9.7%	-		
Latur	Multiple specimens	61.8%	-	-	2011	78
Mumbai	?	20%	20%	-	2011	79
Bijapur	Multiple specimens	31.7%	46.4%	-	2011	80
Belgaum	Multiple specimens	-	74%	-	2011	81
Hubli	Multiple specimens	62.2%	71.9%	-	2011	82
Davangere	Blood	14%	43.9%		2011	83
Bangalore	Multiple specimens	81%	67.1%	-	2011	84
Bangalore	Multiple specimens	67.7%	63.3%	-	2011	85
Bangalore	Urine	42.2%	9.6%	-	2011	86
Anantpur	Multiple specimens	-	43.6%	-	2011	87
Pondichery	Blood	-	97.2%	CTX-M-15 (92%) TEM (82%) SHV (46%) OXA-1 (82%)	2011	88
Pondicherry	Multiple specimens	81%	74%	-	2011	89
Salem	Multiple specimens	79.5%	50%	CTX-M-1 group: EC (88.6%), KP (85.2%)	2011	90
Vellore	Multiple specimens	78%		CTX-M: EC (75%), KP (73.8%)	2011	91

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
				SHV: EC (14.3%), KP (62.3%) TEM: EC (51.8%), KP (62.3%)		
Delhi, Lucknow, Indore, Kolkata, Mumbai, Hyderabad, Bangalore, Chennai, Vellore	Intra-abdominal specimens	79%	70%	-	2011	92
Assam	Urine	13.9%	33.3%	-	2012	93
Kolkata	Multiple specimens	-	58.9%	CTX-M (37%) SHV (45%) TEM (52%) TEM-1, TEM-116, SHV-11, CTX-M-72	2012	94
Aligarh	Multiple specimens	7.3%	0%	CTX-M (100%) TEM (100%)	2012	95
Ujjain	Multiple specimens	69%	41%	-	2012	96

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
Jhalawar	Urine	73.5%	59.1%	-	2012	97
Pune	Urine	23%	-	CTX-M-15, TEM-1, OXA-1 group, OXA-2 group	2012	98
Nagpur	Multiple specimens	23.4%	19.8%	-	2012	99
Ahmedabad	Blood	-	81.5%	-	2012	100
Ahmedabad	Multiple specimens	39.3%		-	2012	101
Pondicherry	Multiple specimens	60.8%	39.2%	CTX-M-1 group: EC (63.7%), KP (36.2%)	2012	102
Vellore	Multiple specimens	89.7%	7.5%	-	2012	103
Amritsar	Multiple specimens	46.4%	52.3%	CTX-M: EC (89.8%), KP (75%) SHV: EC (22%), KP (62.5%) TEM: EC (11.9%), KP (25%)	2013	104
Amritsar	Multiple ICU specimens	56.3%	15.6%	-	2013	105
Chandigarh	Urine	52.6%	-	-	2013	106
Delhi	Blood	75%	60%	CTX-M-15: EC & KP (100%) SHV: EC (0%), KP (67%) TEM: EC (0%), KP (100%)	2013	107

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
				TEM-1, SHV-1, SHV-11, SHV-12 (16.7%), SHV-28		
Delhi	Neonate feces	20.6%		CTX-M-15 (82.5%); CTX-M-8, TEM-136, TEM-149, SHV-28	2013	108
Delhi	Intra-abdominal	61.7%	74.1%	-	2013	109
Nainital	Pus	24%	9%	-	2013	110
Varanasi	Tissue, swabs	75%	-	CTX-M (62.5%) SHV (50%) TEM (56.2%) CTX-M-15, CTX-M-1, CTX-M-3 CTX-M-9, TEM-1, TEM-10, TEM-20, TEM-52, SHV-1, SHV-2, SHV-5, SHV-12, OXA-1		111
Ujjain	Perianal swabs	15.3%	-	CTX-M-15 (74%) TEM-1 (74%) OXA-1 (49%)	2013	112
Ujjain	Feces	9%	-	-	2013	113
Aizawl	Feces	12.3%	-	CTX-M-1 (10.8%) SHV-12 (1.5%)	2013	114

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
Kolkata	Blood	89.5%	92.9%	CTX-M-15, TEM-1, OXA-1	2013	115
Kolkata	Urine	64.3%	-	-	2013	116
Kolkata	Multiple specimens	57.8%	50.6%	TEM: EC & KP (60.3%); TEM-1, TEM-33, TEM-116, TEM-169, TEM-190	2013	117
Jaipur	Multiple specimens	30.8%	15.6%	-	2013	118
Jaipur	Multiple specimens	56.9%	67%	CTX-M: EC (80%), KP (85) SHV: EC (55%), KP (60%) TEM: EC (60%), KP (75%)	2013	119
Pune	Multiple specimens	44%	32%	-	2013	120
Raichur	Urine	47.5%	-	-	2013	121
Bangalore	Pus	40%	63.1%	-	2013	122
Tumkur	Endotracheal aspirate	66.7%	32.2%	CTX-M: EC (63.2%), KP (42.9%) SHV: EC (10.5%), KP (35.7%) TEM: EC (15.8%), KP (7.1%)	2013	123
Baddi	Multiple specimens	89.9%	80%	CTX-M-1, CTX-M-9, TEM-1, TEM-3, TEM-50, SHV-1, SHV-4, SHV-10, SHV-28, OXA-2, OXA-10, OXA-23, OXA-48	2014	124

Center	Specimens	EC	KP	<i>bla</i> genes	Year	Reference
Guwahati	Multiple specimens	73.6%	67.2%	CTX-M: EC (88.7%), KP (62.1%) SHV: EC (13.2%), KP (50.6%) TEM: EC (49%), KP (77.6%)	2014	125
Imphal	Multiple specimens	34.2%	26.2%	-	2014	126
Noida	Multiple specimens	55.5%	-	-	2014	127
Bhopal	Multiple specimens	50.1%	48.3%	-	2014	128
Indore	Urine	41.6%	26%	-	2014	129
Ahmedabad	Multiple specimens	62.5%	65.7%	SHV: EC (4%), KP (96%) TEM: EC (48%), KP (80%)	2014	130
Vadodhara	Endotracheal aspirates	20%	67%	-	2014	131
Secunderabad	Multiple specimens	18.4%	33.7%	-	2014	132
Chitradurga	Multiple specimens	73%	51.4%	-	2014	133
Vellore	Feces	60.3%	-	-	2014	134

EC: *E. coli*; KP: *K. pneumoniae*

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among patients, healthy individuals and in the environment. Indian J Med Microbiol 2014;32:172-4.

ANNEXURE 3:

Nucleotide and amino acid sequences of *bla* genes detected in this study

***bla*TEM-1**

ATGAGTATTCAACATTTTCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTT
CCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGT
TGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGA
GAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTAT
GTGGTGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTGCGCGCAT
ACACTATTCTCAGAATGACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCTT
ACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGAT
AACACTGCTGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACC
GCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGG
AGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAA
TGGCAACAACGTTGCGCAAACCTATTAAGTGGCGAACTACTTACTCTAGCTTCCCG
GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCG
CTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGT
GGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCG
TAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGA
TCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAA

Peptide translation:

MSIQHFRVALIPFFAAFLP VF AHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFR
PEERFPM MSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQN DLVEYSPVTEKHLTDGM
TVRELCSAAITMSDNTAANLLLTTIGGPKELT AFLHNMGD HVTRLDRWEPELNEAIP
NDERDTT MP AAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLR SALPAGWF
IADKSGAGERGSRGIIAALGPDGKPSRIVVIYTTGSQATMDERNRQIAEIGASLIKHW

***bla*TEM-219**

ATGAGTATTCAACATTTTCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTT
CCTGTTTTTGTCTACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGT
TGGGTGCACGAGTGGGTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGA
GAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTAT
GTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTGCGCGCAT
ACACTATTCTCAGAATGACTTGGTTGAGTACTACCAGTCACAGAAAAGCATCTT
ACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGAT
AACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACC
GCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGG
AGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAA
TGGCAACAACGTTGCGCAAACATTAACCTGGCGAACTACTTACTCTAGCTTCCCG
GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCG
CTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGT
GGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCG
TATTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGA
TCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAA

Peptide translation:

MSIQHFRVALIPFFAAFLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFR
PEERFPMMSSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGM
TVRELCSAAITMSDNTAANLLLTIGGPKELTAFLHNMGDHSVTRLDRWEPELNEAIP
NDERDTPMPAAMATTLRKLLTGELLTLASRQQLIDWMEADKVAGPLLRSAIPAGWF
IADKSGAGERGSRGIIAALGPDGKPSRIVFIYTTGSQATMDERNRQIAEIGASLIKHW

blaSHV-1

ATGCGTTATATTGCGCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATA
ACAGCGCCGCCAATCTGCTACTGGCCACCGTCGGCGGCCCGCCGAGGATTGACTGC
CTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAA
CTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCACTACCCCGGCCAGCATG
GCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCGAAC
GGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTC
CGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAGCGGGG
TGCGCGCGGGATTGTGCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACCCCGGCGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPQPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRYQQDLVDYSPVSEKHLADGM
TVGELCAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-2a

ATGCGTTATATTGCGCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAAACAAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACATCTTG
CCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATA
ACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCCGAGGATTGACTGC
CTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAA
CTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCACTACCCCGGCCAGCATG
GCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCGAAC
GGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTC
CGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTAGCGAACGGGG
TGCGCGCGGGATTGTGCGCCCTGCTTGCCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACCCCGCGCAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKQSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRRQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGASERGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-11

ATGCGTTATATTGCGCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAAACAAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGTGCCGCCGCCATTACCATGAGCGATAA
CAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCCGCAGGATTGACTGCC
TTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAAC
TGAATGAGGCGCTTCCCGGCGACGCCCGGACACCACTACCCCGGCCAGCATGG
CCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCAACG
GCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTCC
GTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAACGGGGT
GCGCGCGGGATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATCGTG
GTGATTTATCTGCGGGATACCCCGCGGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKQSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRRQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-12

ATGCGTTATATTGCCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAAACAAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATA
ACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCCGAGATTGACTGC
CTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAA
CTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCACTACCCCGGCCAGCATG
GCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTTCGCAAC
GGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTC
CGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTAGCAAGCGGGG
TGCGCGCGGGATTGTGCGCCCTGCTTGCCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACCCCGCGCAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKQSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRRQDLVDYSPVSEKHLADGM
TVGELCAAATMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGASKRGARGIVALLGPNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-14

ATGCGTTATTTTCGCCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCAGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGTGCCGCCGCCATTACCATGAGCGATAA
CAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCCGCAGGATTGACTGCC
TTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAAC
TGAATGAGGCGCTTCCCGGCGACGCCCGGACACCACTACCCCGGCCAGCATGG
CCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCAACG
GCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTCC
GTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAGCGGGGT
GCGCGCGGGATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACCCCGCGGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYFRLCIISLLATLPLAVHASPQPLEQIKLSESQLSGSVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRYQQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-18

ATGCGTTATTTTCGCCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCAGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGTGCCGCCGCCATTACCATGAGCGATAA
CAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCCGCAGGATTGACTGCC
TTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAAC
TGAATGAGGCGCTTCCCGGCGACGCCCGGACACCACTACCCCGGCCAGCATGG
CCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCAACG
GCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTCC
GTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGCCAAACGGGGT
GCGCGCGGGATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGGATTGTG
GTGATTTATCTGCGGGATACGCCGGCGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYFRLCIISLLATLPLAVHASPQPLEQIKLSESQLSGSVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRYQQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGAAKRGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEH
WQR

blaSHV-26

ATGCGTTATATTGCCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATA
ACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCCGAGATTGACTGC
CTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAA
CTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCACTACCCCGGCCAGCATG
ACCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTTCGCAAC
GGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTC
CGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAGCGGGG
TGCCCGCGGGATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACCCCGCGCAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRRQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMTATLRKLLTSQRLSARSQRQLLQWMVDDRVRVAGPLIRSVLPAGWFI
ADKTGAGERGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-27

ATGCGTTATATTGCGCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACATCTTG
CCGACGGCATGACGGTCGGCGAACTCTGTGCCGCCGCCATTACCATGAGCGATAA
CAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCCGCAGGATTGACTGCC
TTTTTGCGCCAGATCGACGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAAC
TGAATGAGGCGCTTCCCGGCGACGCCCGGACACCACTACCCCGGCCAGCATGG
CCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCAACG
GCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTCC
GTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAGCGGGGT
GCGCGCGGGATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACGCCGGCGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPQPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRYQQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIDDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-28

ATGCGTTTTATTTCGCCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACATCTTG
CCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATA
ACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCCGAGGATTGACTGC
CTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAA
CTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCACTACCCCGGCCAGCATG
GCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTTCGCAAC
GGCAGCTGCTGCAGTGGATGGTGGACGATCGAGTCGCCGGACCGTTGATCCGCTC
CGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAACGGGG
TGCGCGCGGGATTGTGCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATCGTG
GTGATTTATCTGCGGGATACCCCGCGCAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRFIRLCIISLLATLPLAVHASPPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRAD
ERFPMMSTFKVVLGAVLARVDAGDEQLERKIHRYQQDLVDYSPVSEKHLADGMT
VGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALPG
DARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVRVAGPLIRSVLPAGWFI
ADKTGAGERGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-31

ATGCGTTATATTGCGCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAAACAAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGTGCCGCCGCCATTACCATGAGCGATAA
CAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCCGCAGGATTGACTGCC
TTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAAC
TGAATGAGGCGCTTCCCGGCGACGCCCGGACACCACTACCCCGGCCAGCATGG
CCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCAACG
GCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTCC
GTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCAAGCGGGGT
GCGCGCGGGATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACGCCGGCGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKQSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRYQQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGAGKRGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEH
WQR

blaSHV-61

ATGCGTTATATTGCGCGGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAAACAAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATA
ACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCCAGGATTGACTGC
CTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAA
CTGAATGAGGCGCTTCCCGGCGACGCGCGACACCACTACCCCGGCCAGCATG
GCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCGAAC
GGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTC
CGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAGCGGGG
TGCGCGCGGCATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACGCCGGCGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRRCIISLLATLPLAVHASPPLEQIKQSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRRQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-71

ATGCGTTATATTGCGCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAATACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGTGCCGCCGCCATTACCATGAGCGATAA
CAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCTAGGATTGACTGCC
TTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAAC
TGAATGAGGCGCTTCCCGGCGACGCCCGGACACCACTACCCCGGCCAGCATGG
CCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCAACG
GCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTCC
GTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAGCGGGGT
GCGCGCGGGATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACCCCGCGGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRYQQDLVDYSPVSEKYLADGM
TVGELCAAAITMSDNSAANLLLATVGGPVGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-75

ATGCGTTATATTGCGCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACATCTTG
CCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATA
ACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCCGAGGATTGACTGC
CTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAA
CTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCACTACCCCGGCCAGCATG
GCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCGAAC
GGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTC
CGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAACGGGG
TGCGCGCGGGATTGTGCGCCCTGCTTGCCCCGCATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACCCCGCGCAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRRQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPHNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-108

ATGCGTTATATTGCGCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAAACAAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGTGCCGCCGCCATTACCATGAGCGATAA
CAGCGCCGCCAATCTGCTGCTGGCCATCGTCGGCGGCCCGCAGGATTGACTGCC
TTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAAC
TGAATGAGGCGCTTCCCGGCGACGCCCGGACACCACTACCCCGGCCAGCATGG
CCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCAACG
GCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTCC
GTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAACGGGGT
GCGCGCGGGATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACCCCGCGGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKQSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLCGAVLARVDAGDEQLERKIHRRQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLAIVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-168

ATGCGTTATTTTCGCCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGTGCCGCCGCCATTACCATGAGCGATAA
CAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCTAGGATTGACTGCC
TTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAAC
TGAATGAGGCGCTTCCCGGCGACGCCGCGACACCACTACCCCGGCCAGCATGG
CCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTGCAACG
GCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTCC
GTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAGCGGGGT
GCGCGCGGGATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTG
GTGATTTATCTGCGGGATACCCCGCGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYFRLCIISLLATLPLAVHASPPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPM MSTFKVVLGAVLARVDAGDEQLERKIH YRQQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPVGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

blaSHV-185

ATGCGTTATATTGCCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
ACACGCCAGCCCGCAGCCGCTTGAGCAAATTAACTAAGCGAAAGCCAGCTGTC
GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATA
ACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCCGAGATTGACTGC
CTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAA
CTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCACTACCCCGGCCAGCATG
GCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTTCGCAAC
GGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTC
CGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAACGGGG
TGCGCGCGGGATTGTGCGCCCTGCTTGCCCCGAATAACAAAGCAGAGCGCATTGTG
ATGATTTATCTGCGGGATACGCCGGCGAGCATGGCCGAGCGAAATCAGCAAATC
GCCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLGAVLARVDAGDEQLERKIHRYQQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMAATLRKLLTSQRLSARSQRQLLQWMVDDRVAGPLIRSVLPAGWF
IADKTGAGERGARGIVALLGPNNKAERIVMIYLRDTPASMAERNQQIAGIGAALIEH
WQR

blaSHV-186

ATGCGTTATATTGCCTGTGTATTATCTCCCTGTTAGCCACCCTGCCGCTGGCGGT
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GGGCCGCGTAGGCATGATAGAAATGGATCTGGCCAGCGGCCGCACGCTGACCGC
CTGGCGCGCCGATGAACGCTTTCCCATGATGAGCACCTTTAAAGTAGTGCTCTGC
GGCGCAGTGCTGGCGCGGGTGGATGCCGGTGACGAACAGCTGGAGCGAAAGATC
CACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCAGCGAAAAACACCTTG
CCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCATTACCATGAGCGATA
ACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCCCGCCGAGGATTGACTGC
CTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGACCGCTGGGAAACGGAA
CTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCACTACCCCGGCCAGCATG
ACCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCTGAGCGCCCGTTTCGCAAC
GGCAGCTGCTGCAGTGGATGGTGGACGATCGGGTCGCCGGACCGTTGATCCGCTC
CGTGCTGCCGGCGGGCTGGTTTATCGCCGATAAGACCGGAGCTGGCGAGCGGTGT
GCGCGCGGCATTGTCGCCCTGCTTGGCCCGAATAACAAAGCAGAGCGCATTGTGG
TGATTTATCTGCGGGATACGCCGGCGAGCATGGCCGAGCGAAATCAGCAAATCG
CCGGGATCGGCGCGGCGCTGATCGAGCACTGGCAACGCTAA

Peptide translation:

MRYIRLCIISLLATLPLAVHASPPLEQIKLSESQLSGRVGMIEMDLASGRTLTAWRA
DERFPMMSTFKVVLGAVLARVDAGDEQLERKIHRYQQDLVDYSPVSEKHLADGM
TVGELCAAAITMSDNSAANLLLATVGGPAGLTAFLRQIGDNVTRLDRWETELNEALP
GDARDTTTPASMTATLRKLLTSQRLSARSQRQLLQWMVDDRVAGPLIRSVLPAGWFI
ADKTGAGERCARGIVALLGPNNKAERIVVIYLRDTPASMAERNQQIAGIGAALIEHW
QR

***bla*CTX-M-15**

ATGGTTAAAAAATCACTGCGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGC
TGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAAC
TTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACA
CAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAG
CACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACC
GAATCTGTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAAT
CCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCG
GCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTG
GCGGCCCCGGCTAGCGTCACCGCGTTCGCCCCACAGCTGGGAGACGAAACGTTCC
GTCTCGACCGTACCGAGCCGACGTTAAACACCGCCATTCCGGGCGATCCGCGTGA
TACCACTTCACCTCGGGCAATGGCGCAAACCTCTGCGGAATCTGACGCTGGGTAAA
GCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACC
ACCGGTGCAGCGAGCATTACGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATA
AAACCGGCAGCGGTGGCTATGGCACCAACGATATCGCGGTGATCTGGCCAA
AAGATCGTGCGCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCAACCTAAGGC
AGAAAGCCGTCGCGATGTATTAGCGTCGGCGGCTAAAATCGTCACCGACGGTTTG
TAA

Peptide translation:

MVKKSLRQFTLMATATVTLLLGSVPLYAQTADVQQKLAELERQSGGRLGVALINTA
DNSQILYRADERFAMCSTSKVMAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAE
KHVNGTMSLAELSAAALQYSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTE
PTLNTAIPGDPRDTTSPRAMAQTLRNLTGKALGDSQRAQLVTWMKGNTTGAASIQ
AGLPASWVVGDKTGSGGYGTTNDIAVIWPKDRAPLILVTYFTQPQPKAESRRDVLAS
AAKIVTDGL

***bla*CTX-M-155**

ATGGTTAAAAAATCACTGCGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGC
TGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAAC
TTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACA
CAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAG
CACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACC
GAATCTGTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAAT
CCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCG
GCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTG
GCGGCCCGGCTAGCGTCACCGCGTTCGCCCCACAGCTGGGAGACGAAACGTTCC
GTCTCGACCGCACCGCGCCGACGTAAACACCGCCATTCCGGGCGATCCGCGTGA
TACCACTTCACCTCGGGCAATGGCGCAAACCTCTGCGGAATCTGACGCTGGGTAAA
GCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACC
ACCGGTGCAGCGAGCATTCAAGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATA
AAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGCGGTGATCTGGCCAA
AAGATCGTGCGCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCAACCTAAGGC
AGAAAGCCGTCGCGATGTATTAGCGTCGGGCGCTAAAATCGTCACCGACGGTTTG
TAA

Peptide translation:

MVKKSLRQFTLMATATVTLLGSPVLYAQTADVQQKLAELERQSGGRLGVALINTA
DNSQILYRADERFAMCSTSKVMAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAE
KHVNGTMSLAELSAAALQYSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTA
PTLNTAIPGDPRDTTSPRAMAQTLRNLTGKALGDSQRAQLVTWMKGNTTGAASIQ
AGLPASWVVGDKTSGSGYGTTNDIAVIWPKDRAPLILVITYFTQPQPKAESRRDVLAS
GAKIVTDGL

***bla*CTX-M-156**

ATGGTTAAAAAATCACTGCGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGC
TGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAAC
TTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACA
CAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAG
CACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACC
GAATCTGTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAAT
CCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCG
GCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTG
GCGGCCCGGCTAGCGTCACCGCGTTCGCCCCGACAGCTGGGAGACGAAACGTTCC
GTCTCGACCGTACCGAGCCGACGTAAACACCGCCATTCCGGGCGATCCGCGTGA
TACCACTTCACCTCGGGCAATGGCGCAAACCTCTGCGGAATCTGACGCTGGGTAAA
GCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACC
ACCGGTGCAGCGAGCATTACGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATA
AAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGCGGTGATCTGGCCAA
AAGATCGTGCGCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCAACCTAACGC
AGAAAGCCGTCGCGATGTATTAGCGTCGGCGGCTAAAATCGTCACCGACGGTTTG
TAA

Peptide translation:

MVKKSLRQFTLMATATVTLLGSVPLYAQTADVQQKLAELERQSGGRLGVALINTA
DNSQILYRADERFAMCSTSKVMAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAE
KHVNGTMSLAELSAAALQYSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTE
PTLNTAIPGDPRDTTSPRAMAQTLRNLTGKALGDSQRAQLVTWMKGNTTGAASIQ
AGLPASWVVGDKTGSGGYGTTNDIAVIWPKDRAPLILVTYFTQPQPNAESRRDVLAS
AAKIVTDGL

blaCTX-M-157

ATGGTTAAAAAATCACTGCGCCAGTTCACGCTGATGGCGACGGCAACCGTCACGC
TGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACAGCAAAAAC
TTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATTGATTAACA
CAGCAGATAATTCGCAAATACTTTATCGTGCTGATGAGCGCTTTGCGATGTGCAG
CACCAGTAAAGTGATGGCCGCGGCCGCGGTGCTGAAGAAAAGTGAAAGCGAACC
GAATCTGTAAATCAGCGAGTTGAGATCAAAAAATCTGACCTTGTTAACTATAAT
CCGATTGCGGAAAAGCACGTCAATGGGACGATGTCACTGGCTGAGCTTAGCGCG
GCCGCGCTACAGTACAGCGATAACGTGGCGATGAATAAGCTGATTGCTCACGTTG
GCGGCCCGGCTAGCGTCACCGCGTTCGCCCCGACAGCTGGGAGACGAAACGTTCC
GTCTCGACCGTACCGCGCCGACGTAAACACCGCCATTCCGGGCGATCCGCGTGA
TACCACTTCACCTCGGGCAATGGCGCAAACCTCTGCGGAATCTGACGCTGGGTAAA
GCATTGGGCGACAGCCAACGGGCGCAGCTGGTGACATGGATGAAAGGCAATACC
ACCGGTGCAGCGAGCATTACGGCTGGACTGCCTGCTTCCTGGGTTGTGGGGGATA
AAACCGGCAGCGGTGGCTATGGCACCACCAACGATATCGCGGTGATCTGGCCAA
AAGATCGTGCGCCGCTGATTCTGGTCACTTACTTCACCCAGCCTCAACCTAAGGC
AGAAAGCCGTCGCGATGTATTAGCGTCGGCGGCTAAAATCGTCACCGACGGTTTG
TAA

Peptide translation:

MVKKSLRQFTLMATATVTLLGSVPLYAQTADVQQKLAELERQSGGRLGVALINTA
DNSQILYRADERFAMCSTSKVMAAAVLKKSESEPNLLNQRVEIKKSDLVNYNPIAE
KHVNGTMSLAELSAAALQYSDNVAMNKLIAHVGGPASVTAFARQLGDETFRLDRTA
PTLNTAIPGDPRDTTSPRAMAQTLRNLTGKALGDSQRAQLVTWMKGNTTGAASIQ
AGLPASWVVGDKTSGGGYGTTNDIAVIWPKDRAPLILVTYFTQPQKAESRRDVLAS
AAKIVTDGL

ANNEXURE 4

Master chart of all samples tested in this study

SL	LO C	DATE	SPEC	AG E	SE X	IS OL	CA Z	CT X	CT R	A O	SC R	CL SI	CLS I	PB A	RES	C N	CP M	CF C	CP T	CE C	CA C	CM C	IM P	CO N	CA Z	CT X	CT R	ESBL
1	BEL	24-03-11	Urine	22	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	
2	BEL	24-03-11	Urine	32	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S		16	>1 28	64	
3	BEL	24-03-11	Urine	78	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	S	P	16	64	64	
4	BEL	24-03-11	Urine	35	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	S	S	P	16	64	64	
5	BEL	24-03-11	Urine	48	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	
6	BEL	24-03-11	Urine	65	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	S	S	P	32	>1 28	>1 28	
7	BEL	24-03-11	Urine	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	S	S	P	16	12 8	64	
8	BEL	24-03-11	Urine	4	M	EC	S	S	S	S	N				SUS C													
9	BEL	24-03-11	Urine	20	M	EC	S	S	S	S	N				SUS C													
10	BEL	24-03-11	Urine	42	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	S	S	P	16	64	64	
11	BEL	24-03-11	Urine	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	
12	BEL	24-03-11	Urine	55	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	S	S	S	S	P	16	64	64	
13	BEL	24-03-11	Urine	51	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	
14	BEL	24-03-11	Urine	18	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	
15	BEL	24-03-11	Urine	21	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	32	64	64	
16	BEL	24-03-11	Urine	55	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	

17	BEL	26-03-11	Urine	19	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	S	S	P	16	64	64	
18	BEL	26-03-11	Urine	75	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	S	S		16	64	>1 28	
19	BEL	26-03-11	Urine	25	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S		16	>1 28	64	
20	BEL	26-03-11	Urine	40	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	32	>1 28	>1 28	
21	BEL	26-03-11	Urine	44	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	32	>1 28	>1 28	
22	BEL	26-03-11	Urine	8	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	32	>1 28	>1 28	
23	BEL	26-03-11	Urine	10	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S		16	64	64	
24	BEL	26-03-11	Urine	32	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	S	S	P	16	64	64	
25	BEL	26-03-11	Urine	60	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	16	64	64	
26	BEL	26-03-11	Urine	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	
27	BEL	26-03-11	Urine	51	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	12 8	>1 28	
28	BEL	26-03-11	Urine	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	16	>1 28	>1 28	
29	BEL	26-03-11	Urine	31	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	32	64	64	
30	BEL	26-03-11	Urine	60	M	EC	S	S	S	S	N				SUS C													
31	BEL	26-03-11	Urine	26	F	EC	S	S	S	S	N				SUS C													
32	BEL	26-03-11	Urine	18	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	16	64	64	
33	BEL	26-03-11	Urine	20	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	
34	BEL	26-03-11	Urine	32	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	
35	BEL	26-03-11	Urine	65	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	S	S	P	16	64	64	
36	BEL	26-03-11	Urine	20	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		32	64	>1 28	

37	BEL	26-03-11	Sputum	40	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	R	S	S	S		16	16	16	
38	BEL	14-04-11	Sputum	25	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		16	64	64	
39	BEL	14-04-11	Sputum	50	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S		16	64	64	
40	BEL	14-04-11	Sputum	35	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	I	S	P	12 8	64	64	
41	BEL	14-04-11	Sputum	42	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		16	64	64	
42	BEL	14-04-11	Sputum	40	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	R	I	I	S		16	64	>1 28	
43	BEL	14-04-11	Asci Fluid	3	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	I	S	P	16	64	64	
44	BEL	14-04-11	Pus	50	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	16	>1 28	>1 28	
45	BEL	14-04-11	Pus	30	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	I	S		16	64	64	
46	BEL	14-04-11	Pus	22	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S		16	64	>1 28	
47	BEL	14-04-11	Pus	26	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S	P	16	64	>1 28	
48	BEL	14-04-11	Pus	13	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		16	12 8	>1 28	
49	BEL	14-04-11	Pus	26	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	I	S	I	S		16	64	12 8	
50	BEL	14-04-11	Pus	33	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	I	S	P	16	12 8	>1 28	
51	BEL	14-04-11	Pus	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		64	64	64	
52	BEL	14-04-11	Pus	34	M	EC	S	S	S	S	N				SUS C													
53	BEL	14-04-11	Pus	53	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S	P	32	12 8	64	
54	BEL	14-04-11	Pus	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	S	S		32	64	>1 28	
55	BEL	14-04-11	Pus	68	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		16	64	64	
56	BEL	14-04-11	Pus	35	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	I	S	P	16	64	64	

57	BEL	14-04-11	Pus	23	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	I	S		16	64	64	
58	BEL	14-04-11	Pus	32	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	I	S		16	64	>1 28	
59	BEL	14-04-11	Pus	55	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	I	S		16	64	>1 28	
60	BEL	14-04-11	Pus	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	I	S		16	64	>1 28	
61	BEL	14-04-11	Pus	9	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	I	S	P	16	64	>1 28	
62	BEL	14-04-11	Pus	12	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	S	S	P	16	64	>1 28	
63	BEL	14-04-11	Pus	28	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S		16	64	64	
64	BEL	14-04-11	Pus	28	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	R	I	I	S		16	64	64	
65	BEL	14-04-11	Pus	33	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	I	S		16	64	>1 28	
66	BEL	14-04-11	Pus	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	R	I	I	S		16	64	64	
67	BEL	14-04-11	Pus	36	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S		16	64	64	
68	BEL	14-04-11	Blood	10	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	I	S		16	64	64	
69	BEL	14-04-11	Blood	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	I	S	S	S		16	64	64	
70	BEL	14-04-11	Pus	25	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S		32	64	64	
71	BEL	14-04-11	Pus	46	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	16	64	64	
72	BEL	14-04-11	Pus	40	F	EC	S	S	S	S	N				SUS C													
73	BEL	14-04-11	Pus	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	S	S	P	16	64	64	
74	BEL	14-04-11	Pus	32	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S	P	16	64	64	
75	BEL	14-04-11	Pus	20	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	16	64	64	
76	BEL	14-04-11	Blood	9	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S	P	16	>1 28	>1 28	

77	BEL	14-04-11	Urine	21	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	I	S	S	S	P	16	64	64	
78	BEL	14-04-11	Urine	17	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	32	64	64	
79	BEL	14-04-11	Urine	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S	P	32	64	>1 28	
80	BEL	14-04-11	Urine	40	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	I	S	S	S		32	16	>1 28	
81	BEL	14-04-11	Urine	13	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	16	64	64	
82	BEL	14-04-11	Urine	30	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S		32	64	>1 28	
83	BEL	14-04-11	Sputum	28	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S		16	>1 28	64	
84	BEL	19-04-11	Sputum	28	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	32	>1 28	>1 28	
85	BEL	19-04-11	Sputum	25	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	32	>1 28	>1 28	
86	BEL	19-04-11	Urine	41	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S	P	>1 28	>1 28	>1 28	
87	BEL	24-04-11	Urine	20	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	32	>1 28	>1 28	
88	BEL	24-04-11	Urine	20	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	S	P	64	>1 28	>1 28	
89	BEL	24-04-11	Urine	22	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	32	>1 28	>1 28	
90	BEL	24-04-11	Urine	38	M	EC	S	S	S	S	N				SUS C													
91	BEL	30-04-11	Urine	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S	P	32	12 8	12 8	
92	BEL	30-04-11	Urine	40	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S		32	12 8	12 8	
93	BEL	30-04-11	Urine	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	R	S	P	>1 28	>1 28	>1 28	
94	BEL	30-04-11	Urine	50	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	64	12 8	12 8	
95	BEL	30-04-11	Urine	38	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	12 8	>1 28	>1 28	
96	BEL	28-05-11	Urine	25	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	R	I	R	S	P	>1 28	>1 28	>1 28	TEM-1, CTX-M-15

[illegible]

11 7	BEL	28-05- 11	Urine	21	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	R	S		64	>1 28	>1 28	
11 8	BEL	28-05- 11	Urine	54	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S		32	>1 28	>1 28	
11 9	BEL	28-05- 11	Urine	50	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	R	I	S	S		>1 28	32	32	
12 0	BEL	28-05- 11	Urine	70	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	S	S	S	S	P	32	64	>1 28	
12 1	BEL	28-05- 11	Urine	24	F	EC	S	S	S	S	N				SUS C													
12 2	BEL	28-05- 11	Urine	25	F	EC	S	S	S	S	N				SUS C													
12 3	BEL	28-05- 11	Urine	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	32	64	>1 28	CTX-M-15
12 4	BEL	28-05- 11	Urine	40	F	EC	S	S	S	S	N				SUS C													
12 5	BEL	28-05- 11	Urine	60	F	EC	S	S	S	S	N				SUS C													
12 6	BEL	28-05- 11	Urine	55	M	EC	R	R	R	R	P	N		EA	COP R	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
12 7	BEL	28-05- 11	Urine	75	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	R	R	R	R	S	P	>1 28	>1 28	>1 28	
12 8	BEL	28-05- 11	Urine	72	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	I	S	R	S		>1 28	>1 28	>1 28	CTX-M-15
12 9	BEL	31-05- 11	Urine	41	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	I	S	P	>1 28	>1 28	>1 28	
13 0	BEL	31-05- 11	Urine	24	M	KP	S	S	S	S	N				SUS C													
13 1	BEL	31-05- 11	Urine	40	M	EC	S	S	S	S	N				SUS C													
13 2	BEL	31-05- 11	Urine	36	M	EC	R	R	R	R	P	N		A	Am pC	R	R	R	R	R	R	R	S		>1 28	>1 28	>1 28	
13 3	BEL	31-05- 11	Urine	75	M	EC	R	R	R	R	P	N		A	Am pC	R	R	R	R	R	R	R	S	P	>1 28	>1 28	>1 28	
13 4	BEL	31-05- 11	Urine	48	F	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	S	P	>1 28	>1 28	>1 28	
13 5	BEL	31-05- 11	Urine	47	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S		64	>1 28	>1 28	
13 6	BEL	31-05- 11	Urine	3	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	32	>1 28	>1 28	TEM-1, CTX-M-15

137	BEL	31-05-11	Urine	22	M	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	S	P	>128	>128	>128	
138	BEL	31-05-11	Urine	24	F	EC	S	S	S	S	N				SUS C													
139	BEL	31-05-11	Urine	25	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S	P	32	>128	>128	TEM-1, CTX-M-15
140	BEL	31-05-11	Urine	26	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S		>128	>128	>128	
141	BEL	31-05-11	Urine	74	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S	P	>128	>128	>128	
142	BEL	31-05-11	Urine	70	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	R	S	P	>128	>128	>128	CTX-M-15
143	BEL	31-05-11	Urine	50	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	I	S	P	>128	>128	>128	
144	BEL	31-05-11	Urine	75	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S	P	>128	>128	>128	
145	BEL	31-05-11	Urine	50	M	KP	S	S	S	S	N				SUS C													
146	BEL	31-05-11	Urine	40	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S		>128	>128	>128	TEM-1, SHV-28, CTX-M-15
147	BEL	20-06-11	Urine	80	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	I	I	S	R	S	P	>128	>128	>128	
148	BEL	20-06-11	Urine	30	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	R	S	P	64	>128	>128	
149	BEL	20-06-11	Urine	45	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	I	I	S	R	S	P	64	>128	>128	
150	BEL	20-06-11	Urine	75	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	S	S	S	R	R	R	S	P	>128	64	64	
151	BEL	20-06-11	Urine	43	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	S	S	I	S	S	R	S	P	>128	>128	>128	
152	BEL	20-06-11	Urine	66	M	KP	S	S	S	S	N				SUS C													
153	BEL	20-06-11	Urine	5	F	KP	S	S	S	S	N				SUS C													
154	BEL	20-06-11	Urine	18	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	I	R	I	R	S	P	>128	>128	>128	
155	BEL	20-06-11	Urine	55	M	KP	S	S	S	S	N				SUS C													
156	BEL	20-06-11	Urine	45	F	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	I	R	R	R	S	P	>128	>128	>128	

157	BEL	20-06-11	Urine	32	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	I	I	I	R	S	P	>1 28	>1 28	>1 28	
158	BEL	20-06-11	Urine	55	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	I	R	I	R	S		>1 28	>1 28	>1 28	
159	BEL	20-06-11	Urine	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	I	S	R	S	P	>1 28	>1 28	>1 28	
160	BEL	20-06-11	Urine	36	F	EC	R	R	R	R	P	P	Ce		ESB L	R	R	I	R	R	R	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15
161	BEL	20-06-11	Urine	58	M	KP	S	S	S	S	N				SUS C													
162	BEL	24-11-11	Urine	41	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	I	I	R	S		>1 28	>1 28	>1 28	
163	BEL	24-11-11	Urine	27	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	I	I	R	S		64	>1 28	>1 28	TEM-1, CTX-M-15
164	BEL	24-11-11	Urine	28	F	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	S	R	R	R	S		>1 28	>1 28	>1 28	TEM-1, CTX-M-15
165	BEL	24-11-11	Urine	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	R	S		>1 28	12 8	12 8	TEM-1, SHV-1, CTX-M-15
166	BEL	24-11-11	Urine	2	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
167	BEL	26-11-11	Urine	45	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	I	S		64	>1 28	>1 28	TEM-1, SHV-11, CTX-M-15
168	BEL	26-11-11	Urine	30	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	S	R	R	R	S		>1 28	>1 28	>1 28	
169	BEL	26-11-11	Urine	1	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	S	S	R	S	P	>1 28	>1 28	>1 28	CTX-M-15
170	BEL	26-11-11	Urine	56	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	S	R	R	R	S		>1 28	>1 28	>1 28	CTX-M-15
171	BEL	26-11-11	Urine	1	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	I	I	I	S		12 8	>1 28	>1 28	
172	BEL	26-11-11	Urine	20	F	EC	R	R	R	R	P	P	Ce		ESB L	R	I	I	S	R	R	R	S		>1 28	>1 28	12 8	TEM-1, CTX-M-15
173	BEL	26-11-11	Urine	59	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	R	S	P	64	>1 28	>1 28	
174	BEL	26-11-11	Urine	35	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	I	S		12 8	>1 28	>1 28	
175	BEL	26-11-11	Urine	45	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	I	R	S	P	12 8	>1 28	>1 28	TEM-1, CTX-M-15
176	BEL	26-11-11	Urine	35	F	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-12, CTX-M-15

17 7	BEL	26-11- 11	Urine	20	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-12, CTX- M-15
17 8	BEL	26-11- 11	Urine	49	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	I	S	S	S		>1 28	12 8	12 8	CTX-M-15
17 9	BEL	26-11- 11	Urine	26	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	S	R	R	R	S		>1 28	>1 28	>1 28	
18 0	BEL	19-12- 11	Urine	72	M	EC	R	R	R	R	P	N		A	Am pC	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
18 1	BEL	19-12- 11	Urine	58	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	S	S	S	S	P	64	12 8	>1 28	TEM-1, CTX-M-15
18 2	BEL	19-12- 11	Sputum	33	M	KP	S	S	S	S	N				SUS C													
18 3	BEL	19-12- 11	Sputum	45	M	KP	S	S	S	S	N				SUS C													
18 4	BEL	19-12- 11	Sputum	25	M	KP	S	S	S	S	N				SUS C													
18 5	BEL	19-12- 11	Sputum	25	M	KP	S	S	S	S	N				SUS C													
18 6	BEL	19-12- 11	Urine	65	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	S	S	R	S		12 8	>1 28	>1 28	CTX-M-15
18 7	BEL	19-12- 11	Sputum	25	M	KP	S	S	S	S	N				SUS C													
18 8	BEL	19-12- 11	Sputum	30	M	KP	S	S	S	S	N				SUS C													
18 9	BEL	19-12- 11	Sputum	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	I	S		64	>1 28	>1 28	TEM-1, SHV-11, CTX- M-15
19 0	BEL	19-12- 11	Urine	42	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	S	R	R	R	S		>1 28	>1 28	>1 28	
19 1	BEL	19-12- 11	Sputum	65	M	KP	S	S	S	S	N				SUS C													
19 2	BEL	19-12- 11	Urine	16	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	I	S	R	R	R	S		>1 28	>1 28	>1 28	TEM-1, CTX-M-15
19 3	BEL	19-12- 11	Urine	55	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	R	R	R	S		>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
19 4	BEL	19-12- 11	Sputum	45	M	KP	S	S	S	S	N				SUS C													
19 5	BEL	19-12- 11	Urine	30	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	S	R	R	R	S		>1 28	>1 28	>1 28	TEM-1, CTX-M-15
19 6	BEL	19-12- 11	Urine	51	M	EC	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					

19 7	BEL	19-12- 11	Sputum	62	M	KP	S	S	S	S	N				SUS C													
19 8	BEL	19-12- 11	Urine	19	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	I	R	S		12 8	>1 28	>1 28	
19 9	BEL	19-12- 11	Urine	38	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	S	S	I	S		12 8	>1 28	>1 28	TEM-1, SHV-27, CTX- M-15
20 0	BEL	19-12- 11	Urine	22	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	S	I	S	S		>1 28	64	64	SHV-12
20 1	BEL	19-12- 11	Urine	18	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	R	S		12 8	>1 28	>1 28	
20 2	BEL	19-12- 11	Urine	26	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	R	S		>1 28	>1 28	>1 28	CTX-M-15
20 3	BEL	19-12- 11	Urine	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	I	S		12 8	>1 28	>1 28	CTX-M-15
20 4	BEL	19-12- 11	Sputum	33	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S		64	>1 28	>1 28	
20 5	BEL	19-12- 11	Urine	45	F	EC	R	R	R	R	P	P	Ce		ESB L	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
20 6	BEL	19-12- 11	Urine	25	F	EC	R	R	R	R	P	N		A	Am pC	R	R	R	R	R	R	R	S		>1 28	>1 28	>1 28	
20 7	BEL	19-12- 11	Urine	32	M	EC	S	S	S	S	N				SUS C													
20 8	BEL	19-12- 11	Urine	25	F	EC	S	S	S	S	N				SUS C													
20 9	BEL	19-12- 11	Sputum	47	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	I	I	S		12 8	>1 28	>1 28	
21 0	BEL	19-12- 11	Urine	60	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	I	I	S		>1 28	>1 28	>1 28	CTX-M-15
21 1	BEL	19-12- 11	Sputum	65	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	I	R	R	S		>1 28	>1 28	>1 28	
21 2	BEL	19-12- 11	Urine	33	F	EC	R	R	R	R	P	P	Ce		ESB L	R	R	I	S	R	R	R	S		>1 28	>1 28	>1 28	
21 3	BEL	19-12- 11	Sputum	60	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
21 4	BEL	19-12- 11	Urine	50	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	I	S	R	R	R	S		>1 28	>1 28	>1 28	CTX-M-15
21 5	BEL	19-12- 11	Urine	36	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	I	S		16	12 8	>1 28	
21 6	BEL	19-12- 11	Urine	2	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	R	S		>1 28	>1 28	>1 28	

[illegible]

23 7	BEL	22-07- 12	Urine	4	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	S	S	S	S		8	16	8	SHV-18
23 8	BEL	22-07- 12	Urine	36	M	KP	S	S	S	S	N				SUS C													
23 9	BEL	22-07- 12	Urine	70	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	I	R	S	S		>1 28	16	12 8	SHV-28
24 0	BEL	22-07- 12	Sputum	70	M	KP	S	S	S	S	N				SUS C													
24 1	BEL	22-07- 12	Sputum	26	M	KP	S	S	S	S	N				SUS C													
24 2	BEL	22-07- 12	Sputum	60	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	I	S	P	12 8	12 8	>1 28	TEM-1, SHV-108, CTX-M-15
24 3	BEL	22-07- 12	Sputum	60	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	I	S	P	12 8	64	>1 28	SHV-28, CTX-M-15
24 4	BEL	22-07- 12	Sputum	22	M	KP	S	S	S	S	N				SUS C													
24 5	BEL	22-07- 12	Sputum	55	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	S	P	8	64	12 8	TEM-1, SHV-108, CTX-M-15
24 6	BEL	22-07- 12	Sputum	28	M	KP	S	S	S	S	N				SUS C													
24 7	BEL	22-07- 12	Sputum	44	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	S		>1 28	>1 28	>1 28	TEM-1, SHV-18, CTX- M-15
24 8	BEL	22-07- 12	Sputum	36	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	R	S	P	12 8	64	>1 28	
24 9	BEL	22-07- 12	Sputum	48	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S		8	64	>1 28	
25 0	BEL	22-07- 12	Sputum	66	M	KP	S	S	S	S	N				SUS C													
25 1	BEL	22-07- 12	Sputum	60	F	KP	S	S	S	S	N				SUS C													
25 2	BEL	22-07- 12	Sputum	40	M	KP	S	S	S	S	N				SUS C													
25 3	BEL	22-07- 12	Sputum	32	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	S	S	S	S	P	8	64	>1 28	
25 4	BEL	22-07- 12	Sputum	16	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S	P	8	64	>1 28	TEM-1, SHV-?, CTX- M-15
25 5	BEL	22-07- 12	Sputum	25	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	R	R	R	S	P	12 8	>1 28	>1 28	TEM-1, SHV-18, CTX- M-15
25 6	BEL	22-07- 12	Sputum	24	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S		8	16	16	SHV-14

25 7	BEL	22-07- 12	Sputum	70	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R	P	>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX- M-15
25 8	BEL	22-07- 12	Sputum	34	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	I	S	S	S		8	8	64	
25 9	BEL	22-07- 12	Sputum	55	M	KP	S	S	S	S	N				SUS C													
26 0	BEL	22-07- 12	Sputum	60	M	KP	S	S	S	S	N				SUS C													
26 1	BEL	22-07- 12	Sputum	20	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	I	S	S		8	8	8	SHV-14
26 2	BEL	22-07- 12	Sputum	32	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S		8	16	16	SHV-18
26 3	BEL	22-07- 12	Sputum	85	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	I	I	R	R	R	R		>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
26 4	BEL	22-07- 12	Sputum	30	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	I	R	R	R	R	R		>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
26 5	BEL	22-07- 12	Sputum	65	M	KP	S	S	S	S	N				SUS C													
26 6	BEL	22-07- 12	Sputum	18	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	S	S	S	S		8	16	16	
26 7	BEL	01-08- 12	Sputum	36	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	I	R	R	R	R	P	>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
26 8	BEL	01-08- 12	Sputum	70	M	KP	S	S	S	S	N				SUS C													
26 9	BEL	01-08- 12	Sputum	17	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	S	S	S	S		16	16	16	
27 0	BEL	01-08- 12	Sputum	42	M	KP	S	S	S	S	N				SUS C													
27 1	BEL	01-08- 12	Sputum	22	M	KP	S	S	S	S	N				SUS C													
27 2	BEL	01-08- 12	Sputum	36	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	32	64	>1 28	TEM-1, SHV-1, CTX- M-15
27 3	BEL	01-08- 12	Sputum	60	M	KP	S	S	S	S	N				SUS C													
27 4	BEL	01-08- 12	Sputum	51	M	KP	S	S	S	S	N				SUS C													
27 5	BEL	01-08- 12	Sputum	26	M	KP	S	S	S	S	N				SUS C													
27 6	BEL	01-08- 12	Sputum	18	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	I	R	I	R	S		64	12 8	>1 28	

27	BEL	01-08-12	Sputum	26	M	KP	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
27	BEL	01-08-12	Sputum	56	M	KP	S	S	S	S	N				SUS C													
27	BEL	01-08-12	Sputum	54	M	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	I	R	R	S		16	16	8	
28	BEL	01-08-12	Sputum	45	M	KP	S	S	S	S	N				SUS C													
28	BEL	01-08-12	Sputum	64	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	I	S	R	R	R	S	P	>1 28	>1 28	>1 28	
28	BEL	01-08-12	Sputum	32	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	R	S	P	32	12 8	>1 28	SHV-108, CTX-M-15
28	BEL	01-08-12	Sputum	15	M	KP	S	S	S	S	N				SUS C													
28	BEL	01-08-12	Sputum	32	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	S	S	S	I	R	R	S	P	32	16	64	
28	BEL	01-08-12	Sputum	33	F	KP	S	S	S	S	N				SUS C													
28	BEL	01-08-12	Sputum	66	M	KP	S	S	S	S	N				SUS C													
28	BEL	01-08-12	Sputum	33	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	I	S	P	12 8	16	12 8	
28	BEL	01-08-12	Sputum	54	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	I	S		32	64	>1 28	
28	BEL	01-08-12	Asci Fluid	65	M	KP	S	S	S	S	N				SUS C													
29	BEL	01-08-12	Pus	80	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	I	I	S	S		32	16	8	TEM-1, SHV-14, CTX-M-15
29	BEL	01-08-12	Pus	36	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	I	I	S		32	64	>1 28	
29	BEL	01-08-12	Pus	90	F	KP	S	S	S	S	N				SUS C													
29	BEL	01-08-12	Pus	36	M	KP	S	S	S	S	N				SUS C													
29	BEL	01-08-12	Pus	35	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		64	12 8	>1 28	
29	BEL	01-08-12	Pus	53	F	KP	S	S	S	S	N				SUS C													
29	BEL	01-08-12	Pus	26	M	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	I	R	R	S		16	16	16	SHV-14

297	BEL	01-08-12	Pus	40	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	I	S	P	32	64	12 8	
298	BEL	01-08-12	Pus	36	M	KP	S	S	S	S	N				SUS C													
299	BEL	01-08-12	Pus	27	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	I	I	R	R	R	R	P	>1 28	>1 28	>1 28	
300	BEL	01-08-12	Pus	60	F	KP	S	S	S	S	N				SUS C													
301	BEL	01-08-12	Pus	20	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	R	S	S		64	16	12 8	SHV-14
302	BEL	01-08-12	Blood	10	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	I	I	I	S	P	32	64	>1 28	
303	BEL	01-08-12	Blood	44	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	I	S		32	12 8	>1 28	
304	BEL	01-08-12	Blood	5	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R	P	>1 28	12 8	>1 28	
305	BEL	01-08-12	Blood	32	F	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	S	R	R	S		16	16	16	
306	BEL	01-08-12	Blood	49	M	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	I	R	R	S		32	16	16	
307	BEL	01-08-12	Blood	32	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	R	R	S		64	12 8	>1 28	
308	BEL	01-08-12	Blood	6	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	S	I	R	R	R	R	P	>1 28	>1 28	>1 28	
309	BEL	01-08-12	Blood	27	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	R	I	S	S		32	16	64	SHV-?
310	BEL	01-08-12	Urine	20	F	KP	S	S	S	S	N				SUS C													
311	BEL	01-08-12	Urine	4	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	R	R	I	S	P	64	16	64	SHV-28
312	BEL	01-08-12	Urine	6	F	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	I	I	R	S	P	16	8	8	
313	BEL	01-08-12	Urine	60	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	R	I	I	S	P	32	16	64	SHV-18
314	BEL	01-08-12	Urine	65	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	R	I	I	R		32	16	64	
315	BEL	01-08-12	Urine	4	M	KP	S	S	S	S	N				SUS C													
316	BEL	01-08-12	Urine	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	S	S		32	16	64	SHV-28

31 7	BEL	01-08- 12	Urine	23	F	KP	S	S	S	S	N				SUS C														
31 8	BEL	01-08- 12	Urine	33	M	KP	R	R	R	R	P	P	Ce		ESB L	R	I	S	S	I	I	S	S		16	8	16		
31 9	BEL	01-08- 12	Urine	60	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	S	S	S	I	S	S	S		32	16	16		
32 0	BEL	01-08- 12	Urine	46	F	KP	S	S	S	S	N				SUS C														
32 1	BEL	01-08- 12	Urine	38	F	KP	S	S	S	S	N				SUS C														
32 2	BEL	01-08- 12	Urine	19	F	KP	S	S	S	S	N				SUS C														
32 3	BEL	01-08- 12	Urine	4	M	KP	S	S	S	S	N				SUS C														
32 4	BEL	01-08- 12	Sputum	26	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	R	S	S		64	8	64		
32 5	BEL	01-08- 12	Sputum	30	F	KP	S	S	S	S	N				SUS C														
32 6	BEL	01-08- 12	Sputum	36	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	R	I	I	S	P	64	16	64		
32 7	BEL	01-08- 12	Sputum	66	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	I	S		16	32	>1 28	TEM-1, SHV-11, CTX- M-15	
32 8	BEL	01-08- 12	Sputum	48	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	I	R	S	S		16	16	16		
32 9	BEL	01-08- 12	Sputum	32	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	I	R	I	S		12 8	16	12 8	SHV-?	
33 0	BEL	01-08- 12	Sputum	18	M	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	R	R	R	S		8	8	16		
33 1	BEL	01-08- 12	Sputum	75	F	KP	S	S	S	S	N				SUS C														
33 2	BEL	01-08- 12	Sputum	33	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	R	I	S	S		32	16	16		
33 3	BEL	01-08- 12	Sputum	60	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	I	S		32	64	>1 28	SHV-14, CTX-M-15	
33 4	BEL	01-08- 12	Sputum	68	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	I	S	P	16	32	>1 28	TEM-1, SHV-14, CTX- M-15	
33 5	BEL	01-08- 12	Sputum	60	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	S	S	S	S		32	16	16		
33 6	BEL	01-08- 12	Sputum	55	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S	P	12 8	12 8	>1 28		

[illegible]

[illegible]

[illegible]

[illegible]

417	DV G	27-04- 10	CSF	1	F	EC	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
418	DV G	27-04- 10	Urine	1	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S	P	16	>1 28	>1 28	
419	DV G	27-04- 10	Urine	45	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	I	R	S	I	S		32	>1 28	>1 28	
420	DV G	27-04- 10	Pus	33	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	I	R	R	R	S		>1 28	>1 28	>1 28	
421	DV G	27-04- 10	Pus	70	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	R	S	S	S		8	>1 28	>1 28	
422	DV G	27-04- 10	Sputum	60	M	EC	S	S	S	S	N				SUS C													
423	DV G	27-04- 10	Pus	20	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S	P	12 8	>1 28	>1 28	
424	DV G	27-04- 10	Rectal swa	35	M	EC	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
425	DV G	27-04- 10	Rectal swa	28	F	EC	S	S	S	S	N				SUS C													
426	DV G	27-04- 10	Sputum	46	M	KP	S	S	S	S	N				SUS C													
427	DV G	27-04- 10	Urine	21	F	EC	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
428	DV G	27-04- 10	Urine	11	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	16	>1 28	>1 28	
429	DV G	27-04- 10	Sputum	60	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	16	>1 28	>1 28	
430	DV G	27-04- 10	Sputum	65	M	EC	R	R	R	R	P	N		EA	COP R	R	R	I	I	R	R	R	S		>1 28	>1 28	>1 28	
431	DV G	27-04- 10	Urine	5	F	EC	R	R	R	R	P	N			UN KN	R	S	S	S	R	R	R	S		32	>1 28	>1 28	
432	DV G	27-04- 10	ET Tube	20	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		8	>1 28	>1 28	
433	DV G	27-04- 10	Urine	10	F	EC	S	S	S	S	N				SUS C													
434	DV G	27-04- 10	Sputum	70	M	KP	S	S	S	S	N				SUS C													
435	DV G	27-04- 10	Urine	1	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		32	>1 28	>1 28	
436	DV G	27-04- 10	Sputum	24	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	12 8	>1 28	>1 28	

437	DV G	27-04- 10	Urine	45	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		16	>1 28	>1 28	
438	DV G	27-04- 10	Urine	18	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	I	I	S	S	S	P	16	12 8	>1 28	
439	DV G	27-04- 10	Sputum	69	M	KP	S	S	S	S	N				SUS C													
440	DV G	27-04- 10	Rectal swa	2	M	EC	S	S	S	S	N				SUS C													
441	DV G	27-04- 10	Cerv Swab	23	F	EC	S	S	S	S	N				SUS C													
442	DV G	27-04- 10	Sputum	62	M	KP	R	S	S	R	P	N			UN KN	S	S	S	S	S	S	S	S		4	0.5	12 8	
443	DV G	27-04- 10	Urine	40	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	8	12 8	>1 28	
444	DV G	27-04- 10	Sputum	36	M	EC	R	R	R	R	P	N		EA	COP R	R	R	S	S	R	R	R	S		>1 28	>1 28	>1 28	
445	DV G	27-04- 10	Sputum	80	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		16	>1 28	>1 28	
446	DV G	29-04- 10	Pus	63	F	EC	R	R	R	R	P	N		A	Am pC	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
447	DV G	29-04- 10	Pus	70	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		8	>1 28	>1 28	
448	DV G	29-04- 10	Rectal swa	26	F	EC	S	S	S	S	N				SUS C													
449	DV G	29-04- 10	Pus	23	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S	P	>1 28	>1 28	>1 28	TEM-1, CTX-M-15
450	DV G	29-04- 10	Rectal swa	1	M	KP	S	S	S	S	N				SUS C													
451	DV G	29-04- 10	Pus	50	M	EC	R	R	R	R	P	N		A	Am pC	R	R	S	S	R	R	R	S		>1 28	>1 28	>1 28	
452	DV G	29-04- 10	Pus	54	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S	P	>1 28	>1 28	>1 28	
453	DV G	07-05- 10	Pus	22	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	I	I	S		>1 28	>1 28	>1 28	
454	DV G	07-05- 10	Suct tip	36	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	S	S	P	64	>1 28	>1 28	
455	DV G	07-05- 10	Urine	25	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	R	R	R	S	P	>1 28	>1 28	>1 28	TEM-1, CTX-M-15
456	DV G	07-05- 10	Pus	18	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	R	S	S	S		>1 28	>1 28	>1 28	

457	DVG	07-05-10	Pus	10	F	EC	S	S	S	S	N				SUSC																								
458	DVG	07-05-10	Urine	70	M	EC	R	R	R	R	P	P	CaCe		ESBL	R	R	S	S	R	S	S	S		16	>128	>128												
459	DVG	07-05-10	Urine	18	F	EC	R	R	R	R	P	P	CaCe		ESBL	R	R	S	S	R	S	S	S		64	128	>128											TEM-1, CTX-M-15	
460	DVG	07-05-10	Sputum	22	F	EC	S	S	S	S	N				SUSC																								
461	DVG	07-05-10	Pus	70	M	EC	R	R	R	R	P	P	CaCe		ESBL	S	R	S	S	I	S	S	S		16	>128	>128												
462	DVG	07-05-10	Blood	55	M	EC	S	S	S	S	N				SUSC																								
463	DVG	07-05-10	Rectal swa	1	M	EC	R	R	R	R	P	P	CaCe		ESBL	I	R	S	S	I	S	S	S	P	32	>128	>128												
464	DVG	07-05-10	Urine	1	M	EC	R	R	R	R	P	P	CaCe		ESBL	R	R	S	S	I	S	S	S		16	>128	>128												
465	DVG	04-08-10	Sputum	72	M	KP	R	R	R	R	P	P	CaCe		ESBL	R	I	S	S	R	S	S	S	P	64	>128	>128												
466	DVG	04-08-10	Sputum	58	F	KP	R	R	R	R	P	P	CaCe		ESBL	R	R	S	S	R	S	S	S	P	32	>128	>128												
467	DVG	04-08-10	Sputum	58	F	KP	R	R	R	R	P	P	CaCe		ESBL	S	R	S	S	R	R	S	S	P	32	>128	>128												
468	DVG	04-08-10	Urine	27	F	EC	R	R	R	R	P	P	CaCe		ESBL	I	R	S	I	R	I	S	S		16	>128	>128												
469	DVG	04-08-10	Urine	33	F	EC	R	R	R	R	P	P	CaCe		ESBL	I	R	S	S	R	I	S	S		16	>128	>128												
470	DVG	04-08-10	Urine	33	F	EC	R	R	R	R	P	P	CaCe		ESBL	I	R	S	S	R	S	S	S	P	16	>128	>128												
471	DVG	04-08-10	Urine	60	F	EC	S	S	S	S	N				SUSC																								
472	DVG	04-08-10	Throat swa	1	M	EC	R	R	R	R	P	P	CaCe		ESBL	R	R	S	S	R	S	S	S		32	>128	>128												
473	DVG	04-08-10	Pus	38	M	KP	R	R	R	R	P	P	CaCe		ESBL	R	R	I	I	R	I	S	S	P		>128	>128	>128											
474	DVG	04-08-10	Urine	62	M	EC	R	R	R	R	P	P	CaCe		ESBL	R	R	S	S	R	S	S	S		64	>128	>128												
475	DVG	04-08-10	Urine	28	F	EC	R	R	R	R	P	P	CaCe		ESBL	R	R	I	S	R	S	S	S		64	>128	>128											TEM-1, SHV-28, CTX-M-15	
476	DVG	04-08-10	Sputum	17	M	KP	R	R	R	R	P	N			UNKN		R	R	S	R	R	R	R																

47	DV	04-08-10	Blood	23	M	EC	R	R	R	R	P	N		EA	COP R	R	R	R	R	R	R	R	S		>1 28	>1 28	>1 28	
47	DV	08-08-10	Urine	51	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	I	S	S	S	P	16	>1 28	>1 28	CTX-M-15
47	DV	08-08-10	Rectal swa	6	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	I	R	R	R	S		>1 28	>1 28	>1 28	
48	DV	08-08-10	Cerv Swab	23	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		16	>1 28	>1 28	
48	DV	08-08-10	Pus	75	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	R	S		16	>1 28	>1 28	
48	DV	08-08-10	Pus	23	M	EC	R	R	R	R	P	N			UN KN		S	S	S	R	S	I	S					
48	DV	08-08-10	Sputum	23	M	KP	S	S	S	S	N				SUS C													
48	DV	08-08-10	Urine	20	F	EC	S	S	S	S	N				SUS C													
48	DV	08-08-10	Blood	33	F	KP	S	S	S	S	N				SUS C													
48	DV	08-08-10	Urine	22	M	KP	S	S	S	S	N				SUS C													
48	DV	08-08-10	Pus	22	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		64	>1 28	>1 28	
48	DV	16-09-10	Urine	21	M	EC	R	R	R	R	P	N		EA	COP R	R	R	I	S	R	R	R	S	P	>1 28	>1 28	>1 28	
48	DV	23-09-10	Pleural fl	28	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S		8	>1 28	>1 28	
49	DV	23-09-10	Urine	31	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	S	S	S	P	32	>1 28	>1 28	
49	DV	23-09-10	Urine	32	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	16	>1 28	>1 28	
49	DV	23-09-10	Urine	23	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	8	>1 28	>1 28	CTX-M-15
49	DV	23-09-10	Urine	40	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	I	R	R	R	S		>1 28	>1 28	>1 28	
49	DV	23-09-10	Urine	64	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	S	S		64	12 8	>1 28	
49	DV	23-09-10	Pus	65	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	S	S	S	P	16	>1 28	>1 28	
49	DV	23-09-10	Throat swa	40	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		8	>1 28	>1 28	CTX-M-15

[illegible]

517	DVG	23-09-10	Pus	17	M	KP	S	S	S	S	N				SUS C														
518	DVG	23-09-10	Pus	17	M	EC	S	S	S	S	N				SUS C														
519	DVG	04-10-10	Urine	28	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	S	S	P	128	>128	>128		
520	DVG	04-10-10	Urine	40	F	KP	S	S	S	S	N				SUS C														
521	DVG	04-10-10	Sputum	36	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		32	>128	>128		
522	DVG	04-10-10	Pus	26	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	I	S	S	S		16	32	128	TEM-1, SHV-75, CTX-M-15	
523	DVG	04-10-10	Urine	28	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	R	S	P	>128	>128	>128		
524	DVG	04-10-10	Sputum	65	M	KP	S	S	S	S	N				SUS C														
525	DVG	04-10-10	Sputum	85	M	KP	S	S	S	S	N				SUS C														
526	DVG	04-10-10	ET Tube	1	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R	P	>128	>128	>128		
527	DVG	04-10-10	Urine	30	F	EC	R	R	R	R	P	N		A	Am pC	R	R	R	S	R	R	R	S	P	>128	>128	>128		
528	DVG	04-10-10	Sputum	7	M	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	S		>128	>128	>128		
529	DVG	04-10-10	Sputum	18	F	KP	S	S	S	S	N				SUS C														
530	DVG	04-10-10	Sputum	20	F	EC	S	S	S	S	N				SUS C														
531	DVG	04-10-10	Pus	18	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	R	S	P	>128	>128	>128		
532	DVG	04-10-10	Sputum	23	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	64	128	128		
533	DVG	04-10-10	Urine	38	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	I	R	I	R	S	P	>128	>128	>128		
534	DVG	04-10-10	Urine	28	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S	P	64	>128	>128		
535	DVG	04-10-10	Sputum	21	F	EC	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R						
536	DVG	06-11-10	Pus	57	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	S	R	R	R	S		>128	>128	>128		

537	DVG	06-11-10	Sputum	40	F	KP	S	S	S	S	N				SUS C														
538	DVG	06-11-10	Sputum	75	M	EC	R	R	R	R	P	N			UN KN	R	S	S	S	S	I	S	S		32	64	32		
539	DVG	06-11-10	Sputum	46	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	64	>1 28	>1 28		
540	DVG	06-11-10	Sputum	35	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S		8	4	4		
541	DVG	06-11-10	Sputum	14	M	EC	R	R	R	R	P	N		A	Am pC	R	S	S	S	R	R	R	S		4	4	4		
542	DVG	06-11-10	Pus	35	F	EC	S	S	S	S	N				SUS C														
543	DVG	06-11-10	Urine	23	F	EC	R	R	R	R	P	N		EA	COP R	R	R	S	I	R	R	R	S	P	>1 28	>1 28	>1 28		
544	DVG	06-11-10	Urine	49	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S	P	32	>1 28	>1 28		
545	DVG	06-11-10	Sputum	58	F	EC	R	R	R	R	P	N		A	Am pC	R	R	I	I	R	R	R	S	P	>1 28	>1 28	>1 28		
546	DVG	08-11-10	Sputum	55	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	S	S	S	S		>1 28	4	32	TEM-1	
547	DVG	08-11-10	Urine	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	64	12 8	>1 28		
548	DVG	08-11-10	Sputum	20	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	I	I	S	S		64	4	4		
549	DVG	08-11-10	Pus	65	F	EC	S	S	S	S	N				SUS C														
550	DVG	08-11-10	Pus	49	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	I	S	S	S		16	32	32	TEM-1, CTX-M-15	
551	DVG	08-11-10	Urine	24	F	EC	S	S	S	S	N				SUS C														
552	DVG	07-01-11	Sputum	99	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	I	I	R		32	>1 28	>1 28	TEM-?	
553	DVG	07-01-11	Urine	65	F	EC	R	R	R	R	P	N		A	Am pC	R	S	S	S	R	R	R	S		64	32	64		
554	DVG	07-01-11	Sputum	45	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S		12 8	>1 28	>1 28		
555	DVG	07-01-11	Urine	23	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	I	S	S	S		8	>1 28	>1 28		
556	DVG	07-01-11	Urine	16	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	I	S	P	32	12 8	12 8		

55	DV	12-01-										Ca		ESB										>1	>1	>1	
7	G	11	Urine	29	F	EC	R	R	R	P	P	Ce		L	R	S	S	S	S	S	S		28	28	28		
55	DV	21-01-										Ca		ESB										>1	>1	>1	
8	G	11	Blood	7	M	EC	R	R	R	P	P	Ce		L	S	R	S	S	I	S	I	S	32	28	28		
55	DV	21-01-												SUS													
9	G	11	Pus	59	M	KP	S	S	S	S	N			C													
56	DV	21-01-										Ca		ESB										>1	>1	>1	
0	G	11	Urine	57	M	EC	R	R	R	P	P	Ce		L	S	R	S	S	R	S	S	S	32	28	28		
56	DV	21-01-											A	Am									12	12	>1		
1	G	11	Sputum	71	M	EC	R	R	R	P	N			pC	R	S	S	S	R	R	S	P	8	8	28		
56	DV	21-01-										Ca		ESB									12	12	12		
2	G	11	Sputum	71	M	KP	R	R	R	P	P	Ce		L	S	I	S	S	I	S	S	P	32	8	8		
56	DV	21-01-										Ca		ESB									>1	>1	>1		
3	G	11	Urine	30	F	EC	R	R	R	P	P	Ce		L	R	R	S	S	R	S	R	S	28	28	28		
56	DV	21-01-												SUS													
4	G	11	Pus	21	M	KP	S	S	S	S	N			C													
56	DV	21-01-										Ca		ESB										>1	>1	>1	
5	G	11	Sputum	45	F	EC	R	R	R	P	P	Ce		L	R	R	S	S	R	I	I	S	32	28	28		
56	DV	23-01-											EA	COP									>1	>1	>1		
6	G	11	Urine	26	F	EC	R	R	R	P	N			R	R	R	I	I	R	R	R	S	28	28	28		
56	DV	23-01-												SUS													
7	G	11	Sputum	25	M	KP	S	S	S	S	N			C													
56	DV	23-01-												SUS													
8	G	11	Urine	30	F	EC	S	S	S	S	N			C													
56	DV	23-01-												COP									>1	>1	>1		
9	G	11	Urine	21	M	EC	R	R	R	R	P	N		EA	R	R	I	S	R	R	R	S	28	28	28		
57	DV	23-01-										Ca		ESB									32	12	>1		
0	G	11	Sputum	28	M	EC	R	R	R	P	P	Ce		L	R	I	S	S	R	S	S	S	28	8	28		
57	DV	23-01-	Vag									Ca		ESB									12	12	>1		
1	G	11	Swab	22	F	KP	R	R	R	R	P	P	Ce	L	S	R	S	S	R	S	I	S	8	8	28		
57	DV	23-01-										Ca		ESB										>1	>1	>1	
2	G	11	Urine	4	F	EC	R	R	R	R	P	P	Ce	L	R	R	S	S	I	S	S	S	32	28	28		
57	DV	23-01-												SUS													
3	G	11	Sputum	60	F	KP	S	S	S	S	N			C													
57	DV	23-01-												SUS													
4	G	11	Sputum	45	F	EC	S	S	S	S	N			C													
57	DV	23-01-												COP									>1	>1	>1		
5	G	11	Pus	65	M	EC	R	R	R	R	P	N		EA	R	R	I	S	R	R	R	S	28	28	28		
57	DV	23-01-												SUS													
6	G	11	Sputum	70	M	KP	S	S	S	S	N			C													

57	DV	23-01-11	Pus	20	M	EC	R	R	R	R	P	P	Ca		ESB	L	S	R	S	S	R	S	S	S	P	32	12	12	
57	DV	23-01-11	Urine	23	F	EC	R	R	R	R	P	P	Ca		ESB	L	R	R	S	S	I	S	S	S	P	32	>1	>1	
57	DV	23-01-11	Sputum	28	F	EC	S	S	S	S	N				SUS	C													
58	DV	23-01-11	Pus	25	M	EC	S	S	S	S	N				SUS	C													
58	DV	23-01-11	Pus	47	F	EC	R	R	R	R	P	N		EA	COP	R	R	R	I	I	R	R	R	S	P	>1	>1	>1	
58	DV	23-01-11	Pus	50	M	EC	R	R	R	R	P	N		EA	COP	R	R	R	I	S	R	R	R	S	P	>1	>1	>1	
58	DV	23-01-11	Pus	28	F	EC	R	R	R	R	P	P	Ca		ESB	L	I	R	S	S	I	S	S	S		32	>1	12	
58	DV	23-01-11	Urine	65	M	EC	S	S	S	S	N				SUS	C													
58	DV	23-01-11	Sputum	59	F	EC	R	R	R	R	P	P	Ca		ESB	L	S	R	S	S	I	S	I	S		32	>1	>1	
58	DV	23-01-11	Pus	50	M	EC	R	R	R	R	P	P	Ca		ESB	L	I	R	S	S	R	S	I	S	P	64	12	>1	
58	DV	26-03-11	Pus	45	M	EC	R	R	R	R	P	N		EA	COP	R	R	R	S	S	R	R	R	S	P	>1	>1	>1	
58	DV	26-03-11	Sputum	60	M	EC	R	R	R	R	P	P	Ca		ESB	L	I	R	S	S	I	S	S	S	P	16	64	12	CTX-M-15
58	DV	26-03-11	Urine	8	M	EC	R	R	R	R	P	P	Ca		ESB	L	I	R	S	S	R	S	S	S		32	>1	>1	
59	DV	26-03-11	Urine	25	F	EC	R	R	R	R	P	N		EA	COP	R	R	R	S	S	R	R	R	S		>1	>1	>1	
59	DV	26-03-11	Urine	26	F	EC	R	R	R	R	P	P	Ca		ESB	L	I	R	S	S	I	S	S	S		8	64	64	
59	DV	26-03-11	Urine	30	F	EC	R	R	R	R	P	P	Ca		ESB	L	R	R	S	S	R	S	S	S	P	64	>1	>1	
59	DV	26-03-11	Pus	3	M	EC	R	R	R	R	P	P	Ca		ESB	L	I	R	S	S	I	S	S	S	P	16	64	64	CTX-M-15
59	DV	26-03-11	Urine	83	M	EC	R	R	R	R	P	P	Ca		ESB	L	S	S	S	S	S	S	S	S	P	32	8	8	
59	DV	26-03-11	Sputum	70	M	EC	R	R	R	R	P	P	Ca		ESB	L	I	R	S	S	R	I	S	S	P	32	64	64	
59	DV	26-03-11	Pus	42	M	EC	R	R	R	R	P	P	Ca		ESB	L	I	R	S	S	I	S	S	S		16	64	64	TEM-1, SHV-28

597	DVG	30-04-11	Urine	25	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	S	R	R	R	S	P	>128	>128	>128	
598	DVG	30-04-11	Urine	24	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	16	64	64	
599	DVG	30-04-11	Urine	33	F	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	S	P	>128	>128	>128	
600	DVG	30-04-11	Pus	3	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	32	128	64	
601	DVG	30-04-11	Pus	42	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S	P	32	128	64	
602	DVG	30-04-11	Urine	8	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S		64	>128	>128	
603	DVG	30-04-11	Urine	83	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	I	S	S	S	P	64	32	32	
604	DVG	30-04-11	Sputum	70	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S	P	64	>128	>128	
605	DVG	30-04-11	Sputum	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	64	128	128	
606	DVG	30-04-11	Urine	2	M	EC	S	S	S	S	N				SUS C													
607	DVG	30-04-11	Urine	6	M	EC	S	S	S	S	N				SUS C													
608	DVG	30-04-11	Urine	38	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	I	R	R	R	S		>128	>128	>128	TEM-1, CTX-M-15
609	DVG	30-04-11	Pus	1	M	EC	R	R	R	R	P	P	Ca		ESB L	R	R	S	S	R	R	R	S		>128	>128	>128	CTX-M-15
610	DVG	30-04-11	Pus	1	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	R	R	R	S	P	>128	>128	>128	SHV-28, CTX-M-15
611	DVG	30-04-11	Urine	75	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S		64	128	>128	
612	DVG	30-04-11	Urine	1	F	EC	S	S	S	S	N				SUS C													
613	DVG	30-04-11	Urine	26	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	S	R	R	R	S	P	>128	>128	>128	
614	DVG	30-04-11	Urine	28	F	KP	R	R	R	R	P	N		EA	COP R	R	R	R	S	R	R	R	S	P	>128	>128	>128	
615	DVG	30-04-11	Urine	30	F	EC	R	R	R	R	P	N		A	Am pC	R	R	S	R	R	R	S	R	P	>128	>128	>128	
616	DVG	30-04-11	Pus	24	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	I	R	I	I	S		>128	>128	>128	

617	DVG	30-04-11	Pus	34	F	EC	S	S	S	S	N				SUS C														
618	DVG	30-04-11	Pus	67	F	EC	R	R	R	R	P	N		A	Am pC	R	S	S	S	R	R	R	S		>128	64	64		
619	DVG	30-04-11	Pus	55	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	R	S		>128	>128	>128		
620	DVG	30-04-11	Urine	61	F	EC	R	R	R	R	P	N		A	Am pC	R	I	S	S	R	R	R	S	P	>128	>128	>128		
621	DVG	30-04-11	Pus	65	F	KP	S	S	S	S	N				SUS C														
622	DVG	30-04-11	Pus	80	F	KP	S	S	S	S	N				SUS C														
623	DVG	30-04-11	Urine	40	M	EC	R	R	R	R	P	N		A	Am pC	R	R	S	S	R	R	R	S	P	>128	>128	>128		
624	DVG	30-04-11	Sputum	52	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	I	I	S	P	128	>128	>128		
625	DVG	30-04-11	Sputum	50	M	KP	S	S	S	S	N				SUS C														
626	DVG	30-04-11	Sputum	32	F	EC	S	S	S	S	N				SUS C														
627	DVG	30-04-11	Urine	50	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	I	S		128	>128	>128		
628	DVG	31-05-11	Urine	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	I	S		32	>128	>128		
629	DVG	31-05-11	Pus	25	M	KP	R	R	R	R	P	N		EA	COP R	R	R	I	R	R	R	R	S		>128	>128	>128		
630	DVG	31-05-11	Pus	47	M	EC	R	R	R	R	P	N			UN KN	R	R	I	R	R	R	R	S	P	>128	>128	>128		
631	DVG	31-05-11	Urine	17	M	KP	R	R	R	R	P	N		A	Am pC	R	R	I	R	R	R	R	R		>128	>128	>128		
632	DVG	31-05-11	Pus	55	M	EC	R	R	R	R	P	N		EA	COP R	R	R	R	I	R	R	R	S		>128	>128	>128		
633	DVG	31-05-11	Urine	60	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	R	S		>128	>128	>128		
634	DVG	31-05-11	Urine	22	F	KP	S	S	S	S	N				SUS C														
635	DVG	20-06-11	Sputum	34	M	KP	S	S	S	S	N				SUS C														
636	DVG	20-06-11	Pus	53	M	EC	R	R	R	R	P	P	Ce		ESB L	R	R	I	S	R	R	R	S	P	>128	>128	>128	CTX-M-15	

63	DV	20-06-11	Pus	18	M	EC	R	R	R	R	P	P	Ca		ESB	R	S	S	S	R	R	R	S	P	>1	>1	>1	N	
63	DV	20-06-11	Urine	9	M	EC	R	R	R	R	P	P	Ca		ESB	S	R	R	S	I	S	R	S		>1	>1	>1		
63	DV	20-06-11	Urine	32	F	EC	R	R	R	R	P	P	Ca		ESB	I	R	R	I	R	I	R	S	P	64	>1	>1	>1	CTX-M-15
64	DV	20-06-11	Urine	11	M	KP	S	S	S	S	N				SUS														
64	DV	20-06-11	Pus	55	F	KP	S	S	S	S	N				SUS														
64	DV	20-06-11	Sputum	45	M	EC	R	R	R	R	P	P	Ca		ESB	I	R	S	S	S	S	R	S	P	>1	>1	>1		
64	DV	20-06-11	Urine	26	F	KP	R	R	R	R	P	P	Ce		ESB	R	R	I	R	R	R	R	R	P	>1	>1	>1		
64	DV	20-06-11	Sputum	61	M	EC	R	R	R	R	P	P	Ca		ESB	S	R	S	S	S	S	S	S	P	32	>1	>1	>1	
64	DV	20-06-11	Sputum	45	M	EC	R	R	R	R	P	P	Ca		ESB	S	R	S	S	S	S	I	S	P	>1	>1	>1		
64	DV	20-06-11	Sputum	51	M	EC	R	R	R	R	P	P	Ca		ESB	S	R	S	S	S	S	I	S		>1	>1	>1	CTX-M-15	
64	DV	20-06-11	Sputum	29	M	KP	R	R	R	R	P	P	Ca		ESB	I	R	S	I	I	S	R	S	P	>1	>1	>1		
64	DV	20-06-11	Sputum	29	M	EC	R	R	R	R	P	P	Ca		ESB	I	R	R	I	I	I	R	S	P	>1	>1	>1		
64	DV	20-06-11	Urine	20	F	EC	S	S	S	S	N				SUS														
65	DV	20-06-11	Urine	30	M	EC	R	R	R	R	P	P	Ca		ESB	R	R	R	S	R	I	R	S	P	>1	>1	>1	CTX-M-15	
65	DV	20-06-11	Urine	22	F	EC	R	R	R	R	P	P	Ca		ESB	I	R	S	S	S	S	R	S	P	32	>1	>1	>1	CTX-M-15
65	DV	20-06-11	Sputum	61	F	EC	R	R	R	R	P	P	Ca		ESB	S	R	S	S	S	S	S	S	P	32	>1	>1	>1	CTX-M-15
65	DV	20-06-11	Suct tip	28	F	EC	R	R	R	R	P	P	Ca		ESB	I	R	S	S	R	I	R	S	P	>1	>1	>1	TEM-219, SHV-71, CTX-M-15	
65	DV	20-06-11	Urine	35	F	EC	R	R	R	R	P	P	Ca		ESB	I	R	S	S	S	S	R	S	P	>1	>1	>1	CTX-M-15	
65	DV	20-06-11	Urine	38	F	EC	R	R	R	R	P	P	Ca		ESB	R	R	I	I	R	R	R	S	P	>1	>1	>1	SHV-2a, CTX-M-15	
65	DV	20-06-11	Sputum	70	M	EC	R	R	R	R	P	P	Ca		ESB	I	R	S	S	S	S	R	S	P	>1	>1	>1	TEM-1, CTX-M-15	

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67	DV	01-07-12	Pus	45	M	KP	S	S	S	S	N				SUS C														
678	DV	01-07-12	Urine	33	F	KP	S	S	S	S	N				SUS C														
679	DV	01-07-12	Sputum	55	M	KP	S	S	S	S	N				SUS C														
680	DV	01-07-12	Pus	67	M	KP	S	S	S	S	N				SUS C														
681	DV	01-07-12	Sputum	6	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S	S	P	32	32	64	
682	DV	01-07-12	Urine	20	F	KP	S	S	S	S	N				SUS C														
683	DV	01-07-12	Sputum	62	M	KP	S	S	S	S	N				SUS C														
684	DV	01-07-12	ET Tube	23	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S	P	32	64	64		
685	DV	01-07-12	ET Tube	85	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	I	S	P	64	128	128	
686	DV	01-07-12	Sputum	64	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S	P	32	16	64		
687	DV	01-07-12	Sputum	80	M	KP	S	S	S	S	N				SUS C														
688	DV	01-07-12	Sputum	45	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S	P	32	32	64		
689	DV	01-07-12	Pus	32	M	KP	S	S	S	S	N				SUS C														
690	DV	01-07-12	Pus	45	M	KP	S	S	S	S	N				SUS C														
691	DV	01-07-12	Pus	55	F	KP	S	S	S	S	N				SUS C														
692	DV	01-07-12	Gastric La	48	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	R	R	S	P	128	>128	>128		
693	DV	01-07-12	Pus	35	M	KP	S	S	S	S	N				SUS C														
694	DV	22-07-12	Urine	55	F	KP	S	S	S	S	N				SUS C														
695	DV	22-07-12	BAL	55	M	KP	S	S	S	S	N				SUS C														
696	DV	22-07-12	Pus	60	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	S	P	>128	>128	>128		

[illegible]

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77	DV	04-01-13	Sputum	60	F	KP	S	S	S	S	N				SUS C														
778	DV	14-01-13	Pus	54	M	KP	S	S	S	S	N				SUS C														
779	DV	14-01-13	Pus	28	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	R	S		32	>1 28	>1 28	CTX-M-15	
780	DV	14-01-13	Pus	45	F	KP	S	S	S	S	N				SUS C														
781	DV	14-01-13	Pus	50	F	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R						
782	DV	14-01-13	Sputum	53	M	KP	S	S	S	S	N				SUS C														
783	DV	14-01-13	Urine	20	F	KP	S	S	S	S	N				SUS C														
784	DV	14-01-13	Blood	4	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	R	R	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15	
785	DV	14-01-13	Urine	16	F	KP	S	S	S	S	N				SUS C														
786	DV	14-01-13	Pus	59	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	I	I	R	S	P	32	>1 28	>1 28	TEM-1, SHV-27, CTX-M-15	
787	DV	14-01-13	Pus	27	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S		32	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15	
788	DV	14-01-13	Urine	14	F	KP	S	S	S	S	N				SUS C														
789	DV	14-01-13	Pus	2	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	TEM-1, CTX-M-15	
790	DV	14-01-13	Sputum	22	F	KP	S	S	S	S	N				SUS C														
791	DV	14-01-13	ET Tube	1	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	P		>1 28	>1 28	>1 28	TEM-1, SHV-11, CTX-M-15	
792	DV	14-01-13	Pus	38	M	KP	R	R	R	R	P	N		A	Am pC	R	I	S	S	I	S	S	S		>1 28	>1 28	>1 28		
793	DV	14-01-13	Pus	54	M	KP	S	S	S	S	N				SUS C														
794	DV	14-01-13	Pus	47	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-12, CTX-M-15	
795	DV	14-01-13	Pus	22	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R							
796	DV	14-01-13	Pus	54	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	I	R	R	R	S	P	12 8	>1 28	>1 28	TEM-1, SHV-11, CTX-M-155	

797	DVG	14-01-13	Pus	30	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	R	R	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15
798	DVG	14-01-13	Sputum	8	M	KP	S	S	S	S	N				SUS C													
799	DVG	14-01-13	Pus	58	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	I	R	R	R	S	P	12 8	>1 28	>1 28	TEM-1, SHV-11
800	DVG	14-01-13	Sputum	19	F	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
801	DWD	16-09-10	Urine	66	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	8	12 8	32	TEM-1, CTX-M-15
802	DWD	16-09-10	Urine	70	M	EC	R	R	R	R	P	P	Ca		ESB L	R	R	S	S	R	R	R	S	P	>1 28	>1 28	>1 28	
803	DWD	16-09-10	Urine	31	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	I	S	S	S	P	8	64	16	CTX-M-15
804	DWD	16-09-10	Urine	33	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	8	12 8	64	TEM-1, CTX-M-15
805	DWD	16-09-10	Urine	51	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	S	S	S	S	P	8	32	16	
806	DWD	16-09-10	Urine	18	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	S	R	R	R	S	P	>1 28	>1 28	>1 28	
807	DWD	16-09-10	Urine	28	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	8	12 8	>1 28	TEM-1, CTX-M-15
808	DWD	16-09-10	Urine	25	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S	P	8	64	64	
809	DWD	16-09-10	Urine	30	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	S	S	S	S	P	8	64	16	CTX-M-15
810	DWD	16-09-10	Urine	38	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	8	>1 28	64	CTX-M-15
811	DWD	16-09-10	Urine	25	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	8	12 8	16	CTX-M-15
812	DWD	16-09-10	Urine	56	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	S	P	8	64	64	
813	DWD	16-09-10	Urine	48	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	8	64	16	
814	DWD	16-09-10	Urine	27	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	S	P	8	32	64	
815	DWD	16-09-10	Urine	25	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S		8	12 8	>1 28	TEM-1
816	DWD	16-09-10	Urine	25	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	I	S	S	S	P	8	12 8	>1 28	TEM-1, CTX-M-15

[illegible]

837	DW D	06-11-10	Pus	24	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S		32	>1 28	>1 28	TEM-1, CTX-M-15	
838	DW D	06-11-10	Pus	28	M	EC	S	S	S	S	N			SUS C															
839	DW D	06-11-10	Pus	80	F	EC	R	R	R	R	P	N		A	Am pC	R	R	I	I	R	R	R	S	P	>1 28	>1 28	>1 28		
840	DW D	06-11-10	Pus	33	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S		16	>1 28	>1 28		
841	DW D	06-11-10	Pus	28	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	I	S	P	32	>1 28	>1 28		
842	DW D	06-11-10	Sputum	70	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	R	R	I	R	S	P	>1 28	>1 28	>1 28		
843	DW D	06-11-10	Asci Fluid	20	M	EC	R	R	R	R	P	N			UN KN		S	S	S	I	I	S	S						
844	DW D	06-11-10	Sputum	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	I	S		32	>1 28	>1 28		
845	DW D	06-11-10	Pus	66	M	EC	R	R	R	R	P	N		EA	COP R	R	R	I	S	R	R	R	S	P	>1 28	>1 28	>1 28		
846	DW D	06-11-10	Pus	17	M	EC	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	S	S	S	S		12 8	>1 28	>1 28		
847	DW D	06-11-10	Vag Swab	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	I	S	S	S		8	32	64	CTX-M-15	
848	DW D	06-11-10	Pus	83	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	R	S	S	S		8	64	64		
849	DW D	06-11-10	Pus	75	M	EC	R	R	R	R	P	N		A	Am pC	R	I	I	I	R	R	R	S		>1 28	>1 28	>1 28		
850	DW D	06-11-10	Pus	10	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	R	S	P	64	>1 28	>1 28		
851	DW D	12-01-11	Throat swa	57	M	KP	S	S	S	S	N				SUS C														
852	DW D	12-01-11	Pus	50	M	KP	S	S	S	S	N				SUS C														
853	DW D	12-01-11	Pus	30	M	KP	S	S	S	S	N				SUS C														
854	DW D	12-01-11	Urine	23	F	EC	S	S	S	S	N				SUS C														
855	DW D	12-01-11	ET Tube	45	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S	P	>1 28	>1 28	>1 28		
856	DW D	12-01-11	Urine	66	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	S	S	S	S	S	S	S	P	12 8	32	32	TEM-1, SHV-12	

[illegible]

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897	DW D	30-04- 11	Urine	18	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S	P	64	12 8	>1 28	
898	DW D	30-04- 11	Pus	49	M	EC	S	S	S	S	N				SUS C													
899	DW D	30-04- 11	Sputum	72	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S	P	16	32	64	CTX-M-15
900	DW D	30-04- 11	Urine	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S		64	>1 28	>1 28	CTX-M-15
901	DW D	30-04- 11	Urine	26	F	EC	S	S	S	S	N				SUS C													
902	DW D	30-04- 11	Urine	36	F	EC	R	R	R	R	P	N		A	Am pC	R	R	I	R	R	R	R	S	P	>1 28	>1 28	>1 28	
903	DW D	30-04- 11	Urine	30	F	EC	S	S	S	S	N				SUS C													
904	DW D	30-04- 11	Urine	45	F	EC	S	S	S	S	N				SUS C													
905	DW D	30-04- 11	Pus	60	M	EC	S	S	S	S	N				SUS C													
906	DW D	30-04- 11	Cerv Swab	34	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	R	S	P	>1 28	>1 28	>1 28	
907	DW D	30-04- 11	Pus	47	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	64	>1 28	>1 28	CTX-M-15
908	DW D	30-04- 11	Pus	14	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	64	>1 28	>1 28	
909	DW D	30-04- 11	Pus	40	M	EC	R	R	R	R	P	N		EA	COP R	R	R	R	I	R	R	R	S	P	>1 28	>1 28	>1 28	
910	DW D	30-04- 11	Pus	70	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	I	S	P	64	12 8	>1 28	
911	DW D	30-04- 11	Pus	45	F	EC	S	S	S	S	N				SUS C													
912	DW D	07-05- 11	Blood	84	M	EC	S	S	S	S	N				SUS C													
913	DW D	07-05- 11	Blood	1	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	R	S	S	S	P	12 8	>1 28	>1 28	
914	DW D	07-05- 11	Blood	1	M	EC	S	S	S	S	N				SUS C													
915	DW D	07-05- 11	Blood	50	M	EC	S	S	S	S	N				SUS C													
916	DW D	07-05- 11	Urine	71	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	I	R	S	P	>1 28	>1 28	>1 28	

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937	DW D	07-05- 11	Urine	70	F	EC	S	S	S	S	N				SUS C														
938	DW D	07-05- 11	Asci Fluid	70	M	EC	S	S	S	S	N				SUS C														
939	DW D	07-05- 11	Pus	72	M	EC	R	R	R	R	P	N		A	Am pC	R	R	I	R	R	R	R	S	P	>1 28	>1 28	>1 28		
940	DW D	07-05- 11	Vag Swab	80	F	EC	S	S	S	S	N				SUS C														
941	DW D	07-05- 11	Pus	35	M	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	S		>1 28	>1 28	>1 28		
942	DW D	07-05- 11	Urine	24	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	I	S	P	>1 28	>1 28	>1 28		
943	DW D	07-05- 11	Urine	42	F	EC	S	S	S	S	N				SUS C														
944	DW D	07-05- 11	Urine	61	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	R	S	P	12 8	>1 28	>1 28	CTX-M-15	
945	DW D	07-05- 11	Urine	30	F	EC	S	S	S	S	N				SUS C														
946	DW D	07-05- 11	Asci Fluid	1	M	EC	S	S	S	S	N				SUS C														
947	DW D	07-05- 11	Urine	25	F	EC	S	S	S	S	N				SUS C														
948	DW D	07-05- 11	Urine	32	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	S	R	R	R	S	P	>1 28	>1 28	>1 28		
949	DW D	07-05- 11	Urine	65	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	64	>1 28	>1 28	CTX-M-15	
950	DW D	07-05- 11	Urine	50	F	EC	S	S	S	S	N				SUS C														
951	DW D	07-05- 11	Urine	22	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	I	S	P	>1 28	>1 28	>1 28	CTX-M-15	
952	DW D	07-05- 11	Urine	50	F	EC	S	S	S	S	N				SUS C														
953	DW D	07-05- 11	Urine	21	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	R	S	P	>1 28	>1 28	>1 28		
954	DW D	07-05- 11	Urine	42	F	EC	S	S	S	S	N				SUS C														
955	DW D	07-05- 11	Urine	19	F	EC	S	S	S	S	N				SUS C														
956	DW D	07-05- 11	Pus	33	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	S	R	R	R	S	P	>1 28	>1 28	>1 28		

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[illegible]

10 37	DW D	10-07- 11	Pus	25	F	KP	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
10 38	DW D	10-07- 11	Sputum	58	M	KP	S	S	S	S	N				SUS C														
10 39	DW D	10-07- 11	Vag Swab	20	F	KP	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
10 40	DW D	10-07- 11	ET Tube	23	M	KP	S	S	S	S	N				SUS C														
10 41	DW D	10-07- 11	ET Tube	57	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	S	S	S	S	P	64	>1 28	>1 28		
10 42	DW D	10-07- 11	Throat swa	28	M	KP	S	S	S	S	N				SUS C														
10 43	DW D	10-07- 11	Pus	40	M	KP	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
10 44	DW D	10-07- 11	Suct tip	38	F	KP	S	S	S	S	N				SUS C														
10 45	DW D	10-07- 11	Pus	20	M	KP	S	S	S	S	N				SUS C														
10 46	DW D	10-07- 11	Throat swa	40	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	S	S	I	S		64	>1 28	>1 28		
10 47	DW D	10-07- 11	Pus	80	F	KP	S	S	S	S	N				SUS C														
10 48	DW D	10-07- 11	ET Tube	75	M	KP	S	S	S	S	N				SUS C														
10 49	DW D	10-07- 11	Pus	27	M	KP	S	S	S	S	N				SUS C														
10 50	DW D	10-07- 11	Sputum	61	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	I	I	R	S		>1 28	>1 28	>1 28		
10 51	DW D	10-07- 11	Pus	64	M	KP	S	S	S	S	N				SUS C														
10 52	DW D	10-07- 11	Pus	41	M	KP	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
10 53	DW D	10-07- 11	Sputum	38	M	KP	S	S	S	S	N				SUS C														
10 54	DW D	10-07- 11	Vag Swab	36	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	I	S	I	S	P	64	>1 28	>1 28		
10 55	DW D	10-07- 11	Pus	6	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S	P	>1 28	>1 28	>1 28		
10 56	DW D	10-07- 11	Throat swa	52	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	I	S	I	R	R	S	P	>1 28	>1 28	>1 28		

1057	DW D	10-07-11	Pus	54	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	S	S	R	S	P	128	>128	>128	
1058	DW D	10-07-11	Pus	60	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	S	S	R	S	P	128	>128	>128	CTX-M-15
1059	DW D	10-07-11	Pus	19	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	S	S	R	S	P	128	>128	>128	CTX-M-15
1060	DW D	10-07-11	Pus	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	S	S	S	S		32	64	>128	
1061	DW D	10-07-11	Sputum	40	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	S	I	R	S	P	128	>128	>128	CTX-M-15
1062	DW D	10-07-11	Pus	67	M	EC	S	S	S	S	N				SUS C													
1063	DW D	10-07-11	Pus	48	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	S	S	I	S	P	32	>128	>128	CTX-M-15
1064	DW D	10-07-11	Pus	26	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	S	I	I	R	S	P	>128	>128	>128	CTX-M-15
1065	DW D	10-07-11	Vag Swab	50	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	R	S		128	>128	>128	TEM-1, CTX-M-15
1066	DW D	10-07-11	Pus	19	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S		128	8	64	
1067	DW D	10-07-11	Asci Fluid	42	F	EC	S	S	S	S	N				SUS C													
1068	DW D	10-07-11	Pus	58	M	EC	S	S	S	S	N				SUS C													
1069	DW D	10-07-11	Pus	55	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	R	S	P	64	128	>128	CTX-M-15
1070	DW D	10-07-11	Pus	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	I	R	S		32	>128	>128	CTX-M-15
1071	DW D	10-07-11	Pus	4	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	I	S		64	>128	>128	CTX-M-15
1072	DW D	10-07-11	Pus	73	M	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	R		>128	>128	>128	
1073	DW D	10-07-11	Pus	74	M	EC	S	S	S	S	N				SUS C													
1074	DW D	10-07-11	Pus	21	F	EC	S	S	S	S	N				SUS C													
1075	DW D	10-07-11	Pus	54	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	I	I	R	S	P	32	>128	>128	
1076	DW D	10-07-11	ET Tube	42	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	I	S	P	32	>128	>128	CTX-M-15

[illegible]

10 97	DW D	24-11- 11	Pus	72	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	I	R	R	S		>1 28	>1 28	>1 28	
10 98	DW D	24-11- 11	Pus	51	M	KP	S	S	S	S	N				SUS C													
10 99	DW D	24-11- 11	Throat swa	17	M	KP	S	S	S	S	N				SUS C													
11 00	DW D	24-11- 11	ET Tube	33	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
11 01	DW D	24-11- 11	Pus	45	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
11 02	DW D	24-11- 11	Pus	65	F	KP	R	R	R	R	P	N		A	Am pC	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
11 03	DW D	24-11- 11	Pus	33	F	KP	S	S	S	S	N				SUS C													
11 04	DW D	24-11- 11	ET Tube	18	M	KP	S	S	S	S	N				SUS C													
11 05	DW D	24-11- 11	Sputum	70	M	KP	S	S	S	S	N				SUS C													
11 06	DW D	24-11- 11	Suct tip	33	M	KP	S	S	S	S	N				SUS C													
11 07	DW D	24-11- 11	Sputum	1	M	KP	S	S	S	S	N				SUS C													
11 08	DW D	24-11- 11	ET Tube	70	F	KP	S	S	S	S	N				SUS C													
11 09	DW D	24-11- 11	ET Tube	31	F	KP	S	S	S	S	N				SUS C													
11 10	DW D	24-11- 11	Pus	66	M	KP	S	S	S	S	N				SUS C													
11 11	DW D	24-11- 11	Pus	29	M	KP	S	S	S	S	N				SUS C													
11 12	DW D	24-11- 11	Pus	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	I	I	I	S		>1 28	>1 28	>1 28	
11 13	DW D	24-11- 11	Throat swa	22	F	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
11 14	DW D	24-11- 11	Pus	60	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R	P	>1 28	>1 28	>1 28	
11 15	DW D	24-11- 11	ET Tube	45	M	KP	S	S	S	S	N				SUS C													
11 16	DW D	24-11- 11	Throat swa	45	F	KP	R	R	R	R	P	N		A	Am pC	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	

11 17	DW D	24-11- 11	ET Tube	24	M	KP	S	S	S	S	N				SUS C													
11 18	DW D	24-11- 11	Throat swa	1	F	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	S	R	R	S		12 8	64	64	
11 19	DW D	24-11- 11	Pus	72	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	I	R	R	R	S		>1 28	>1 28	>1 28	
11 20	DW D	24-11- 11	Sputum	44	F	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
11 21	DW D	24-11- 11	Sputum	43	M	KP	S	S	S	S	N				SUS C													
11 22	DW D	24-11- 11	Pus	22	F	KP	S	S	S	S	N				SUS C													
11 23	DW D	24-11- 11	Sputum	35	M	KP	S	S	S	S	N				SUS C													
11 24	DW D	24-11- 11	Sputum	43	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	I	I	R	S		12 8	>1 28	>1 28	SHV-1, CTX-M-15
11 25	DW D	24-11- 11	Pus	67	M	KP	S	S	S	S	N				SUS C													
11 26	DW D	24-11- 11	ET Tube	30	F	KP	S	S	S	S	N				SUS C													
11 27	DW D	24-11- 11	Sputum	33	M	KP	S	S	S	S	N				SUS C													
11 28	DW D	24-11- 11	Pus	40	F	KP	S	S	S	S	N				SUS C													
11 29	DW D	24-11- 11	ET Tube	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	I	I	R	S		12 8	>1 28	>1 28	
11 30	DW D	24-11- 11	Pus	34	M	KP	S	S	S	S	N				SUS C													
11 31	DW D	24-11- 11	Pus	18	M	KP	S	S	S	S	N				SUS C													
11 32	DW D	24-11- 11	Pus	58	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	S	S	I	S	P	>1 28	>1 28	>1 28	
11 33	DW D	24-11- 11	Sputum	70	F	KP	S	S	S	S	N				SUS C													
11 34	DW D	24-11- 11	Sputum	2	F	KP	S	S	S	S	N				SUS C													
11 35	DW D	24-11- 11	Sputum	31	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	I	I	I	S	P	>1 28	>1 28	>1 28	
11 36	DW D	24-11- 11	Pus	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	I	I	R	S	P	>1 28	>1 28	>1 28	

11 37	DW D	24-11- 11	Sputum	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S	P	64	>1 28	>1 28	
11 38	DW D	01-07- 12	Urine	80	M	KP	S	S	S	S	N				SUS C													
11 39	DW D	01-07- 12	Urine	55	F	KP	S	S	S	S	N				SUS C													
11 40	DW D	01-07- 12	Urine	70	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		64	>1 28	12 8	TEM-1, SHV-11, CTX- M-15
11 41	DW D	01-07- 12	Urine	64	M	KP	S	S	S	S	N				SUS C													
11 42	DW D	01-07- 12	Sputum	60	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		12 8	>1 28	>1 28	
11 43	DW D	01-07- 12	Pus	40	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	P		>1 28	>1 28	>1 28	TEM-1, SHV-11, CTX- M-15
11 44	DW D	01-07- 12	Pus	58	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R						
11 45	DW D	01-07- 12	ET Tube	42	M	KP	S	S	S	S	N				SUS C													
11 46	DW D	01-07- 12	Pus	18	M	KP	S	S	S	S	N				SUS C													
11 47	DW D	01-07- 12	Pus	30	M	KP	S	S	S	S	N				SUS C													
11 48	DW D	01-07- 12	Pus	50	M	KP	S	S	S	S	N				SUS C													
11 49	DW D	01-07- 12	ET Tube	40	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	R	I	I	S		32	16	64	
11 50	DW D	01-07- 12	Pus	55	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		12 8	>1 28	>1 28	TEM-1, SHV-11, CTX- M-15
11 51	DW D	01-07- 12	Pus	84	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	TEM-1, SHV-11, CTX- M-15
11 52	DW D	01-07- 12	ET Tube	48	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R						
11 53	DW D	01-07- 12	Pus	58	M	KP	S	S	S	S	N				SUS C													
11 54	DW D	01-07- 12	Asci Fluid	45	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	I	I	R	S	P	64	12 8	12 8	CTX-M-15
11 55	DW D	01-07- 12	Sputum	40	M	KP	S	S	S	S	N				SUS C													
11 56	DW D	01-07- 12	Sputum	60	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R						

11 57	DW D	01-07- 12	Pus	47	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	R	I	R	S		64	>1 28	>1 28	TEM-1, SHV-108, CTX-M-15
11 58	DW D	01-07- 12	Pus	50	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	R	I	I	S	P	64	>1 28	>1 28	TEM-1, SHV-108, CTX-M-15
11 59	DW D	01-07- 12	Pus	1	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	R	R	R	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
11 60	DW D	01-07- 12	Sputum	70	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	I	S	I	S	P	64	>1 28	>1 28	TEM-1, SHV-11, CTX- M-15
11 61	DW D	01-07- 12	Pus	59	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
11 62	DW D	01-07- 12	Pleural fl	45	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S	P	12 8	>1 28	>1 28	TEM-1, SHV-168, CTX-M-15
11 63	DW D	01-07- 12	ET Tube	21	M	KP	S	S	S	S	N				SUS C													
11 64	DW D	01-07- 12	ET Tube	27	M	KP	S	S	S	S	N				SUS C													
11 65	DW D	01-07- 12	Pus	47	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	S	S	S	S	P	32	12 8	64	TEM-1, SHV-28, CTX- M-15
11 66	DW D	01-07- 12	Pus	51	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
11 67	DW D	01-07- 12	ET Tube	55	F	KP	S	S	S	S	N				SUS C													
11 68	DW D	01-07- 12	Pus	47	M	KP	S	S	S	S	N				SUS C													
11 69	DW D	01-07- 12	Sputum	55	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
11 70	DW D	01-07- 12	Pus	59	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
11 71	DW D	01-07- 12	ET Tube	40	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	I	I	R	S	P	64	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
11 72	DW D	01-07- 12	ET Tube	65	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
11 73	DW D	01-07- 12	Pus	47	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-168, CTX-M-15
11 74	DW D	01-07- 12	Sputum	62	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
11 75	DW D	01-07- 12	ET Tube	19	M	KP	S	S	S	S	N				SUS C													
11 76	DW D	01-07- 12	Pus	63	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					

11 77	DW D	01-07- 12	Sputum	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	S	I	R	S	P	32	>1 28	12 8	TEM-1, SHV-108, CTX-M-15
11 78	DW D	01-07- 12	Pus	19	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	SHV-11, CTX-M-15
11 79	DW D	01-07- 12	Sputum	15	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	TEM-1, SHV-11, CTX- M-156
11 80	DW D	01-07- 12	Pus	12	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	I	I	R	S	P	64	12 8	12 8	TEM-1, SHV-108, CTX-M-15
11 81	DW D	01-07- 12	Sputum	45	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	I	S	I	S	P	32	>1 28	>1 28	TEM-1, SHV-108, CTX-M-15
11 82	DW D	01-07- 12	Pus	12	F	KP	S	S	S	S	N				SUS C													
11 83	DW D	01-07- 12	Pus	24	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R	P	>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
11 84	DW D	01-07- 12	Pus	33	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	R	R	R	S		12 8	>1 28	>1 28	
11 85	DW D	01-07- 12	Urine	30	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
11 86	DW D	01-07- 12	ET Tube	18	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	I	S	P	32	12 8	64	TEM-1, SHV-11, CTX- M-15
11 87	DW D	01-07- 12	Pus	50	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
11 88	DW D	01-07- 12	Pus	55	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
11 89	DW D	01-07- 12	Pus	65	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	R	I	S	S		32	32	12 8	
11 90	DW D	01-07- 12	Pus	35	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		12 8	12 8	12 8	
11 91	DW D	01-07- 12	ET Tube	55	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	R	I	S	S	P	32	>1 28	>1 28	SHV-108, CTX-M-15
11 92	DW D	01-07- 12	Pus	27	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	I	S	R	S	P	32	>1 28	12 8	TEM-1, SHV-108, CTX-M-15
11 93	DW D	01-07- 12	Pus	66	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
11 94	DW D	01-07- 12	Pus	65	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	I	S	S		64	32	64	TEM-1, SHV-28, CTX- M-15
11 95	DW D	01-07- 12	Pus	32	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	P		>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
11 96	DW D	01-07- 12	ET Tube	59	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		>1 28	>1 28	12 8	

11 97	DW D	01-07- 12	Pus	30	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R							
11 98	DW D	01-07- 12	Pus	56	M	KP	S	S	S	S	N				SUS C															
11 99	DW D	01-07- 12	Pus	46	F	KP	S	S	S	S	N				SUS C															
12 00	DW D	01-07- 12	Pus	85	M	KP	S	S	S	S	N				SUS C															
12 01	KO L	30-10- 10	Urine	35	F	EC	R	R	R	R	P	P	Ca		ESB L	R	S	S	S	S	R	R	R	S	P	12 8	32	32		
12 02	KO L	30-10- 10	Pus	28	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	I	S	S	S	P	16	64	64		
12 03	KO L	30-10- 10	Urine	8	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	S	I	S	S	S	P	64	12 8	64	CTX-M-15	
12 04	KO L	30-10- 10	Urine	40	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	S	S	S	S			16	64	64		
12 05	KO L	30-10- 10	Urine	90	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	R	I	R	S			>1 28	>1 28	>1 28		
12 06	KO L	30-10- 10	Pus	27	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	S	S	S	S	P		16	32	64		
12 07	KO L	30-10- 10	Pus	3	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	S	S	S	S	P		16	32	32		
12 08	KO L	30-10- 10	Pus	77	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	I	S	S	S		32	12 8	12 8	CTX-M-15	
12 09	KO L	30-10- 10	Pus	75	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S			16	64	64		
12 10	KO L	30-10- 10	Asci Fluid	24	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	I	S	S	S	P		12 8	12 8	>1 28	CTX-M-15
12 11	KO L	30-10- 10	Urine	45	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	I	S	S	S			64	>1 28	>1 28	SHV-?
12 12	KO L	30-10- 10	Urine	23	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	I	S	S	S			16	64	64	CTX-M-15
12 13	KO L	30-10- 10	Pus	53	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	I	S	S	S			32	12 8	64	CTX-M-15
12 14	KO L	30-10- 10	Pus	46	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	I	S	S	S			64	>1 28	>1 28	CTX-M-15
12 15	KO L	30-10- 10	Urine	20	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	I	S	S	S			32	64	12 8	
12 16	KO L	30-10- 10	Urine	11	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	S	S			32	>1 28	12 8	

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12 57	KO L	30-10- 10	Urine	28	M	EC	S	S	S	S	N				SUS C														
12 58	KO L	30-10- 10	Urine	28	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	32	64	64		
12 59	KO L	30-10- 10	Urine	59	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	R	S	S	S	P	32	12 8	12 8	CTX-M-15	
12 60	KO L	30-10- 10	Pleural fl	1	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S		32	>1 28	>1 28	CTX-M-15	
12 61	KO L	30-10- 10	Asci Fluid	23	M	EC	R	R	R	R	P	N		A	Am pC	R	R	S	S	R	R	R	S	P	>1 28	>1 28	>1 28		
12 62	KO L	30-10- 10	Urine	36	F	EC	R	R	R	R	P	N		A	Am pC	R	R	I	S	R	R	R	S		>1 28	12 8	>1 28		
12 63	KO L	30-10- 10	Urine	40	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S	P	16	64	64		
12 64	KO L	30-10- 10	Urine	37	M	EC	R	R	R	R	P	N		A	Am pC	R	R	I	I	R	R	R	S		>1 28	>1 28	>1 28		
12 65	KO L	30-10- 10	Urine	63	M	EC	R	R	R	R	P	N		A	Am pC	R	R	I	S	R	R	R	S		>1 28	>1 28	>1 28		
12 66	KO L	30-10- 10	Urine	45	F	EC	R	R	R	R	P	N		A	Am pC	R	R	I	I	R	R	R	S	P	>1 28	>1 28	>1 28		
12 67	KO L	30-10- 10	Urine	44	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	I	S	S	S	P	32	>1 28	>1 28		
12 68	KO L	30-10- 10	Urine	22	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	I	S	R	S	I	S		64	>1 28	>1 28		
12 69	KO L	30-10- 10	Urine	34	M	EC	S	S	S	S	N				SUS C														
12 70	KO L	30-10- 10	Urine	45	F	EC	S	S	S	S	N				SUS C														
12 71	KO L	30-10- 10	Urine	60	F	EC	S	S	S	S	N				SUS C														
12 72	KO L	30-10- 10	Urine	20	F	EC	S	S	S	S	N				SUS C														
12 73	KO L	30-10- 10	Urine	40	M	EC	S	S	S	S	N				SUS C														
12 74	KO L	30-10- 10	Urine	72	F	EC	S	S	S	S	N				SUS C														
12 75	KO L	30-10- 10	Urine	60	M	EC	S	S	S	S	N				SUS C														
12 76	KO L	30-10- 10	Pus	47	F	EC	R	R	R	R	P	N		EA	COP R	R	R	I	I	R	R	R	S		>1 28	>1 28	>1 28		

1277	KO L	30-10-10	Pus	65	F	EC	R	R	R	R	P	N		A	Am pC	R	R	I	I	R	R	R	S	P	>128	>128	>128	
1278	KO L	30-10-10	Pus	47	M	EC	S	S	S	S	N				SUS C													
1279	KO L	30-10-10	Pus	32	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S	P	32	>128	128	
1280	KO L	30-10-10	Pus	50	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S	P	32	>128	>128	
1281	KO L	30-10-10	Pus	48	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	32	>128	>128	
1282	KO L	24-03-11	Throat swa	2	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	>128	>128	>128	
1283	KO L	24-03-11	Pus	58	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	>128	>128	>128	
1284	KO L	24-03-11	Pus	60	M	KP	S	S	S	S	N				SUS C													
1285	KO L	24-03-11	Pus	7	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	>128	>128	>128	
1286	KO L	24-03-11	Urine	28	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S	P	>128	>128	>128	
1287	KO L	24-03-11	Sputum	60	F	KP	S	S	S	S	N				SUS C													
1288	KO L	24-03-11	Urine	60	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	>128	>128	>128	
1289	KO L	24-03-11	Blood	1	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S	P	>128	>128	>128	
1290	KO L	24-03-11	Urine	18	M	KP	S	S	S	S	N				SUS C													
1291	KO L	24-03-11	Pus	36	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	>128	>128	>128	
1292	KO L	24-03-11	Sputum	45	M	KP	S	S	S	S	N				SUS C													
1293	KO L	24-03-11	Urine	39	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S	P	>128	>128	>128	
1294	KO L	24-03-11	Urine	80	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	I	S	P	>128	>128	>128	
1295	KO L	24-03-11	Blood	1	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	>128	>128	>128	
1296	KO L	24-03-11	ET Tube	10	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	>128	>128	>128	

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13 17	KO L	14-04- 11	Blood	1	F	KP	S	S	S	S	N				SUS C															
13 18	KO L	14-04- 11	Pus	60	F	KP	S	S	S	S	N				SUS C															
13 19	KO L	14-04- 11	Sputum	80	M	KP	S	S	S	S	N				SUS C															
13 20	KO L	14-04- 11	Sputum	31	M	KP	S	S	S	S	N				SUS C															
13 21	KO L	14-04- 11	Blood	8	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	I	S	S	S	P	>1 28	>1 28	>1 28			
13 22	KO L	14-04- 11	Blood	2	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	I	I	I	S	P	>1 28	>1 28	>1 28			
13 23	KO L	14-04- 11	Urine	93	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	R	I	I	S	P	>1 28	>1 28	>1 28			
13 24	KO L	14-04- 11	Pus	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	R	I	R	I	I	S	P	>1 28	>1 28	>1 28			
13 25	KO L	14-04- 11	Urine	53	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	R	S	P	>1 28	>1 28	>1 28			
13 26	KO L	14-04- 11	Pus	6	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	R	I	I	S		>1 28	>1 28	>1 28	SHV-11, CTX-M-15		
13 27	KO L	14-04- 11	Urine	75	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	R	I	I	S		>1 28	>1 28	>1 28	SHV-18, CTX-M-15		
13 28	KO L	14-04- 11	Pus	35	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	I	I	I	S	P	>1 28	>1 28	>1 28			
13 29	KO L	14-04- 11	Urine	62	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	R	S	R	I	I	S		>1 28	>1 28	>1 28			
13 30	KO L	14-04- 11	Urine	3	M	KP	S	S	S	S	N				SUS C															
13 31	KO L	14-04- 11	Urine	4	M	KP	S	S	S	S	N				SUS C															
13 32	KO L	14-04- 11	Pus	56	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	I	S	I	S		>1 28	>1 28	>1 28	SHV-11, CTX-M-15		
13 33	KO L	19-04- 11	Urine	38	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S	P	>1 28	>1 28	>1 28			
13 34	KO L	19-04- 11	Urine	80	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	R	I	R	S	P	>1 28	>1 28	>1 28			
13 35	KO L	19-04- 11	Urine	62	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	I	S	P	>1 28	>1 28	>1 28			
13 36	KO L	19-04- 11	Urine	11	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	S	R	S	P	>1 28	>1 28	>1 28			

[illegible]

[illegible]

13 77	KO L	19-04- 11	Urine	9	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S		>1 28	>1 28	>1 28	CTX-M-15
13 78	KO L	19-04- 11	Urine	78	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	I	S		32	16	16	CTX-M-15
13 79	KO L	19-04- 11	Urine	73	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S	P	>1 28	>1 28	>1 28	CTX-M-15
13 80	KO L	19-04- 11	Urine	50	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	R	S	P	>1 28	>1 28	>1 28	
13 81	KO L	19-04- 11	Urine	73	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S	P	>1 28	>1 28	>1 28	CTX-M-15
13 82	KO L	19-04- 11	Urine	4	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	R	S	P	>1 28	>1 28	>1 28	CTX-M-15
13 83	KO L	19-04- 11	Urine	42	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S	P	>1 28	>1 28	>1 28	CTX-M-15
13 84	KO L	19-04- 11	Urine	50	F	EC	S	S	S	S	N				SUS C													
13 85	KO L	19-04- 11	Urine	23	F	EC	S	S	S	S	N				SUS C													
13 86	KO L	19-04- 11	Urine	65	F	EC	S	S	S	S	N				SUS C													
13 87	KO L	19-04- 11	Urine	70	M	EC	S	S	S	S	N				SUS C													
13 88	KO L	19-04- 11	Urine	4	F	EC	S	S	S	S	N				SUS C													
13 89	KO L	19-04- 11	Urine	20	F	EC	S	S	S	S	N				SUS C													
13 90	KO L	19-04- 11	Pus	28	M	EC	S	S	S	S	N				SUS C													
13 91	KO L	19-04- 11	Pus	13	F	EC	S	S	S	S	N				SUS C													
13 92	KO L	19-04- 11	Pus	32	F	EC	S	S	S	S	N				SUS C													
13 93	KO L	19-04- 11	Pus	45	M	EC	S	S	S	S	N				SUS C													
13 94	KO L	19-04- 11	Pus	38	F	EC	S	S	S	S	N				SUS C													
13 95	KO L	24-04- 11	Urine	28	M	KP	S	S	S	S	N				SUS C													
13 96	KO L	24-04- 11	Urine	7	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	R	S	P	>1 28	>1 28	>1 28	

[illegible]

14 17	KO L	24-04- 11	Pus	46	M	EC	S	S	S	S	N				SUS C														
14 18	KO L	24-04- 11	Pus	50	M	EC	S	S	S	S	N				SUS C														
14 19	KO L	24-04- 11	Pus	19	M	EC	S	S	S	S	N				SUS C														
14 20	KO L	24-04- 11	Pus	18	F	EC	S	S	S	S	N				SUS C														
14 21	KO L	24-04- 11	Pus	25	M	EC	S	S	S	S	N				SUS C														
14 22	KO L	24-04- 11	Pus	50	F	EC	S	S	S	S	N				SUS C														
14 23	KO L	24-04- 11	Pus	25	M	EC	S	S	S	S	N				SUS C														
14 24	KO L	24-04- 11	Pus	65	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	I	S	P	>1 28	>1 28	>1 28	CTX-M-15	
14 25	KO L	24-04- 11	Pus	37	M	EC	S	S	S	S	N				SUS C														
14 26	KO L	24-04- 11	Pus	21	F	EC	S	S	S	S	N				SUS C														
14 27	KO L	24-04- 11	Blood	38	M	EC	S	S	S	S	N				SUS C														
14 28	KO L	24-04- 11	Blood	38	M	EC	S	S	S	S	N				SUS C														
14 29	KO L	24-04- 11	Urine	52	M	EC	S	S	S	S	N				SUS C														
14 30	KO L	24-04- 11	Urine	32	F	EC	S	S	S	S	N				SUS C														
14 31	KO L	24-04- 11	Urine	35	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S	P	>1 28	>1 28	>1 28	CTX-M-15	
14 32	KO L	24-04- 11	Pleural fl	45	M	EC	S	S	S	S	N				SUS C														
14 33	KO L	24-04- 11	Asci Fluid	32	F	EC	S	S	S	S	N				SUS C														
14 34	KO L	24-04- 11	Pus	33	M	EC	S	S	S	S	N				SUS C														
14 35	KO L	24-04- 11	Pus	35	M	EC	S	S	S	S	N				SUS C														
14 36	KO L	30-04- 11	Urine	24	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	I	R	R	R	S		>1 28	>1 28	>1 28		

[illegible]

[illegible]

[illegible]

[illegible]

15 17	KO L	10-10- 12	Pus	51	M	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	R					
15 18	KO L	10-10- 12	Pus	18	M	KP	S	S	S	S	N				SUS C														
15 19	KO L	10-10- 12	Pus	60	F	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	R					
15 20	KO L	10-10- 12	Pus	87	M	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	R					
15 21	KO L	10-10- 12	Pus	60	M	KP	S	S	S	S	N				SUS C														
15 22	KO L	10-10- 12	Urine	20	F	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	R					
15 23	KO L	10-10- 12	Pus	44	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX- M-15
15 24	KO L	10-10- 12	Urine	11	F	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	R					
15 25	KO L	10-10- 12	Pus	18	F	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	S					
15 26	KO L	10-10- 12	Pus	22	F	KP	S	S	S	S	N				SUS C														
15 27	KO L	10-10- 12	Pus	40	M	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	R					
15 28	KO L	10-10- 12	Pus	60	M	KP	S	S	S	S	N				SUS C														
15 29	KO L	10-10- 12	Pus	48	F	KP	S	R	S	S	P	N			UN KN			R	R	R	R	R	R	R					
15 30	KO L	10-10- 12	Pus	23	F	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	R					
15 31	KO L	10-10- 12	Blood	4	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	S	S		12 8	>1 28	>1 28		
15 32	KO L	10-10- 12	Urine	42	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	R	S		32	12 8	>1 28	TEM-1, SHV-185, CTX-M-15	
15 33	KO L	10-10- 12	Pus	43	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	I	S		32	>1 28	>1 28		
15 34	KO L	10-10- 12	Pus	65	F	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	S					
15 35	KO L	10-10- 12	Pus	32	M	KP	R	R	R	R	P	N			UN KN			R	R	R	R	R	R	R					
15 36	KO L	10-10- 12	Pus	65	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	I	R	S	P	16	64	12 8		

15 37	KO L	10-10- 12	Pus	55	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	I	S		32	12 8	12 8	
15 38	KO L	10-10- 12	Pus	60	M	KP	S	S	S	S	N				SUS C													
15 39	KO L	10-10- 12	Pus	28	F	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
15 40	KO L	10-10- 12	Pus	46	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	S	S		64	>1 28	>1 28	
15 41	KO L	10-10- 12	Urine	68	M	KP	S	S	S	S	N				SUS C													
15 42	KO L	10-10- 12	Blood	1	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S	P	32	64	12 8	
15 43	KO L	10-10- 12	Urine	27	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	I	S	P	12 8	>1 28	>1 28	
15 44	KO L	10-10- 12	Urine	76	M	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
15 45	KO L	10-10- 12	Urine	85	F	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
15 46	KO L	10-10- 12	Urine	12	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	I	S	P	32	12 8	>1 28	
15 47	KO L	10-10- 12	Urine	60	F	KP	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R					
15 48	KO L	10-10- 12	Urine	60	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-185, CTX-M-15
15 49	KO L	10-10- 12	Urine	54	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	S	P	12 8	12 8	>1 28	
15 50	KO L	10-10- 12	Sputum	40	M	KP	S	S	S	S	N				SUS C													
15 51	KO L	10-10- 12	Sputum	67	M	KP	S	S	S	S	N				SUS C													
15 52	KO L	10-10- 12	Urine	68	M	KP	S	R	S	S	P	N			UN KN		R	R	R	R	R	R	R					
15 53	KO L	10-10- 12	Pus	40	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-61, CTX- M-15
15 54	KO L	10-10- 12	Pus	45	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	R	S	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX- M-15
15 55	KO L	10-10- 12	Urine	55	F	KP	R	R	S	R	P	N			UN KN		S	S	S	I	S	S	S					
15 56	KO L	10-10- 12	Vag Swab	22	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S		>1 28	>1 28	>1 28	TEM-1, SHV-?, CTX- M-156

[illegible]

1577	KO L	14-01-13	ET Tube	75	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	R	S	P	32	>1 28	>1 28	TEM-1, SHV-11, CTX-M-15
1578	KO L	14-01-13	ET Tube	36	F	KP	S	S	S	S	N				SUS C													
1579	KO L	14-01-13	Blood	21	F	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	R	R	R	S		32	8	32	SHV-27
1580	KO L	14-01-13	Pus	23	M	KP	S	S	S	S	N				SUS C													
1581	KO L	14-01-13	Pus	33	F	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	R	R	R	S		>1 28	8	64	SHV-27
1582	KO L	14-01-13	Pus	36	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S		32	>1 28	>1 28	SHV-1, CTX-M-15
1583	KO L	14-01-13	Sputum	47	M	KP	R	R	R	R	P	N		A	Am pC	R	S	S	S	R	R	R	S		>1 28	>1 28	>1 28	
1584	KO L	14-01-13	ET Tube	61	M	KP	S	S	S	S	N				SUS C													
1585	KO L	14-01-13	ET Tube	35	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S	P	32	>1 28	>1 28	TEM-1, CTX-M-15
1586	KO L	14-01-13	ET Tube	55	M	KP	S	S	S	S	N				SUS C													
1587	KO L	14-01-13	Pus	25	F	KP	S	S	S	S	N				SUS C													
1588	KO L	14-01-13	Sputum	75	M	KP	R	R	R	R	P	N			UN KN		R	S	S	I	S	S	S					
1589	KO L	14-01-13	Asci Fluid	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	I	I	R	S	P	32	>1 28	>1 28	CTX-M-15
1590	KO L	14-01-13	Pus	63	F	KP	S	S	S	S	N				SUS C													
1591	KO L	14-01-13	Asci Fluid	63	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S	P	32	>1 28	>1 28	TEM-1, SHV-?, CTX-M-15
1592	KO L	14-01-13	ET Tube	55	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-11, CTX-M-15
1593	KO L	14-01-13	Sputum	76	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S	P	32	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15
1594	KO L	14-01-13	Pus	40	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	I	S	I	I	R	S	P	32	>1 28	>1 28	TEM-1, SHV-11, CTX-M-15
1595	KO L	14-01-13	Urine	45	M	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	TEM-?,SHV-1, CTX-M-15
1596	KO L	14-01-13	ET Tube	35	M	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	I	R	R	S		>1 28	8	64	SHV-27

15 97	KO L	14-01- 13	Pus	53	M	KP	S	S	S	S	N				SUS C														
15 98	KO L	14-01- 13	Pus	19	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	S	R	I	R	S		32	>1 28	>1 28	TEM-1, SHV-11, CTX- M-15	
15 99	KO L	14-01- 13	Suct tip	55	M	KP	S	S	S	S	N				SUS C														
16 00	KO L	14-01- 13	Sputum	60	F	KP	S	S	S	S	N				SUS C														
16 01	MN G	08-08- 10	Urine	4	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	I	R	I	I	S		32	>1 28	>1 28		
16 02	MN G	08-08- 10	Urine	50	M	EC	R	R	R	R	P	N			UN KN		R	R	R	R	R	R	R						
16 03	MN G	08-08- 10	Urine	21	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		>1 28	>1 28	>1 28		
16 04	MN G	08-08- 10	Urine	6	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	I	S	P	32	>1 28	>1 28		
16 05	MN G	08-08- 10	Urine	70	M	EC	R	R	R	R	P	N			UN KN	I	R	S	S	R	S	S	S		2	4	8		
16 06	MN G	08-08- 10	Urine	45	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		16	>1 28	>1 28		
16 07	MN G	08-08- 10	Pus	50	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	R	S		8	>1 28	>1 28		
16 08	MN G	08-08- 10	Urine	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S	P	32	>1 28	>1 28		
16 09	MN G	08-08- 10	Urine	50	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	I	R	R	R	S		8	12 8	12 8		
16 10	MN G	08-08- 10	Vag Swab	24	F	KP	R	R	R	R	P	N			UN KN	R	S	S	S	R	I	S	S	P	2	12 8	12 8		
16 11	MN G	08-08- 10	Urine	50	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	I	S		32	>1 28	>1 28		
16 12	MN G	08-08- 10	Urine	55	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	R	S		>1 28	>1 28	>1 28		
16 13	MN G	08-08- 10	Urine	75	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S	P	8	>1 28	>1 28		
16 14	MN G	08-08- 10	Pus	28	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	I	S		64	>1 28	>1 28	CTX-M-15	
16 15	MN G	08-08- 10	Urine	13	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	R	R	I	I	R		64	>1 28	>1 28		
16 16	MN G	08-08- 10	Pus	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	R	I	I	R		16	>1 28	>1 28		

16	MN	08-08-10	Urine	1	M	KP	R	S	S	R	P	N			UN			S	S	S	S	S	S	S					
16	MN	08-08-10	Pus	31	F	KP	R	R	R	R	P	P	Ca		ESB		I	R	S	S	R	I	S	I		8	12	>1	
18	G												Ce		L											8	8	28	
16	MN	08-08-10	Pus	1	M	EC	R	R	R	R	P	N			UN			S	S	S	R	S	S	S					
19	G														KN														
16	MN	08-08-10	Urine	65	M	KP	R	R	R	R	P	P	Ca		ESB		R	R	I	S	R	R	S	S		8	16	64	
20	G												Ce		L														
16	MN	08-08-10	Urine	45	M	KP	R	R	R	R	P	N			UN			S	I	S	R	S	S	S					
21	G														KN														
16	MN	08-08-10	Urine	28	F	EC	R	R	R	R	P	P	Ca		ESB		R	R	S	S	R	S	S	S		16	>1	>1	
22	G												Ce		L											>1	>1	>1	
16	MN	08-08-10	Urine	12	F	EC	R	R	R	R	P	P	Ca		ESB		I	R	S	R	R	S	I	S		32	>1	>1	
23	G												Ce		L											>1	>1	>1	
16	MN	08-08-10	Urine	22	F	EC	R	R	R	R	P	P	Ca		ESB		I	I	S	S	R	S	S	S		8	>1	>1	
24	G												Ce		L											>1	>1	>1	
16	MN	08-08-10	Urine	47	M	EC	R	R	R	R	P	P	Ca		ESB		R	R	S	S	R	S	R	S	P	>1	>1	>1	
25	G												Ce		L										28	28	28		
16	MN	08-08-10	Urine	25	F	EC	R	R	R	R	P	N			UN			S	S	S	R	S	S	S					
26	G														KN														
16	MN	08-08-10	Urine	32	M	EC	R	R	R	R	P	P	Ca		ESB		R	R	S	S	R	S	S	S	P	8	>1	>1	
27	G												Ce		L											28	28	28	
16	MN	08-08-10	Urine	1	M	EC	R	R	R	R	P	P	Ca		ESB		R	R	S	S	R	S	S	S	P	64	>1	>1	
28	G												Ce		L											28	28	28	CTX-M-15
16	MN	08-08-10	Pus	25	F	EC	R	R	R	R	P	P	Ca		ESB		R	R	R	S	R	I	R	S		>1	>1	>1	
29	G												Ce		L											28	28	28	
16	MN	08-08-10	Sputum	36	M	KP	R	R	R	R	P	P	Ca		ESB		R	R	S	I	R	I	I	S		32	>1	>1	
30	G												Ce		L											>1	>1		
16	MN	08-08-10	Urine	30	F	EC	R	R	R	R	P	P	Ca		ESB		R	R	I	R	R	R	R	P	16	>1	>1		
31	G												Ce		L											28	28	28	
16	MN	08-08-10	Urine	23	F	EC	R	R	R	R	P	P	Ca		ESB		R	S	S	S	R	S	S	I		16	>1	>1	
32	G												Ce		L											28	28	28	
16	MN	08-08-10	Urine	12	M	KP	S	S	S	S	N				SUS														
33	G														C														
16	MN	08-08-10	Pus	28	F	KP	S	S	S	S	N				SUS														
34	G														C														
16	MN	08-08-10	Sputum	52	F	KP	S	S	S	S	N				SUS														
35	G														C														
16	MN	08-08-10	Urine	50	F	EC	R	R	R	R	P	P	Ca		ESB		I	R	S	S	R	S	I	S	P	8	>1	>1	
36	G												Ce		L											28	28	28	

16 37	MN G	08-08- 10	Pus	1	F	EC	R	R	R	R	P	N			UN KN		S	S	S	R	S	S	S					
16 38	MN G	08-08- 10	Pus	38	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		8	>1 28	>1 28	
16 39	MN G	08-08- 10	Urine	46	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	I	S	P	32	>1 28	>1 28	
16 40	MN G	08-08- 10	Urine	24	F	EC	R	R	R	R	P	N			UN KN		S	S	S	R	S	S	S					
16 41	MN G	08-08- 10	Pus	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		8	>1 28	>1 28	
16 42	MN G	08-08- 10	Urine	40	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		16	>1 28	>1 28	
16 43	MN G	08-08- 10	Urine	20	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	I	R	I	S	S		16	>1 28	>1 28	
16 44	MN G	08-08- 10	Urine	25	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	I	S		32	>1 28	>1 28	
16 45	MN G	08-08- 10	Urine	24	F	EC	R	R	R	R	P	N			UN KN		S	S	S	R	S	S	S					
16 46	MN G	08-08- 10	Urine	1	F	EC	S	S	S	S	N				SUS C													
16 47	MN G	08-08- 10	Pus	50	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	I	R	I	I	S		16	>1 28	>1 28	
16 48	MN G	08-08- 10	Urine	20	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	R	I	S	S		16	>1 28	>1 28	N
16 49	MN G	08-08- 10	Urine	28	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		32	>1 28	>1 28	
16 50	MN G	08-08- 10	Urine	52	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	R	R	R	R	S		>1 28	>1 28	>1 28	
16 51	MN G	08-08- 10	Pus	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	S	R	I	I	S	P	64	>1 28	>1 28	
16 52	MN G	08-08- 10	Pus	28	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	R	R	R	S	P	12 8	>1 28	>1 28	SHV-?
16 53	MN G	08-08- 10	Pus	80	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S	P	8	>1 28	>1 28	
16 54	MN G	08-08- 10	Pus	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	R	S	S	S		32	>1 28	>1 28	
16 55	MN G	08-08- 10	Urine	3	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		16	>1 28	>1 28	
16 56	MN G	08-08- 10	Pus	1	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	R	R	R	R	S		64	>1 28	>1 28	

16 57	MN G	08-08- 10	Urine	26	F	EC	R	R	S	S	P	N			UN KN		S	S	S	R	S	S	S					
16 58	MN G	08-08- 10	Pus	47	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		32	>1 28	>1 28	
16 59	MN G	08-08- 10	Pus	2	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		16	64	>1 28	
16 60	MN G	12-12- 10	Urine	30	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	R	S	P	>1 28	>1 28	>1 28	CTX-M-15
16 61	MN G	12-12- 10	Blood	1	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	I	S		>1 28	64	64	
16 62	MN G	12-12- 10	Urine	40	M	EC	R	R	R	R	P	N		EA	COP R	R	R	I	I	R	R	R	S		>1 28	>1 28	>1 28	
16 63	MN G	12-12- 10	Urine	30	M	EC	R	R	R	R	P	N		A	Am pC	R	R	R	R	R	R	R	P		>1 28	>1 28	>1 28	
16 64	MN G	12-12- 10	Pus	35	F	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	P		>1 28	>1 28	>1 28	
16 65	MN G	12-12- 10	Urine	27	F	EC	R	R	R	R	P	N		EA	COP R	R	R	R	I	R	R	R	R		>1 28	>1 28	>1 28	
16 66	MN G	12-12- 10	Urine	48	F	EC	R	R	R	R	P	N		EA	COP R	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
16 67	MN G	12-12- 10	Urine	38	M	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	P		>1 28	>1 28	>1 28	
16 68	MN G	12-12- 10	Urine	50	M	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	P		>1 28	>1 28	>1 28	
16 69	MN G	12-12- 10	Urine	35	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S		32	>1 28	>1 28	TEM-1, CTX-M-15
16 70	MN G	12-12- 10	Urine	11	F	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	P		>1 28	>1 28	>1 28	
16 71	MN G	12-12- 10	Urine	60	M	EC	R	R	R	R	P	N		EA	COP R	R	R	I	S	R	R	R	S		>1 28	>1 28	>1 28	
16 72	MN G	12-12- 10	Urine	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	I	S		32	>1 28	>1 28	
16 73	MN G	12-12- 10	Urine	1	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	I	I	S	P	64	>1 28	>1 28	
16 74	MN G	12-12- 10	Urine	65	M	EC	R	R	R	R	P	N		EA	COP R	R	R	R	I	R	R	R	S	P	>1 28	>1 28	>1 28	
16 75	MN G	12-12- 10	Urine	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	64	>1 28	>1 28	
16 76	MN G	12-12- 10	Urine	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	I	I	S	P	8	64	64	

1677	MN G	12-12-10	Urine	48	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	I	R	R	R	S	P	>1 28	>1 28	>1 28	
1678	MN G	12-12-10	Urine	25	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S		>1 28	>1 28	>1 28	
1679	MN G	12-12-10	Urine	38	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	I	S		32	>1 28	>1 28	
1680	MN G	12-12-10	Urine	65	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	I	I	S	P	64	>1 28	>1 28	
1681	MN G	12-12-10	Pus	55	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	I	S	P	64	>1 28	>1 28	
1682	MN G	12-12-10	Urine	75	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	I	R	I	R	S		>1 28	>1 28	>1 28	
1683	MN G	12-12-10	Urine	67	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	I	R	R		64	>1 28	>1 28	
1684	MN G	12-12-10	Pus	32	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	I	I	S		64	>1 28	>1 28	
1685	MN G	12-12-10	Urine	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		64	>1 28	>1 28	
1686	MN G	12-12-10	Urine	46	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	I	S		32	>1 28	>1 28	TEM-1, SHV-?, CTX-M-15
1687	MN G	12-12-10	Urine	25	F	EC	S	S	S	S	N				SUS C													
1688	MN G	12-12-10	Urine	19	F	EC	S	S	S	S	N				SUS C													
1689	MN G	12-12-10	Blood	30	F	EC	S	S	S	S	N				SUS C													
1690	MN G	12-12-10	Urine	25	F	EC	S	S	S	S	N				SUS C													
1691	MN G	12-12-10	Pus	40	M	EC	S	S	S	S	N				SUS C													
1692	MN G	12-12-10	Urine	21	F	EC	S	S	S	S	N				SUS C													
1693	MN G	12-12-10	Urine	23	F	EC	S	S	S	S	N				SUS C													
1694	MN G	12-12-10	Urine	26	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	I	I	S		64	>1 28	>1 28	CTX-M-15
1695	MN G	12-12-10	Blood	1	F	EC	R	R	R	R	P	N			UN KN	R	I	I	I	R	I	R	R		4	8	8	
1696	MN G	12-12-10	Urine	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S		12 8	>1 28	>1 28	N

1697	MN G	12-12-10	Urine	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S		64	>1 28	>1 28	
1698	MN G	12-12-10	Urine	13	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	I	S		64	>1 28	>1 28	CTX-M-15
1699	MN G	12-12-10	Urine	1	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	16	>1 28	>1 28	SHV-11, CTX-M-15
1700	MN G	12-12-10	Urine	24	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S		64	>1 28	>1 28	TEM-1, CTX-M-15
1701	MN G	12-12-10	Urine	8	F	EC	R	R	R	R	P	N		A	Am pC	R	R	R	S	R	R	R	S		>1 28	>1 28	>1 28	
1702	MN G	12-12-10	Urine	26	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	I	S	P	64	>1 28	>1 28	
1703	MN G	12-12-10	Urine	35	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S		32	>1 28	>1 28	
1704	MN G	12-12-10	Urine	30	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S		12 8	>1 28	12 8	
1705	MN G	12-12-10	Urine	60	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S	P	32	>1 28	>1 28	CTX-M-15
1706	MN G	12-12-10	Urine	68	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	I	R	R	R	S		>1 28	>1 28	>1 28	
1707	MN G	12-12-10	Urine	38	F	EC	S	S	S	S	N				SUS C													
1708	MN G	12-12-10	Blood	45	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S	P	16	>1 28	>1 28	
1709	MN G	12-12-10	Urine	60	M	EC	R	R	R	R	P	N		A	Am pC	R	R	S	S	R	R	R	S		>1 28	>1 28	>1 28	
1710	MN G	12-12-10	Urine	48	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S		64	>1 28	>1 28	CTX-M-15
1711	MN G	12-12-10	Urine	46	M	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	R	P	>1 28	>1 28	>1 28	
1712	MN G	12-12-10	Urine	30	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S	P	32	>1 28	>1 28	
1713	MN G	12-12-10	Pus	70	M	EC	R	R	R	R	P	N		A	Am pC	R	R	I	S	R	R	R	S	P	12 8	>1 28	>1 28	
1714	MN G	12-12-10	Urine	36	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S		>1 28	>1 28	>1 28	
1715	MN G	12-12-10	Blood	12	M	EC	S	S	S	S	N				SUS C													
1716	MN G	12-12-10	Urine	23	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		64	>1 28	>1 28	

17 17	MN G	12-12- 10	Urine	36	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	S	S		64	>1 28	>1 28	CTX-M-15
17 18	MN G	12-12- 10	Urine	21	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	R	R	S		>1 28	>1 28	>1 28	
17 19	MN G	12-12- 10	Urine	1	M	EC	S	S	S	S	N				SUS C													
17 20	MN G	12-12- 10	Urine	68	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	I	S		16	>1 28	>1 28	CTX-M-15
17 21	MN G	12-12- 10	Urine	20	M	EC	R	R	R	R	P	P	Ca		ESB L	R	R	S	S	R	R	R	S		>1 28	>1 28	>1 28	TEM-1, CTX-M-15
17 22	MN G	12-12- 10	Urine	23	F	EC	S	S	S	S	N				SUS C													
17 23	MN G	12-12- 10	Urine	26	F	EC	S	S	S	S	N				SUS C													
17 24	MN G	19-12- 10	Urine	20	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	S		32	>1 28	>1 28	
17 25	MN G	19-12- 10	Pus	24	F	KP	S	S	S	S	N				SUS C													
17 26	MN G	19-12- 10	Urine	48	F	EC	S	S	S	S	N				SUS C													
17 27	MN G	19-12- 10	Pus	12	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S		>1 28	64	64	
17 28	MN G	19-12- 10	Urine	24	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	R	R	R	S		32	>1 28	64	
17 29	MN G	19-12- 10	Blood	13	F	KP	S	S	S	S	N				SUS C													
17 30	MN G	19-12- 10	Urine	23	F	KP	S	S	S	S	N				SUS C													
17 31	MN G	19-12- 10	Urine	24	F	KP	S	S	S	S	N				SUS C													
17 32	MN G	19-12- 10	Pus	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	I	R	I	I	S		>1 28	>1 28	>1 28	
17 33	MN G	19-12- 10	Urine	40	M	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
17 34	MN G	19-12- 10	Urine	70	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		>1 28	>1 28	>1 28	CTX-M-15
17 35	MN G	19-12- 10	Urine	14	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S		64	>1 28	>1 28	
17 36	MN G	19-12- 10	Urine	49	F	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	

17	MN	19-12-												ESB														
37	G	10	Urine	36	M	EC	R	R	R	R	P	P	Ca Ce		L	R	R	S	S	R	I	R	S		8	>1 28	>1 28	
17	MN	19-12-												SUS														
38	G	10	Urine	40	M	EC	S	S	S	S	N			C														
17	MN	19-12-												ESB														
39	G	10	Pus	40	M	KP	R	R	R	R	P	P	Ca Ce		L	I	R	S	S	S	S	S	S		>1 28	>1 28	>1 28	
17	MN	19-12-												UN														
40	G	10	Urine	21	M	EC	R	R	R	R	P	N		KN	R	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
17	MN	19-12-												UN														
41	G	10	Urine	60	F	EC	R	R	R	R	P	N		KN	R	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	
17	MN	19-12-												SUS														
42	G	10	Pus	55	M	EC	S	S	S	S	N			C														
17	MN	19-12-												ESB														
43	G	10	Urine	18	F	EC	R	R	R	R	P	P	Ca Ce		L	R	R	R	S	R	R	R	S		>1 28	>1 28	>1 28	
17	MN	19-12-												ESB														
44	G	10	Urine	36	M	EC	R	R	R	R	P	P	Ca Ce		L	R	R	S	S	R	I	R	S		>1 28	>1 28	>1 28	CTX-M-15
17	MN	19-12-												ESB														
45	G	10	Urine	12	M	EC	R	R	R	R	P	P	Ca Ce		L	I	R	S	S	R	S	I	S		>1 28	>1 28	>1 28	
17	MN	19-12-												ESB														
46	G	10	Urine	60	F	EC	R	R	R	R	P	P	Ca Ce		L	I	R	S	S	R	S	S	S		16	>1 28	64	
17	MN	19-12-												ESB														
47	G	10	Urine	13	M	EC	R	R	R	R	P	P	Ca Ce		L	I	R	S	S	R	I	I	S	P	64	>1 28	>1 28	TEM-1, CTX-M-15
17	MN	19-12-												ESB														
48	G	10	Urine	50	M	KP	R	R	R	R	P	P	Ca Ce		L	I	R	S	S	I	S	S	S	P	64	64	>1 28	
17	MN	19-12-												SUS														
49	G	10	Urine	65	F	KP	S	S	S	S	N			C														
17	MN	19-12-												SUS														
50	G	10	Pus	40	F	KP	S	S	S	S	N			C														
17	MN	19-12-												ESB														
51	G	10	Blood	12	M	EC	R	R	R	R	P	P	Ce															
17	MN	19-12-												ESB														
52	G	10	Urine	4	F	EC	R	R	R	R	P	P	Ca Ce		L	I	R	S	S	R	S	S	S	P	16	32	32	
17	MN	19-12-												ESB														
53	G	10	Urine	45	M	EC	R	R	R	R	P	P	Ca Ce		L	I	R	S	S	R	S	S	S		16	64	64	
17	MN	19-12-												ESB														
54	G	10	Urine	26	F	EC	R	R	R	R	P	P	Ca Ce		L	I	I	S	S	R	I	I	S		8	32	32	N
17	MN	19-12-												ESB														
55	G	10	Blood	32	M	EC	R	R	R	R	P	P	Ca Ce		L	R	R	S	S	R	I	S	S	P	16	64	64	
17	MN	19-12-												SUS														
56	G	10	Urine	27	F	EC	S	S	S	S	N			C														

17 57	MN G	19-12- 10	Urine	70	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	I	S		32	>1 28	>1 28	
17 58	MN G	19-12- 10	Urine	60	M	EC	R	R	R	R	P	N		EA	COP R	R	R	R	I	R	R	R	S	P	>1 28	>1 28	>1 28	
17 59	MN G	19-12- 10	Urine	60	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	S	S	P	16	12 8	12 8	
17 60	MN G	19-12- 10	Urine	12	M	EC	S	R	S	R	P	N			UN KN		S	S	S	S	S	S	S					
17 61	MN G	19-12- 10	Urine	1	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S		32	64	12 8	
17 62	MN G	19-12- 10	Urine	73	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		32	>1 28	>1 28	TEM-1, CTX-M-15
17 63	MN G	07-01- 11	Sputum	40	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	I	S	S		64	>1 28	>1 28	CTX-M-15
17 64	MN G	07-01- 11	Urine	23	F	KP	S	S	S	S	N				SUS C													
17 65	MN G	07-01- 11	Urine	1	M	KP	S	S	S	S	N				SUS C													
17 66	MN G	07-01- 11	Urine	28	F	KP	S	S	S	S	N				SUS C													
17 67	MN G	07-01- 11	Urine	61	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S		16	>1 28	>1 28	CTX-M-15
17 68	MN G	07-01- 11	Urine	20	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S		32	>1 28	>1 28	CTX-M-15
17 69	MN G	07-01- 11	Urine	60	F	EC	S	S	S	S	N				SUS C													
17 70	MN G	07-01- 11	Blood	1	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	R	S	S	S		16	32	64	
17 71	MN G	07-01- 11	Urine	1	M	EC	S	S	S	S	N				SUS C													
17 72	MN G	07-01- 11	Urine	11	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	I	S	P	32	>1 28	>1 28	
17 73	MN G	07-01- 11	Urine	28	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	I	S	S	S		16	12 8	64	SHV-11, CTX-M-15
17 74	MN G	07-01- 11	Urine	44	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S		32	>1 28	>1 28	
17 75	MN G	07-01- 11	Urine	30	F	EC	S	S	S	S	N				SUS C													
17 76	MN G	07-01- 11	Pleural fl	45	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	S	S	S	I	S	S	S		16	64	64	

177	MN G	07-01-11	Urine	22	F	KP	S	S	S	S	N				SUS C													
1778	MN G	07-01-11	Urine	25	F	EC	R	R	R	R	P	N			UN KN		S	S	S	R	I	S	R					
1779	MN G	07-01-11	Urine	23	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	S	S		32	>1 28	>1 28	
1780	MN G	07-01-11	Urine	23	F	KP	S	S	S	S	N				SUS C													
1781	MN G	07-01-11	Pus	47	M	EC	R	R	R	R	P	N		A	Am pC	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
1782	MN G	07-01-11	Urine	24	F	KP	S	S	S	S	N				SUS C													
1783	MN G	07-01-11	Urine	32	F	EC	R	R	R	R	P	N		EA	COP R	R	R	R	I	R	R	R	S	P	>1 28	>1 28	>1 28	
1784	MN G	07-01-11	Urine	73	F	EC	S	S	S	S	N				SUS C													
1785	MN G	07-01-11	Blood	1	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	I	R	I	R	S	P	64	>1 28	>1 28	
1786	MN G	09-01-11	Pus	50	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	S	S	S	I	R	R	S	P	>1 28	32	32	
1787	MN G	09-01-11	Urine	30	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	S	S		64	>1 28	>1 28	
1788	MN G	09-01-11	Sputum	44	M	KP	S	S	S	S	N				SUS C													
1789	MN G	09-01-11	Urine	32	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	R	R	R	R	S	P	64	>1 28	>1 28	
1790	MN G	09-01-11	Urine	55	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	8	4	4	
1791	MN G	09-01-11	Urine	45	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	64	>1 28	>1 28	
1792	MN G	09-01-11	Urine	2	F	EC	S	S	S	S	N				SUS C													
1793	MN G	23-01-11	Urine	52	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S		>1 28	>1 28	>1 28	
1794	MN G	23-01-11	Pus	36	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	S	S	S	S	P	64	32	64	
1795	MN G	23-01-11	Pus	30	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	32	12 8	12 8	TEM-1, SHV-28, CTX-M-15
1796	MN G	23-01-11	Urine	20	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S	P	32	12 8	12 8	TEM-1, SHV-28, CTX-M-15

1797	MN G	13-05-11	Urine	24	F	EC	S	S	S	S	N				SUS C																
1798	MN G	13-05-11	Pus	20	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S	P	64	>128	>128	CTX-M-15			
1799	MN G	13-05-11	Urine	35	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	S	S	P	64	>128	128				
1800	MN G	13-05-11	Urine	65	F	EC	S	S	S	S	N				SUS C																
1801	MN G	13-05-11	Urine	60	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	I	S	P	>128	>128	>128				
1802	MN G	13-05-11	Urine	45	F	EC	R	R	R	R	P	N			UN KN	R	R	R	R	R	R	R	S		>128	>128	>128				
1803	MN G	13-05-11	Urine	30	M	EC	S	S	S	S	N				SUS C																
1804	MN G	13-05-11	Urine	25	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S		64	>128	128	CTX-M-15			
1805	MN G	13-05-11	Urine	14	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	S	S	P	32	>128	128				
1806	MN G	13-05-11	Urine	60	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S	P	64	>128	>128				
1807	MN G	13-05-11	Urine	25	F	EC	S	S	S	S	N				SUS C																
1808	MN G	13-05-11	Urine	85	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	I	S	S	S	S	S	S	P	16	64	64				
1809	MN G	13-05-11	Urine	45	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S	P	16	>128	>128				
1810	MN G	13-05-11	Urine	3	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	S	I	S	P	128	>128	>128	TEM-1, SHV-28, CTX-M-15			
1811	MN G	13-05-11	Urine	38	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	I	S	P	>128	>128	>128				
1812	MN G	13-05-11	Urine	50	F	EC	S	S	S	S	N				SUS C																
1813	MN G	13-05-11	Urine	51	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	S	S	P	64	>128	>128				
1814	MN G	13-05-11	Urine	26	F	EC	S	S	S	S	N				SUS C																
1815	MN G	13-05-11	Urine	25	F	EC	S	S	S	S	N				SUS C																
1816	MN G	13-05-11	Pus	16	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	S	S	P	64	>128	>128				

[illegible]

18 37	MN G	28-05- 11	Urine	24	F	EC	S	S	S	S	N				SUS C														
18 38	MN G	28-05- 11	Urine	63	M	EC	S	S	S	S	N				SUS C														
18 39	MN G	28-05- 11	Urine	38	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	I	S		32	>1 28	>1 28	CTX-M-15	
18 40	MN G	28-05- 11	Urine	26	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S	P	32	>1 28	>1 28	CTX-M-15	
18 41	MN G	28-05- 11	Urine	23	F	EC	S	S	S	S	N				SUS C														
18 42	MN G	28-05- 11	Urine	49	F	EC	S	S	S	S	N				SUS C														
18 43	MN G	28-05- 11	Urine	40	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	S	I	S		32	>1 28	>1 28		
18 44	MN G	28-05- 11	Urine	44	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	S	S	S		32	>1 28	>1 28		
18 45	MN G	28-05- 11	Urine	40	F	EC	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	S	S		32	>1 28	>1 28		
18 46	MN G	28-05- 11	Urine	30	F	EC	R	R	R	R	P	N		EA	COP R	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28		
18 47	MN G	28-05- 11	Urine	32	M	EC	R	R	R	R	P	N		EA	COP R	R	R	S	S	R	R	R	S		>1 28	>1 28	>1 28		
18 48	MN G	28-05- 11	Urine	46	M	EC	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	R	S	I	S	P	32	64	>1 28	SHV-11, CTX-M-15	
18 49	MN G	20-06- 11	Urine	33	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	I	I	I	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-31, CTX-M-15	
18 50	MN G	20-06- 11	Urine	38	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	R	I	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15	
18 51	MN G	20-06- 11	Pus	40	M	KP	S	S	S	S	N				SUS C														
18 52	MN G	20-06- 11	Urine	73	M	KP	S	S	S	S	N				SUS C														
18 53	MN G	20-06- 11	Pus	55	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	S	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15	
18 54	MN G	20-06- 11	Urine	3	F	KP	S	S	S	S	N				SUS C														
18 55	MN G	20-06- 11	Pus	25	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	R	I	R	S		>1 28	>1 28	>1 28		
18 56	MN G	20-06- 11	Pus	45	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	I	R	R	R	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15	

18	MN	20-06-												ESB																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							

1877	MN G	20-06- 11	Pus	52	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	R	I	R	S	P	>1 28	>1 28	>1 28	TEM-1, CTX-M-15
1878	MN G	20-06- 11	Pus	62	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	S	I	S	P	>1 28	>1 28	>1 28	TEM-1, SHV-11, CTX- M-15
1879	MN G	20-06- 11	Pus	28	F	KP	S	S	S	S	N				SUS C													
1880	MN G	20-06- 11	Sputum	35	M	KP	S	S	S	S	N				SUS C													
1881	MN G	20-06- 11	Urine	39	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	R	R	R	R	S	P	>1 28	>1 28	>1 28	
1882	MN G	20-06- 11	Pus	50	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	I	R	S	P	>1 28	>1 28	>1 28	CTX-M-15
1883	MN G	20-06- 11	Urine	80	F	KP	S	S	S	S	N				SUS C													
1884	MN G	20-06- 11	Pus	58	F	KP	S	S	S	S	N				SUS C													
1885	MN G	20-06- 11	Urine	37	F	KP	S	S	S	S	N				SUS C													
1886	MN G	20-06- 11	Urine	40	F	KP	S	S	S	S	N				SUS C													
1887	MN G	20-06- 11	Urine	22	F	KP	S	S	S	S	N				SUS C													
1888	MN G	20-06- 11	Urine	25	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	I	I	R	S	P	>1 28	>1 28	>1 28	
1889	MN G	20-06- 11	Sputum	65	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	I	R	R	I	R	S		>1 28	>1 28	>1 28	
1890	MN G	20-06- 11	Urine	24	F	KP	S	S	S	S	N				SUS C													
1891	MN G	20-06- 11	Pus	60	F	KP	S	S	S	S	N				SUS C													
1892	MN G	20-06- 11	Pleural fl	44	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	I	S	I	S	P	>1 28	>1 28	>1 28	
1893	MN G	20-06- 11	Pus	50	M	KP	S	S	S	S	N				SUS C													
1894	MN G	20-06- 11	Pus	51	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	I	S	S	I	S	S	S	P	>1 28	16	16	
1895	MN G	20-06- 11	Gastric La	60	F	KP	S	S	S	S	N				SUS C													
1896	MN G	20-06- 11	Urine	1	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	I	S	I	I	I	S		>1 28	>1 28	>1 28	

[illegible]

19	MN	20-06-11	Pus	60	F	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	50	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX-M-15	
19	MN	26-11-11	Urine	23	F	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	8	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	I	R	R	R	R	S	P	>1 28	>1 28	>1 28	SHV-11, CTX-M-15	
19	MN	26-11-11	Urine	40	M	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	40	M	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	40	M	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	23	M	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	18	M	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	84	F	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	45	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	S	S	S	S	P	64	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15	
19	MN	26-11-11	Urine	24	F	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	16	F	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	40	M	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	26	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	I	S		12 8	>1 28	>1 28		
19	MN	26-11-11	Urine	26	F	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	45	M	KP	S	S	S	S	N				SUS C														
19	MN	26-11-11	Urine	70	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	S	R	S	S	S	S	I	S		64	>1 28	>1 28		
19	MN	26-11-11	Urine	20	F	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	R	S	S	S	I	I	S		12 8	>1 28	>1 28		
19	MN	26-11-11	Urine	41	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28		

19	MN	26-11-												ESB														
37	G	11	Urine	60	M	KP	R	R	R	R	P	P	Ca Ce		L	I	R	S	S	S	S	I	S		64	>1 28	>1 28	
19	MN	26-11-												ESB														
38	G	11	Urine	60	M	KP	R	R	R	R	P	P	Ca Ce		L	S	R	S	S	S	S	I	S		12 8	>1 28	>1 28	TEM-1, SHV-1, CTX- M-15
19	MN	26-11-												ESB														
39	G	11	Urine	25	F	KP	R	R	R	R	P	P	Ca Ce		L	R	R	I	S	I	I	R	S	P	>1 28	>1 28	>1 28	SHV-1, CTX-M-15
19	MN	26-11-												ESB														
40	G	11	Urine	31	F	KP	R	R	R	R	P	P	Ca Ce		L	R	R	R	I	R	R	R	S		>1 28	>1 28	>1 28	
19	MN	26-11-												ESB														
41	G	11	Urine	25	F	KP	R	R	R	R	P	P	Ca Ce		L	S	R	I	S	S	I	I	S		12 8	>1 28	>1 28	
19	MN	26-11-												ESB														
42	G	11	Urine	25	F	KP	R	R	R	R	P	P	Ca Ce		L	S	R	S	S	S	S	S	S		64	>1 28	>1 28	
19	MN	26-11-												ESB														
43	G	11	Pus	43	F	KP	R	R	R	R	P	P	Ca Ce		L	S	R	S	S	S	S	I	S		12 8	>1 28	>1 28	SHV-1, CTX-M-15
19	MN	26-11-												SUS														
44	G	11	Sputum	45	M	KP	S	S	S	S	N			C														
19	MN	26-11-												SUS														
45	G	11	Sputum	45	M	KP	S	S	S	S	N			C														
19	MN	26-11-												SUS														
46	G	11	Pus	65	M	KP	S	S	S	S	N			C														
19	MN	26-11-												ESB														
47	G	11	Gastric La	5	F	KP	R	R	R	R	P	P	Ce		L	R	R	S	S	S	R	R	S	P	>1 28	>1 28	>1 28	
19	MN	26-11-												SUS														
48	G	11	Pleural fl	38	M	KP	S	S	S	S	N			C														
19	MN	26-11-												SUS														
49	G	11	Pleural fl	48	M	KP	S	S	S	S	N			C														
19	MN	26-11-												ESB														
50	G	11	Sputum	30	M	KP	R	R	R	R	P	P	Ca Ce		L	R	R	S	S	I	S	S	S		64	>1 28	>1 28	
19	MN	26-11-												SUS														
51	G	11	ET Tube	1	F	KP	S	S	S	S	N			C														
19	MN	26-11-												ESB														
52	G	11	Pus	28	F	KP	R	R	R	R	P	P	Ca Ce		L	I	R	S	S	S	S	S	S	P	64	>1 28	>1 28	
19	MN	26-11-												SUS														
53	G	11	ET Tube	1	F	KP	S	S	S	S	N			C														
19	MN	26-11-												SUS														
54	G	11	Sputum	40	M	KP	S	S	S	S	N			C														
19	MN	26-11-												ESB														
55	G	11	Sputum	50	M	KP	R	R	R	R	P	P	Ca Ce		L	R	R	S	S	S	S	S	S		>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX- M-15
19	MN	26-11-												SUS														
56	G	11	Sputum	33	M	KP	S	S	S	S	N			C														

19	MN	26-11-												SUS																
57	G	11	Pus	23	F	KP	S	S	S	S	N			C																
19	MN	26-11-												SUS																
58	G	11	ET Tube	30	F	KP	S	S	S	S	N			C																
19	MN	26-11-												SUS																
59	G	11	ET Tube	65	M	KP	S	S	S	S	N			C																
19	MN	26-11-												ESB	I	R	S	S	S	S	I	S			12	>1	>1			
60	G	11	ET Tube	60	F	KP	R	R	R	R	P	P	Ca Ce	L										8	28	28				
19	MN	26-11-												ESB	R	R	R	R	R	R	R	R			12	>1	>1			
61	G	11	Pus	45	F	KP	R	R	R	R	P	P	Ce	L										8	28	28				SHV-1, CTX-M-15
19	MN	26-11-												SUS																
62	G	11	Pus	10	F	KP	S	S	S	S	N			C																
19	MN	26-11-												SUS																
63	G	11	Pus	50	M	KP	S	S	S	S	N			C																
19	MN	26-11-												SUS																
64	G	11	Pus	40	F	KP	S	S	S	S	N			C																
19	MN	26-11-												SUS																
65	G	11	Pus	30	M	KP	S	S	S	S	N			C																
19	MN	26-11-												SUS																
66	G	11	Pus	25	F	KP	S	S	S	S	N			C																
19	MN	26-11-												ESB	I	R	S	S	I	S	I	S	P		>1	>1	>1			
67	G	11	Pus	30	F	KP	R	R	R	R	P	P	Ca Ce	L										28	28	28				
19	MN	26-11-												SUS																
68	G	11	Sputum	67	M	KP	S	S	S	S	N			C																
19	MN	26-11-												SUS																
69	G	11	Pus	24	F	KP	S	S	S	S	N			C																
19	MN	26-11-												SUS																
70	G	11	Sputum	70	M	KP	S	S	S	S	N			C																
19	MN	26-11-												Am	R	R	R	R	R	R	R	R			>1	>1	>1			
71	G	11	Pus	1	F	KP	R	R	R	R	P	N		pC										28	28	28				
19	MN	19-12-												ESB	I	R	S	S	S	S	I	S			12	>1	>1			
72	G	11	Urine	21	F	KP	R	R	R	R	P	P	Ca Ce	L										8	28	28				
19	MN	19-12-												Am	R	R	R	R	R	R	R	R			>1	>1	>1			
73	G	11	Pus	25	M	KP	R	R	R	R	P	N		pC										28	28	28				
19	MN	19-12-												SUS																
74	G	11	Pus	22	F	KP	S	S	S	S	N			C																
19	MN	19-12-												SUS																
75	G	11	ET Tube	1	M	KP	S	S	S	S	N			C																
19	MN	19-12-												SUS																
76	G	11	Pus	30	M	KP	S	S	S	S	N			C																

1977	MN G	19-12-11	Sputum	21	M	KP	S	S	S	S	N				SUS C														
1978	MN G	19-12-11	Pus	40	F	KP	S	S	S	S	N				SUS C														
1979	MN G	19-12-11	Pus	42	M	KP	S	S	S	S	N				SUS C														
1980	MN G	22-07-12	Blood	1	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	I	I	R	R	R	R	P	>1 28	>1 28	>1 28	TEM-1, SHV-28, CTX-M-15	
1981	MN G	22-07-12	Blood	55	M	KP	R	R	R	R	P	P	Ce		ESB L	I	I	S	S	R	I	S	S		>1 28	16	12 8	SHV-28	
1982	MN G	22-07-12	Blood	7	F	KP	S	S	S	S	N				SUS C														
1983	MN G	22-07-12	Urine	55	M	KP	S	S	S	S	N				SUS C														
1984	MN G	22-07-12	Urine	1	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	R	R	R	R	R		>1 28	>1 28	>1 28	TEM-1, SHV-11, CTX-M-15	
1985	MN G	22-07-12	Urine	35	F	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	S	I	R	S		8	16	16	N	
1986	MN G	22-07-12	Urine	23	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	I	I	R	R	R	R	P	>1 28	>1 28	>1 28	TEM-1, SHV-1, CTX-M-15	
1987	MN G	22-07-12	Urine	18	F	KP	S	S	S	S	N				SUS C														
1988	MN G	22-07-12	Urine	30	F	KP	R	R	R	R	P	P	Ce		ESB L	R	S	S	S	S	R	R	S	P	64	16	16	SHV-14	
1989	MN G	22-07-12	Urine	3	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	R	S	S	I	I	S	S	P	12 8	12 8	>1 28	SHV-1, CTX-M-15	
1990	MN G	22-07-12	Urine	47	M	KP	S	S	S	S	N				SUS C														
1991	MN G	22-07-12	Urine	50	F	KP	R	R	R	R	P	P	Ce		ESB L	R	R	R	S	R	R	R	S	P	>1 28	>1 28	>1 28	TEM-1, CTX-M-15	
1992	MN G	22-07-12	Urine	8	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	I	S	S	S	S	I	S	S		32	16	16	SHV-18	
1993	MN G	22-07-12	Sputum	70	F	KP	S	S	S	S	N				SUS C														
1994	MN G	22-07-12	Pus	20	M	KP	S	S	S	S	N				SUS C														
1995	MN G	22-07-12	Urine	38	F	KP	S	S	S	S	N				SUS C														
1996	MN G	22-07-12	Sputum	60	M	KP	R	R	R	R	P	P	Ca Ce		ESB L	R	I	S	S	I	I	I	S		>1 28	16	>1 28	SHV-?	

[illegible]

ANNEXURE 5

Email communication with Dr. George Jacoby, who designated the novel bla types on request.



Jacoby, George A. <George.A.Jacoby@lahey.org>

to me ▾

Jun 5 ☆



Dear Dr. Rao,

Thank you for sending the amino acid sequences.

DV2249 is novel TEM-219

U-1206 and U966 are novel SHV-185

M3K-122 is novel SHV-186

B-195 is SHV-75

D5-3119 and D5-3222 are SHV-168

B5-21 is SHV-108

V5066 and V5606 are CTX-M-155

M193-K and D5-3313 are CTX-M-156

P1014-K is CTX-M-157

Let us know when GenBank accession numbers are available or publications about these enzymes. Also are the TEM or SHV enzymes ESBLs or inhibitor resistant?

From: Sridhar Rao [mailto:microrao@gmail.com]

Sent: Wednesday, June 04, 2014 1:47 PM

To: Jacoby, George A.

Cc: Bush, Karen



Jacoby, George A. <George.A.Jacoby@lahey.org>

to me ▾

Jun 6 ☆



Dear Dr. Rao,

Sure, submit the novel sequences to GenBank and include CTX-M-155, 156, and 157. I fear my e-mail didn't make clear that they are novel as well.

George Jacoby

ANNEXURE 6

Sequences submitted to NCBI's GenBank with Accession Numbers of novel beta-lactamases

Klebsiella pneumoniae strain KV15 CTX-M-155 beta-lactamase (bla) gene, complete cds

GenBank: KM211508.1

[FASTA](#) [Graphics](#) [PopSet](#)

Go to: 

LOCUS KM211508 876 bp DNA linear BCT 12-NOV-2014
DEFINITION Klebsiella pneumoniae strain KV15 CTX-M-155 beta-lactamase (bla) gene, complete cds.
ACCESSION KM211508
VERSION KM211508.1 GI:694179057
KEYWORDS .
SOURCE Klebsiella pneumoniae
ORGANISM [Klebsiella pneumoniae](#)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 876)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Genotypic distribution of ESBLs in E. coli and K. pneumoniae isolates across Karnataka, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 876)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Direct Submission
JOURNAL Submitted (18-JUL-2014) Microbiology, JJM Medical College, Davangere, Davangere, Karnataka 577004, India
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
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Location/Qualifiers
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RDVLASGAKIVTDGL"
ORIGIN
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121 gagcggcagt cgggaggcag actgggtgtg gcattgatta acacagcaga taattcgcaa
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241 gcrcgggtgc tgaagaaaag tgaagcgaa ccgaatctgt taaatcagcg agttgagatc
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361 tcactggctg agcttagcgc ggccgcgcta cagtacagcg ataactggcg gatgaataag
421 ctgattgctc agttggcgg cccggctagc gtcaccgctg tcgcccgaca gctgggagac
481 gaaacgticc gtcctgaccg caccgcgccg acgttaaaac ccgccattcc gggcgatccg
541 cgtgatacca cttcacctcg ggcaatggcg caaactctgc ggaatctgac gctgggtaaa
601 gcattgggcg acagccaacg ggcgacgctg gtgacatgga tgaagggcaa taccaccggt
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721 ggtggctatg gcaccaccaa cgatatcgcg gtgactcggc caaagatcg tgcgccctg
781 atttcgtgta cttacttcac ccagcctcaa ctaaggcag aaagccgctg cgatgtatta
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//

Klebsiella pneumoniae strain KK08 CTX-M-156 beta-lactamase (bla) gene, complete cds

GenBank: KM211509.1

[FASTA](#) [Graphics](#) [PopSet](#)

Go to: ☐

LOCUS KM211509 876 bp DNA linear BCT 12-NOV-2014
DEFINITION Klebsiella pneumoniae strain KK08 CTX-M-156 beta-lactamase (bla)
gene, complete cds.
ACCESSION KM211509
VERSION KM211509.1 GI:694179059
KEYWORDS .
SOURCE Klebsiella pneumoniae
ORGANISM [Klebsiella pneumoniae](#)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 876)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Genotypic distribution of ESBLs in E. coli and K. pneumoniae
isolates across Karnataka, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 876)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Direct Submission
JOURNAL Submitted (18-JUL-2014) Microbiology, JJM Medical College,
Davangere, Davangere, Karnataka 577004, India
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
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121 gagcggcagt cgggaggcag actgggtgtg gcattgatta acacagcaga taattcgcaa
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301 aaaaaatctg accttgtaa ctataatcgc attcgggaaa agcacgtcaa tgggacgatg
361 tcactggctg agcttagcgc ggcgcgcgta cagtacagcg ataactggcg gatgaataag
421 ctgattgctc acgttgccgg cccggctagc gtcaccgcgt tcgcccgaca gctgggagac
481 gaaacgttcc gtcctgaccg taccgagccg acgttaaaca cgcaccattcc gggcgatccg
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781 attctggta cttacttcac ccagcctcaa cctaacgcag aaagccgtcg cgatgtatta
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Klebsiella pneumoniae strain KK20 CTX-M-157 beta-lactamase (bla) gene, complete cds

GenBank: KM211510.1

[FASTA](#) [Graphics](#) [PopSet](#)

Go to: ☒

LOCUS KM211510 876 bp DNA linear BCT 12-NOV-2014
DEFINITION Klebsiella pneumoniae strain KK20 CTX-M-157 beta-lactamase (bla)
gene, complete cds.
ACCESSION KM211510
VERSION KM211510.1 GI:694179061
KEYWORDS .
SOURCE Klebsiella pneumoniae
ORGANISM [Klebsiella pneumoniae](#)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 876)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Genotypic distribution of ESBLs in E. coli and K. pneumoniae
isolates across Karnataka, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 876)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Direct Submission
JOURNAL Submitted (18-JUL-2014) Microbiology, JJM Medical College,
Davangere, Davangere, Karnataka 577004, India
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
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121 gagcggcagt cgggaggcag actgggtgtg gcattgatta acacagcaga taattcgcaa
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481 gaaacgttcc gtctcgaccg taccgcgcgc acgttaaaca cgcaccattcc gggcgatccg
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721 ggtggctatg gcaccaccaa cgatatcgcg gtgatctggc caaaagatcg tgcgccctg
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//

Klebsiella pneumoniae strain KK19 SHV-185 beta-lactamase gene, complete cds

GenBank: KM233164.1

[FASTA](#) [Graphics](#) [PopSet](#)

Go to: ☐

LOCUS KM233164 861 bp DNA linear BCT 12-NOV-2014
DEFINITION Klebsiella pneumoniae strain KK19 SHV-185 beta-lactamase gene, complete cds.
ACCESSION KM233164
VERSION KM233164.1 GI:694179373
KEYWORDS .
SOURCE Klebsiella pneumoniae
ORGANISM [Klebsiella pneumoniae](#)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 861)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Genotypic distribution of ESBLs in E. coli and K. pneumoniae isolates across Karnataka, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 861)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Direct Submission
JOURNAL Submitted (24-JUL-2014) Microbiology, JJM Medical College, Davangere, Davangere, Karnataka 577004, India
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
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Klebsiella pneumoniae strain KM18 SHV-186 beta-lactamase gene, complete cds

GenBank: KM233165.1

[FASTA](#) [Graphics](#) [PopSet](#)

Go to: ☐

LOCUS KM233165 861 bp DNA linear BCT 12-NOV-2014
DEFINITION Klebsiella pneumoniae strain KM18 SHV-186 beta-lactamase gene, complete cds.
ACCESSION KM233165
VERSION KM233165.1 GI:694179375
KEYWORDS .
SOURCE Klebsiella pneumoniae
ORGANISM [Klebsiella pneumoniae](#)
Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Klebsiella.
REFERENCE 1 (bases 1 to 861)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Genotypic distribution of ESBLs in E. coli and K. pneumoniae isolates across Karnataka, India
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 861)
AUTHORS Rao,S.P.N. and Subba Rama,P.
TITLE Direct Submission
JOURNAL Submitted (24-JUL-2014) Microbiology, JJM Medical College, Davangere, Davangere, Karnataka 577004, India
COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
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Escherichia coli strain EV25 beta-lactamase TEM-219 gene, complete cds

GenBank: KM114268.1

[FASTA](#) [Graphics](#)

Go to: ☐

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JOURNAL	Unpublished				
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AUTHORS	Rao,S.P.N. and Subba Rama,P.				
TITLE	Direct Submission				
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Publication

Extended-spectrum Beta-lactamases Producing *Escherichia coli* and *Klebsiella pneumoniae*: A Multi-centric Study Across Karnataka

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ABSTRACT

Background: There are sporadic reports on detection of extended-spectrum beta-lactamases (ESBL) producers from Karnataka; hence, this is a first multicentric study across Karnataka state to determine the prevalence of ESBL production among clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae*.

Aims and objectives: To determine the prevalence of ESBL producing clinical isolates of *E. coli* and *K. pneumoniae* from five geographically distributed centers across Karnataka, to study the susceptibility of ESBL producing isolates to other beta-lactam and beta-lactam-beta-lactamase inhibitors and to demonstrate transferability of plasmids coding for ESBL phenotype.

Materials and Methods: Two hundred isolates of *E. coli* and *K. pneumoniae* each were collected from each of the five centers (Bellary, Dharwad, Davangere, Kolar and Mangalore). They were screened for resistance to screening agents (ceftazidime, cefotaxime, ceftriaxone, aztreonam) and positive isolates were confirmed for ESBL production by test described by Clinical and Laboratory Standards Institute. Co-production of ESBL and AmpC beta-lactamase was identified by using amino-phenylboronic acid disk method. Susceptibility of ESBL producers to beta-lactam antibiotics and beta-lactamase inhibitors was performed. Transferability of plasmids was performed by conjugation experiment.

Results: Overall prevalence of ESBL production among *E. coli* and *K. pneumoniae* across five centers of the state was 57.5%. ESBL production was found to be 61.4% among *E. coli* and 46.2% among *K. pneumoniae*. ESBL production was significantly more among *E. coli* than *K. pneumoniae*. Significant variations in distribution of ESBL across the state was observed among *E. coli* isolates, but not among *K. pneumoniae* isolates. All ESBL producers demonstrated minimum inhibitory concentration levels ≥ 2 μ g/ml towards cefotaxime, ceftazidime and ceftriaxone.

Conclusion: Overall prevalence of ESBL production among clinical isolates of *E. coli* and *K. pneumoniae* across Karnataka state was high. The prevalence of ESBL production was significantly higher with *E. coli* than *K. pneumoniae* isolates. Higher rates of resistance to ceftriaxone and cefotaxime than to ceftazidime suggests the possibility of presence of CTX-M type ESBLs. Of all the beta-lactam/beta-lactamase inhibitor combinations tested, cefepime-tazobactam demonstrated highest *in-vitro* activity against ESBL producers. There was no statistical difference in the transferability of plasmids among *E. coli* and *K. pneumoniae*.

Key words: Beta-lactamase, beta-lactamase inhibitor, extended-spectrum beta-lactamases

INTRODUCTION

Treatment of infections caused by Gram-negative bacilli is becoming increasingly

difficult because of antibiotic resistance. Various mechanisms such as enzymatic inactivation of antibiotics, altered target sites, decreased porin permeability and active efflux pumps are known to produce drug resistance. One such mechanism is the production of extended-spectrum beta-lactamase (ESBL) enzymes by these bacteria. ESBLs are known to hydrolyze all penicillins, early cephalosporins, oxymino-cephalosporins and monobactams, but they lack hydrolytic activity on cephamycins and carbapenems. ESBLs are inhibited by beta-lactamase

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inhibitors such as clavulanic acid, tazobactam and sulbactam.^[1] Treatment failures after instituting beta-lactam antibiotic therapy for infections caused by ESBL producing Gram-negative bacilli have been reported.^[2] ESBL producing bacteria have been isolated from healthy subjects, health care workers, food of animal origin, animals, hospital environment, vegetation and sewage.^[3-5] As the genes coding for ESBLs are mainly plasmid borne, ESBLs have rapidly disseminated among the bacterial communities, within and across the species. ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* have known to cause outbreaks in hospital settings.^[6]

Although there are a few reports of ESBL producing bacteria from Karnataka, data from multiple centers across the state is currently lacking. Hence, this is the first multi-centric study that was conducted with the following objectives: (a) To determine the prevalence of ESBL producing clinical isolates of *E. coli* and *K. pneumoniae* from five geographically distributed centers across Karnataka (b) to study the susceptibility of ESBL producing isolates to other beta-lactam and beta-lactam-beta-lactamase inhibitors and (c) to demonstrate transferability of plasmids coding for ESBL phenotype.

MATERIALS AND METHODS

Collection of specimen

Between May 2009 and September 2012, 2000 clinical isolates comprising of 1000 isolates each of *E. coli* and *K. pneumoniae* were collected from hospitals attached to Medical Colleges at Davangere (DVG), Kolar (KOL), Mangalore (MNG), Dharwad (DWD) and Bellary (BEL). 200 isolates each of *E. coli* and *K. pneumoniae* from each center were randomly collected over a period of 3 years and 3 months. The samples yielding these isolates were as follows: Urine-857, pus-595, sputum-290, blood-92, endotracheal tube-67, throat swab-20, ascitic fluid-19, vaginal swab-14, pleural fluid-13, suction tip-12, rectal swab-8, cervical swab-6, gastric lavage-3 and two each from cerebrospinal fluid and bronchoalveolar lavage samples. Isolation of *E. coli* and *K. pneumoniae* from these samples is shown in Table 1. Ethical clearance was obtained from the Institutional Ethical Committee.

Screening for resistance to oxyimino-cephalosporins

Isolates were screened for resistance to three oxyimino-cephalosporins: Cefotaxime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg) and the monobactam:

Aztreonam (30 µg) by disk diffusion test. Isolates that displayed resistance to one or more of these were considered positive for screening test.

Phenotypic detection of ESBL production

Presence of ESBL among isolates positive on screening was confirmed by using both ceftazidime/ceftazidime-clavulanic acid (CAZ/CAC) (30/10 µg) and ceftotaxime/cefotaxime-clavulanic acid (CTX/CEC) (30/10 µg) disks according to phenotypic confirmatory test (PCT). An increase in zone diameter by ≥5 mm around disks with cephalosporin and clavulanic acid versus disks with cephalosporin alone was interpreted as positive as per Clinical and Laboratory Standards Institute (CLSI) 2010 guidelines.^[7]

Detection of ESBL in the presence of AmpC

Isolates that were positive upon screening but negative by PCT were tested for co-production of ESBL and AmpC enzymes by using amino-phenylboronic acid (APB) disk method as described earlier.^[8] Briefly, 400 µg/ml APB acid was added to disks containing cefotaxime/clavulanic acid (30/10 µg) and cefotaxime (30 µg). Plain disks with APB acid were used as controls. Interpretation of the test was made as shown in Table 2.

Table 1: Isolation of *E. coli* and *K. pneumoniae* from various clinical samples

Specimen	Number of samples n=2000	<i>E. coli</i> n=1000	<i>K. pneumoniae</i> n=1000
Urine	857	614	243
Pus	595	242	353
Sputum	290	61	229
Blood	92	35	57
ET tube	67	3	64
Throat swab	20	4	16
Ascitic fluid	19	13	6
Pleural fluid	13	5	8
Vaginal swab	14	9	5
Suction tip	12	2	10
Miscellaneous	21	12	9

E. coli: *Escherichia coli*, ET: Endotracheal

Table 2: Interpretation of amino-phenylboronic acid disk method for co-production of ESBL and AmpC beta-lactamase

CTX	CTX+CLA	CTX+CLA+APB	CTX+APB	Interpretation
R	+	NA	NA	ESBL only
R	-	+	+	AmpC only
R	-	+	-	ESBL and AmpC

CTX: Cefotaxime, CLA: Clavulanic acid, APB: Amino-phenylboronic acid, R: Resistant, NA: Not applicable, +: ≥5 mm, -: <5 mm, ESBL: Extended-spectrum beta-lactamase

Determination of Minimum inhibitory concentration values

The MIC values for cefoxitin, ceftazidime, cefotaxime and ceftriaxone against isolates identified as ESBL producers were obtained by agar dilution method using a dilution range of 128-0.25 µg/ml on Mueller Hinton agar. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as controls.

Additional susceptibility testing

ESBL producing isolates were further tested for susceptibility to cefepime (30 µg), cefepime-tazobactam (30/10 µg), cefepime-clavulanic acid (30/10 µg) and imipenem (10 µg) by disk diffusion method.

Transfer of resistance by conjugation

Isolates phenotypically identified as ESBL producers were tested for transferability of the plasmid by conjugation (mating) experiment as described earlier.^[9] Test strains and the recipient strain were grown overnight separately in Luria Bertani broth at 37°C. Cultures of test strain and recipient strains were mixed in a separate tube at 1:10 ratio and incubated at 37°C overnight. A volume of 50 µl of the mixture was placed on Mueller Hinton agar with 2 µg/ml cefotaxime and 200 µg/ml sodium azide and incubated at 37°C for up to 48 h. Growth on this medium was interpreted as successful conjugation and such colonies were confirmed for ESBL production by PCT. The recipient strain (*E. coli* J53 Az^R) was kindly provided by George Jacoby.

Statistical methods applied

Using an approximate prevalence rate of 50%, confidence interval of 95%, precision of 5% and using the formula $n = (Z_{1-\alpha})^2(P(1-P)/D^2)$, a sample size of 385 was calculated. It was rounded off to 400 samples per center including 200 of *E. coli* and 200 of *K. pneumoniae*. The power of study was set at 80%. Categorical data was analyzed by Chi-square test whereas χ^2 test for proportion was used to determine the relationship between groups. $P \leq 0.05$ was considered to be statistically significant.

Culture media, antibiotic disks, APB acid, dimethylsulphoxide, ATCC strains were procured from Hi-Media laboratories, Mumbai, India. Pure antibiotic powders for MIC determination were procured from Sigma-Aldrich, Bangalore, India.

RESULTS

Screening test

A total of 1276 isolates were considered as screen positive. Of the 1,000 *E. coli* isolates, 740 (74%) were found resistant to one or more the screening agents. Among them 738 (99.7%) were resistant to all the four screening agents. Of the 1,000 *K. pneumoniae* isolates, 536 (53.6%) were found resistant to one or more the screening agents. Among them 529 (98.7%) were resistant to all the four screening agents.

Phenotypic detection of ESBL

Of the 1276 isolates that were positive in the screening test, ESBL production was confirmed by PCT in 1076 (84.3%) isolates, which included 614 (61.4%) *E. coli* and 462 (46.2%) *K. pneumoniae*, indicating that the prevalence of ESBL production is 61.4% in *E. coli* (ESBL-EC) and 46.2% in *K. pneumoniae* (ESBL-KP) across Karnataka. Co-production of ESBL and AmpC beta-lactamase were detected in 58 (2.9%) isolates, which included 56 (5.6%) *E. coli* and 2 (0.2%) *K. pneumoniae*. AmpC production was noted in 35 (3.5%) isolates of *E. coli* and 9 (0.9%) isolates of *K. pneumoniae*. Neither AmpC nor ESBL production could be accounted for cephalosporin resistance in the remaining 35 isolates of *E. coli* and 63 isolates of *K. pneumoniae*.

Distribution of ESBL producers across the state

The distribution of ESBL producing *E. coli* and *K. pneumoniae* from various centers is as shown in Table 3 and Figure 1. The prevalence of ESBL-EC was noticed to be highest from Bellary center (83.5%) followed by Mangalore and Davangere (63.5% each) and lesser from Dharwad (49.5%) and Kolar (47%) centers. Differences in the prevalence of ESBL-EC across Karnataka was significant ($P < 0.001$); on the other hand, the prevalence of ESBL-KP across Karnataka was not significant ($P = 0.2$).

Table 3: Distribution of ESBL producing EC and KP from various centers

Location	Screening test positive n (%)		ESBL positive n (%)	
	EC	KP	EC	KP
Bellary	175 (87.5)	103 (51.5)	167 (83.5)	98 (49)
Davangere	171 (85.5)	110 (55)	127 (63.5)	95 (47.5)
Dharwad	124 (62)	102 (51)	99 (49.5)	77 (38.5)
Kolar	105 (52.5)	120 (60)	94 (47)	96 (48)
Mangalore	165 (82.5)	101 (50.5)	127 (63.5)	96 (48)
Total	740 (74)	536 (53.6)	614 (61.4)	462 (46.2)

EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, ESBL: Extended-spectrum beta-lactamase

MIC values of ESBL producers

MIC values of ESBL-EC and ESBL-KP isolates to ceftazidime, cefotaxime and ceftriaxone are displayed in Table 4. All the ESBL producers had MIC levels of $\geq 2 \mu\text{g/ml}$ to each of the third generation cephalosporin tested, thus confirming resistance. At the concentration of $\geq 128 \mu\text{g/ml}$ of ceftriaxone and cefotaxime, 493 (80.3%) and 486 (79.1%) isolates of ESBL-EC respectively were resistant; however at a similar concentration of ceftazidime only 228 (37.1%) isolates of ESBL-EC showed resistance. These differences in resistance pattern at the concentration $\geq 128 \mu\text{g/ml}$ were statistically significant

($P < 0.001$). This observation was also true with ESBL-KP. At the concentration of $\geq 128 \mu\text{g/ml}$ of ceftriaxone and cefotaxime, 382 (82.7%) and 343 (74.2%) isolates of ESBL-KP respectively were resistant; however at a similar concentration of ceftazidime only 253 (54.8%) of ESBL-KP showed resistance.

Resistance pattern to other beta-lactams, beta-lactam-beta-lactamase inhibitors

Table 5 presents resistance pattern of the ESBL producing isolates to certain beta-lactam antibiotics other than those used for testing and beta-lactam/beta-lactamase

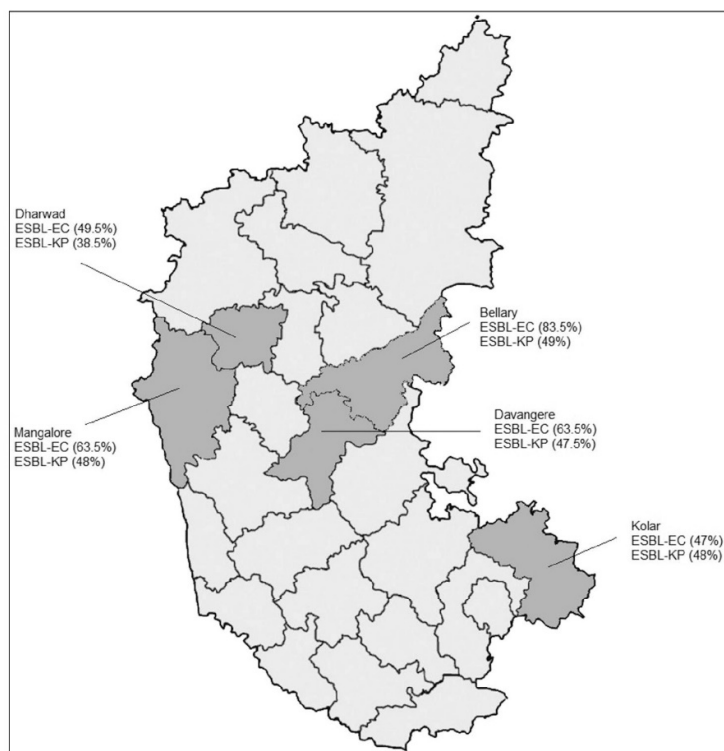


Figure 1: Distribution of extended-spectrum beta-lactamase producers from five centers across Karnataka

Table 4: MIC values of ESBL producers to ceftazidime, cefotaxime and ceftriaxone

MIC range ($\mu\text{g/ml}$)	Ceftriaxone n (%)		Cefotaxime n (%)		Ceftazidime n (%)	
	EC	KP	EC	KP	EC	KP
≥ 128	493 (80.3)	382 (82.7)	486 (79.1)	343 (74.2)	228 (37.1)	253 (54.8)
16-64	119 (19.4)	70 (15.1)	123 (20)	102 (22.1)	331 (53.9)	176 (38.1)
2-8	2 (0.3)	10 (2.2)	5 (0.8)	17 (3.7)	55 (8.9)	33 (7.1)

EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, MIC: Minimum inhibitory concentration, ESBL: Extended-spectrum beta-lactamase

inhibitor combinations. Resistance to the combination of cefotaxime and clavulanic acid was almost the same in case of ESBL-EC (53.4%) and ESBL-KP (49.6%); however, 32.2% ESBL-KP and 9.1% of ESBL-EC were resistant to the combination of ceftazidime and clavulanic acid. This difference was found to be significant ($P < 0.001$). It was observed that 89.1% of ESBL-EC and 79.9% of ESBL-KP were resistant to cefepime. This resistance was reduced considerably (from 89.1% to 2.9%) when cefepime was combined with tazobactam or clavulanic acid. Of all the combinations of beta-lactam/beta-lactamase inhibitor tested, cefepime-tazobactam had the least resistance. Cefoxitin resistance was observed both in ESBL-EC (38.6%) and ESBL-KP (48.5%). Only 2 (0.3%) ESBL-EC and 11 (2.4%) ESBL-KP were found resistant to imipenem.

Plasmid transfer by conjugation

Plasmid mediated resistance transfer was successfully demonstrated in 362/614 (59%) isolates of ESBL-EC and 249/462 (53.9%) isolates of ESBL-KP.

DISCUSSION

In a multi-centric study conducted as part of India SENTRY surveillance, the prevalence of ESBL production was reported to be 84%.^[10] Here we found that 61.4% of *E. coli* and 46.2% of *K. pneumoniae* isolates collected across five centers of Karnataka were ESBL producers. The prevalence of ESBL-EC was significantly more ($P < 0.001$) than ESBL-KP.

ESBL production among *Enterobacteriaceae* members vary widely from region to region and sometimes within the state. We found that the prevalence of ESBL producing bacteria varied across Karnataka with respect to *E. coli* but not with *K. pneumoniae*. In the case of *E. coli* it was highest at Bellary (87.5%) and lowest at Kolar (52.5%). However, it was almost the same with respect to *K. pneumoniae* except from Dharwad. These differences could be due to varied degree exposure of these organisms to beta-lactam antibiotics or due to varied transferability of plasmids in nature among them. However, our plasmid transmission studies conducted in the laboratory did not reveal a significant difference ($P = 0.09$) in the transferability of plasmid in ESBL-EC and ESBL-KP.

Earlier studies from Karnataka found that the detection rates of ESBL-EC has varied from 23.6% (Raichur) to 86.7% (Shimoga). The detection rates of ESBL-KP from different studies across Karnataka has been reported to

vary from 9.6% (Bangalore) to 81.8% (Mangalore).^[11-14] Reports of ESBL detection among clinical isolates of *E. coli* range between 20% and 80.6% and those among *K. pneumoniae* ranges between 20% and 86.7% across the country; these findings are summarized in Table 6. The variation in the detection rates within and across the states could be due to the differences in the methodology used in these studies. We made our study stringent by using four beta-lactam antibiotics for screening and two beta-lactam and beta-lactamase inhibitor combination disks for confirmation of ESBL as per the CLSI guidelines, thus, bringing uniformity in testing.

Co-production of AmpC beta-lactamase and ESBL among the isolates in this study has been only minimal (2.9%), but was observed to be higher among *E. coli* (5.6%) isolates than *K. pneumoniae* (0.2%) isolates. Our findings contrasts with that reported from Bangalore and Manipal. The study from Manipal reported a co-production rate of 58.5% among *E. coli*; a study from Bangalore reported a co-production rate of 30.1% for *E. coli* and 30.3% for *Klebsiella* spp.^[23,24] These variations could be due to differences in the methodologies adopted.

In the present study reported, none of the ESBL producing bacteria had MIC of $<2 \mu\text{g/ml}$ towards ceftazidime, cefotaxime or ceftiraxone. The ESBL producers exhibited significantly higher MIC levels ($\geq 128 \mu\text{g/ml}$) to cefotaxime and ceftiraxone than to ceftazidime. This difference suggests

Table 5: Antibiotic resistance to other beta-lactam antibiotics and beta-lactamase inhibitors

Antibiotics tested	<i>Escherichia coli</i> n (%)	<i>Klebsiella pneumoniae</i> n (%)
Cefotaxime-clavulanic acid	328 (53.4)	229 (49.6)
Ceftazidime-clavulanic acid	56 (9.1)	149 (32.2)
Cefepime	547 (89.1)	369 (79.9)
Cefepime-clavulanic acid	53 (8.6)	94 (20.3)
Cefepime-tazobactam	18 (2.9)	48 (10.4)
Cefoxitin	237 (38.6)	224 (48.5)
Imipenem	2 (0.3)	11 (2.4)

Table 6: Reports of ESBL production in EC and KP across the country

Location	ESBL-EC %	ESBL-KP %	Reference
Mumbai	20	20	Vaidya ^[25]
Chandigarh	70	60	Sharma et al. ^[26]
Pondicherry	60.8	39.2	Mohamudha Parveen et al. ^[27]
Amritsar	46.4	52.3	Kaur and Aggarwal ^[28]
Salem	79.5	50	Priyadharsini et al. ^[29]
Guwahati	43	67	Sarma et al. ^[30]
Ujjain	69	41	Pathak et al. ^[31]
Indore	80.6	86.7	Chitnis et al. ^[32]

EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, ESBL-EC: ESBL producing *E. coli*, ESBL-KP: ESBL producing *K. pneumoniae*

the presence of CTX-M type ESBLs that hydrolyse cefotaxime and ceftriaxone more efficiently than ceftazidime.^[25]

Cefoxitin, which is a cephamycin is not hydrolyzed by ESBLs, but in our study we have observed resistance in both *E. coli* (38.5%) and *K. pneumoniae* (48.5%) isolates. Earlier studies have suggested that cefoxitin resistance could be due to altered membrane porins.^[26] Cefepime resistance among the ESBL producers was also found to be high among both *E. coli* (89.1%) and *K. pneumoniae* (79.9%). Similarly, high resistance to the tune 97.4% was observed among ESBL-EC isolates from Bangalore.^[23] Resistance to cefepime could be attributed to the high prevalence of CTX-M type ESBLs in these isolates, some of which are capable of hydrolyzing cefepime.^[27] Cefepime-razobactam combination was found to be more effective than cefepime-clavulanic acid combination against ESBL producing isolates.

Although all ESBL producing isolates were resistant to ceftazidime and cefotaxime in the screening test, addition of clavulanic acid did not render all the isolates susceptible to the cephalosporins. Ceftazidime-clavulanic acid combination had better inhibitory effect on ESBL-EC and ESBL-KP than cefotaxime-clavulanic acid. This difference in susceptibilities also highlights the possible presence of CTX-M type enzymes in these isolates. ESBLs have no hydrolytic activity on imipenem, but resistance was noted in two *E. coli* and 11 *K. pneumoniae* isolates. Presence of other beta-lactamases or alterations in porin channels might account for this resistance.^[28] As the co-resistance to imipenem in ESBL producers is low, it continues to be effective in treating diseases caused by them.

Presence of ESBL genes on plasmids was demonstrated successfully by mating experiment in 56.8% isolates. *E. coli* isolates were found to readily transfer the plasmids than *K. pneumoniae*, although this observation was statistically not significant. Presence of *bla* gene on non-transmissible plasmids can account for failure in conjugation experiments. Recently, a study was conducted at Mumbai, which evaluated horizontal transfer rates of ESBL carrying plasmids by conjugation. The author reported transfer of resistance under laboratory and environmental conditions at a frequency of $3-4 \times 10^{-5}$, which is high.^[15] The promiscuity of the isolates in transferring the plasmid containing *bla* gene does not seem to be very high in our study.

CONCLUSION

Prevalence of ESBL production among *E. coli* was 61.4% and 46.2% among *K. pneumoniae* across Karnataka state. The

prevalence of ESBL production was significantly higher with *E. coli* than *K. pneumoniae* isolates. There was significant variation in the distribution of ESBL-EC across Karnataka, but not so with ESBL-KP. Isolates that co-produced ESBL and AmpC enzymes were marginally low in both the species. All ESBL producers had MIC levels ≥ 2 µg/ml toward cefotaxime, ceftriaxone and ceftazidime. High level resistance was more apparent with cefotaxime and ceftriaxone than with ceftazidime. Susceptibility pattern indicates possible presence of CTX-M type ESBLs amongst ESBL producers. Cefepime-tazobactam has highest *in-vitro* activity against ESBL producers than cefotaxime-clavulanic acid, ceftazidime-clavulanic acid or cefepime-clavulanic acid. This study, which has shown conjugation rate of 58.8% indicates that conjugation is perhaps only moderate but not rampant.

Strength and weakness of the study

This is the first study to undertake a multi-centric study on prevalence of ESBL-EC and ESBL-KP across Karnataka, which has also demonstrated unusually high levels of resistance to cefoxitin and cefepime. A state-wide surveillance not only reveals the prevalence and the extent of distribution of ESBLs among *E. coli* and *K. pneumoniae* but also helps in monitoring degree of resistances to beta-lactam antibiotics and beta-lactam inhibitor combinations. Although other combinations of beta-lactams and beta-lactamase inhibitors were tested, other antimicrobials like aminoglycosides, fluoroquinolones were not tested since it was beyond the scope of this study. Genotypic study on beta-lactamase gene using polymerase chain reaction would have given better picture on the prevalent ESBL type. Even though, samples were from five geographically distinct location, the results cannot be applied to the entire state.

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