BACTERIOLOGICAL PROFILE OF BLOOD CULTURES IN TERTIARY CARE HOSPITAL.

BY

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IN

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UNDER THE GUIDANCE OF

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SRI DEVARAJ URS MEDICAL COLLEGE, KOLAR

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LIST OF ABBREVIATIONS

ARF	-	Acute Renal Failure
AST	-	Antibiotic Susceptibility Testing
BSI	-	Blood Stream Infection
BBC	-	Blood Culture Broth Bottle
CA-BSI	-	Community Acquired BSI
CLSI	-	Clinical and Laboratory Standard Institute
CRP	-	C-Reactive Protein
CPU	-	Colony Forming Units
CONS	-	Coagulase Negative Staphylococcus
DIC	-	Disseminated Intravascular Coagulation
DM	-	Diabetes Mellitus
EDTA	-	Ethylene Diamine Tetraacetic Acid
ESBL	-	Extended Spectrum Beta Lactamases
HCA-BSI	-	Health Care Associated BSI
ICU	-	Intensive Care Unit
INF	-	Interferons
LBW	-	Low Birth Weight
LPS	-	Lipopolysaccharide
MDR	-	Multi Drug Resistant
MRSA	-	Methicillin Resistant Staphylococcus Aureus
MSSA	-	Methicillin Sesitive Staphylococcus Aureus
MODS	-	Multi Organ Dysfunction Syndrome
NAA	-	Nucleic Acid Amplification
NNPD	-	National Neonatal Perinatal Database
NEC	-	Necrotising Enterocolitis
PAMP	-	Pathogen Associated Molecular Patterns

PROM	-	Prolonged Rupture of Membrane
PCR	-	Polymerase Chain Reaction
PCT	-	Procalcitonin
SIRS	-	Systemic Inflammatory Response Syndrome
SPS	-	Sodium Polyanethol Sulfonate
SSSS	-	Staphylococcal Skin Scalded Syndrome
TLR	-	Toll Like Receptors
TNF	-	Tumor Necrosis Factor
UTI	-	Urinary Tract Infection
VRE	-	Vancomycin Resistant Enterococci
WHO	-	World Health Organization

ABSTRACT

TITLE OF THE STUDY: "BACTERIOLOGICAL PROFILE OF BLOOD CULTURES IN TERTIARY CARE HOSPITAL"

Bacteremia is one of the leading causes of death and the age adjusted death rate has risen by 78% over the past two decades. Due to the high morbidity and mortality associated with bacteremia, the rapid detection and subsequent identification of microorganisms from blood remain critical for clinical microbiology laboratory. Blood culture remains the most valuable tool in the diagnosis of blood stream infections. Many remarkable improvements have been made in an attempt to reduce the time to isolate pathogens from blood. Advancements in the use of liquid media linked with automation technology have enhanced the ability of laboratories to provide faster blood culture results. Following the introduction of automated BacT/Alert blood culture system in our laboratory, this study was undertaken to detect the performance of automated system in terms of microbial recovery and reduced turnaround time.

OBJECTIVES OF THE STUDY:

1. To identify the common pathogens causing bacteremia in different age groups.
2. To determine the antibiotic sensitivity pattern among the isolates.
3. To identify the risk factors associated with bacteremia.
4. To study the outcome of antibiotic therapy of patients admitted with bacteremia.

MATERIALS AND METHODS:

A total of 300 paired blood samples were collected from patients admitted at R.L. Jalappa Hospital, with signs and symptoms of sepsis and pyrexia of unknown origin

between Febraury 2012 - August 2013. Blood samples were collected with strict aseptic precautions before administration of antibiotics and were incubated in automated BacT/Alert system. Once the growth signal was given by the machine, the samples were processed and identified according to the standard laboratory techniques and were subjected to antibiotic susceptibility testing by Kirby Bauer disc diffusion method.

RESULTS:

Out of 300 paired samples processed, 115 (38.3%) were identified as pathogen and 6 (2%) as contaminants. Among the 115 culture positives, bacteremia was common among males (68%) than females (32%) and in pediatric patients (63.5%) than in adults (36.5%). The positive cultures were more among neonates (71.2%). CRP, tachypnea among pediatrics patients and tachycardia among adults were the predominant sepsis screening parameters in culture positives, whereas birth asphyxia, preterm, Low birth weight in pediatrics patients and respiratory infections, hypertension, elderly patients >65 years and GIT infections among adults were the common risk factors observed.

Among the culture positives, gram negative organisms accounted for 47%, gram positive organisms were 33% and Candida species were 26.1%. Gram positive organisms especially S.aureus was highly resistant to commonly used antibiotics and sensitive only to chloramphenicol, linezolid and tetracycline. Similarly Gram negative organisms showed resistance to commonly used antibiotics, with variable percentage of sensitivity to cabapenems, amikacin, chloramphenicol, levofloxacin, amoxyclav and pipericillin-tazobactam. Candida species were sensitive to fluconazole and itraconazole. The mortality rate observed in pediatric patients was 13.7% and 54.8% in adults.

LIST OF TABLES

Table No.	Topic	Page Nos.
1.	Common organisms isolated from blood	7
2.	Definitions	8
3.	Organisms isolated by different studies in India	10
4.	Organisms isolated by different studies worldwide	11
5.	Candida species isolated in different studies	12
6.	Organ dysfunction and shock	19
7.	Risk factors for Neonatal septicemia	22
8.	Volume of blood for blood culture	30
9.	Clinical signs and symptoms which predict sepsis	43
10.	Blood culture results from processed samples	54
11	Organisms isolated in paired samples	55
12	Time duration for culture positivity	56
13	Percentage of culture positivity among patients	58
14	Sepsis screening parameters in culture positive pediatric patients	59

15	Sepsis screening parameters in culture positive adult patients	60
16	Risk factors observed among culture positive pediatric patients	61
17	Risk factors for candidemia in pediatric patients	62
18	Risk factors observed among culture positive adult patients	63
19	Risk factors for candidemia in adult patients	64
20	Type of organism isolated from blood culture	64
21	Organisms isolated from pediatric patients	65
22	Clinical outcome of culture positive pediatric patients	66
23	Organisms isolated from adult patients	67
24	Clinical outcome of culture positive adult patients	68
25	Resistance pattern of Gram positive organisms	69
26	Resistance pattern of Gram negative organisms	70
27	Distribution of MDR strains among bacterial isolates	72
28	Sensitivity pattern of Candida species	76

LIST OF FIGURES

Figure No.	Topic	Page Nos.
1.	Pathophysiology of septic process	20
2.	Risk factors for bacteremia	21
3.	BacT/Alert 3D system	46
4.	BacT/Alert PF bottles	46
5.	Flow chart for sample processing	47
6.	Gram positive cocci in clusters	50
7.	Gram positive cocci in chains	50
8.	Gram negative bacilli	50
9.	Gram positive budding yeast cells	50
10.	Detection of MSSA	52
11.	Detection of MRSA with inducible clindamycin resistance	52
12.	ESBL production	52
13.	Inducible Amp C production	52
14.	Detection of carbapenemase production	53
15.	Modified hodge test	53
16.	Different species of candida on SDA and chrome agar	53
17.	Antifungal susceptibility testing	53

LIST OF CHARTS

Chart No.	Topic	Page Nos.
1.	Blood culture results from processed samples	54
2.	Time duration for culture positivity	56
3.	Gender distribution among culture positive cases	57
4.	Percentage of culture positivity among patients	58
5.	Sepsis screening parameters in culture positive pediatric patients	59
6.	Sepsis screening parameters in culture positive adult patients	60
7.	Risk factors observed among culture positive pediatric patients	61
8.	Risk factors for candidemia in pediatric patients	62
9.	Risk factors observed among culture positive adult patients	63
10.	Type of organism isolated from blood culture	64
11.	Distribution of MDR strains among bacterial isolates	72
12.	Antibiotic susceptibility pattern among MRSA	73
13.	Antibiotic susceptibility pattern among ESBL producers	74
14.	Antibiotic susceptibility pattern among AMP C producers	75
15.	Sensitivity pattern of candida species	76

TABLE OF CONTENTS

Sl N0.	CONTENTS	PAGE NO.
1	INTRODUCTION	1
2	OBJECTIVES	2
3	REVIEW OF LITERATURE	3
4	MATERIAL AND METHODS	43
5	RESULTS	54
6	DISCUSSION	77
7	CONCLUSION	91
8	SUMMARY	93
9	BIBLIOGRAPHY	96
10	ANNEXURES	107

INTRODUCTION:

Bacteremia is the presence of viable bacteria in the blood, as determined by its growth in a blood culture.[1] Bacteria may enter the bloodstream either from a focus of infection within the body or from a surface site with a normal flora or from the material contaminated with the environmental source leading to bacteremia.[2] Microorganisms when present in the circulating blood continuously, intermittently, or transiently, are a threat to every organ in the body.[3] Bacteremia is one of the leading causes of death and the age adjusted death rate has risen by 78% over the past two decades.[4,5]

Bacterial invasion of the blood stream can have serious immediate consequences, including shock, multiple organ failure, disseminated intravascular coagulation and death.[3,6] Septicemia is the condition where bacteria circulate and multiply in the blood and form toxic products.[7] It is a life threatening condition which is a reaction of the human immune system to a severe bacterial infection [8]

Blood culture forms a critical part of the evaluation of patients with suspected sepsis. The detection of microorganisms in a patient's blood, not only has great diagnostic and prognostic significance, but also provides essential information for the evaluation of a variety of diseases like endocarditis, pneumonia, pyrexia of unknown origin and others.[9] Isolation of the pathogens and determination of antimicrobial susceptibility pattern of the isolates remain the main stay of definitive diagnosis and management of BSIs.[10] Early detection and appropriate treatment can make the difference between life and death.[11]

Many remarkable improvements have been made in an attempt to reduce the time to isolate pathogens from blood. Advancements in the use of liquid media linked with automation technology have enhanced the ability of laboratories to provide faster blood culture results. Following the introduction of automated BacT/Alert 3D (bioMerieux) blood culture system in our laboratory, this study is undertaken to detect the performance of automated system in terms of microbial recovery and reduced turnaround time and improve patient management.

OBJECTIVES OF THE STUDY:

1. To identify the common pathogens causing bacteremia in different age groups.
2. To determine the antibiotic sensitivity pattern among the isolates.
3. To identify the risk factors associated with bacteremia.
4. To study the outcome of antibiotic therapy of patients admitted with bacteremia.

REVIEW OF LITERATURE:

INCIDENCE:

Brill reported the first case of bacteremia (due to *Bacillus pyocyaneus*, now *Pseudomonas aeruginosa*) in 1899. Ten years later fewer than 40 cases were reported worldwide, with fewer than 30 additional cases in the following 15 years. Between 1950 and 2003, however the mortality rate due to septicemia increased almost 40-fold.[1]

Sepsis is derived from the original Greek word "σηψις,'' (sepos) which means decomposition of the animal or vegetable matter. It is one of the oldest and most elusive

syndromes in medicine. With the confirmation of germ theory, sepsis was recast as a systemic infection.[12] Septicemia is the 10th leading cause of death in adults and eighth leading cause of death among infants.[1] Neonatal sepsis is a significant cause of morbidity and mortality among neonates worldwide. World Health organization has estimated that 1.6 million deaths occur globally every year due to neonatal infections and 40% of these neonatal deaths occur in developing countries[13] The incidence varies from one institution to another and from time to time in the same institution and depends on various factors predisposing to infections like low birth weight, prematurity, obstetric and nursery practices, nutritional status of the mother, environmental conditions in nurseries and hospital or community infections.[14] According to the National Neonatal Perinatal Database (NNPD) report 2002-2003, the incidence of neonatal septicemia in tertiary care institutions has been reported to be 14.5 per 1000 live births (2.3%) and ranked the second most common cause of neonatal mortality, contributes to 16% of all mortalities among the hospital born neonates.[15]

In the pre-antibiotic era, prior to 1937 the mortality from neonatal septicemia was as high as 90%. Since the advent of antibiotics and early recognition of the nonspecific signs of septicemia the morality rate has fallen significantly to 13%-45% in the 1980s and 1990s.[16] Sepsis is an increasingly common cause of mortality and morbidity even in elderly, particularly immune compromised and critically ill patients. Approximately 25-35% of patients with severe sepsis and 40-55% of patients with septic shock die within 30 days.[17]

ETIOLOGY AND ITS CHANGING PATTERN:

Over the past 25 years, the pattern of organisms responsible for bacteremia has shifted[1]. During 1930s and early 1940s, Group A β-hemolytic *Streptococcus* was the major organism isolated from septicemic newborns, which was controlled with the introduction of penicillin.[18] There has been a striking increase in the gram negative bacteremia and decrease in streptococcal and pneumococcal bacteremias over the last few decades.[2]

Throughout the 1960s and 1970s, gram negative organisms were most frequently isolated from patients with BSI.[19] In the 1980s and 1990s the pattern shifted so that most bacteremia were due to gram positive organisms, although gram negative organisms still represented a large proportion of cases.[1] Recent studies stated that gram negative organisms are more commonly isolated than gram positive organisms in both adults and neonates. Among gram negative organisms, the common organisms are *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter*, *Acinetobacter*, *Pseudomonas*, *Salmonella* and *Haemophilus influenza*. Among the gram positive organisms, Group B *streptococci*, *Coagulase negative staphylococcus*, *Staphylococcus aureus* and *Listeria monocytogens* are common.[13,17]

In addition, antibiotic resistance rates have been rising during the past 2 decades for all predominant organisms.[20] Bacteremia caused by *methicillin-resistant Staphylococcus aureus (MRSA)*, a rare entity in the 1970s, now accounts for more than 40% of all hospitalizations for *S.aureus* bacteremia and the incidence of bacteremia caused by vancomycin-resistant *Enterococci* (VRE) has also increased.[1] Among the gram negative organism, *E.coli* was the commonest organism in the past, which is being

replaced by many multi drug resistant (MDR) bacteria like *Klebsiella* , *Enterobacter* , *Salmonella, Citrobacter, Pseudomonas, Acinetobacter*.[21] In addition, gram-negative organisms expressing *extended-spectrum beta-lactamases(ESBL)* have become more prevalent, complicating the therapy of patients with bacteremia resulting from these organisms. [1] The increase incidence of MDR organism in recent years, results in longer hospitalization, greater mortality and require treatment with more expensive antibiotics.[21] Since the early 1980s, there has been an increase in the incidence and prevalence of fungal infections worldwide.[22] The frequency of candidemia has increased dramatically over the past few decades accounting for 8-10% of all HCA blood stream infections.[23] More recently, in addition to bacterial invasion of blood stream, cases attributable to fungal invasion of the blood stream (fungemia) caused by organisms such as *Candida albicans* have become increasingly important.[1] Candidemia is defined as the presence of positive blood culture for *Candida species* which was confirmed by another blood culture obtained from a different site.[24] Recent reports from our country indicate a trend towards an increasing prevalence of non-albicans candidemia. *Candida species* possess a number of virulence factors which enable the organism to cause disseminated infections in susceptible hosts with increased morbidity and mortality. Such infections are mostly observed in hospitals, mainly from intensive care units (ICUs), oncology units, organ transplants units etc., where most patients are subjected to heavy therapeutic protocols and are immunodeficient.[23]

The microbiology of bacteremia is also marked by an increasing incidence of polymicrobial bacteremia. In the 1930s, virtually every case of bacteremia involved a single organism. By the early 1990s, 10% of bacteremia involved more than one

organism. Polymicrobial bacteremia is generally associated with a higher mortality than monomicrobial bacteremia.[1] The spectrum of microorganisms invading the bloodstream has been systematically reviewed and evaluated in several studies.[25] Changes in the incidence, epidemiology and spectrum of the infecting organisms subject to geographic variations have been noted.[26]

Clinical studies of blood stream infections over the years have led to the proposal of various clinical and laboratory tools designed to aid in differentiating pathogens from contaminants. However Hall and Lyman (2006) warn that despite the high likelihood that certain organisms usually represent contaminants when isolated from blood and it can be difficult to determine the likelihood of true pathogen. It is recommended that a minimum of two sets of blood cultures should be taken at separate times from separate sites to help rule out potentially contaminated samples.[27]

Automation in microbiology first occurred in the early 1970s, which was further improved in 1990s by introducing automated continuous monitoring blood culture systems which accelerated the trend away from conventional methods like brain heart infusion broth, soybean casein digest broth, bile broth and glucose broth where the minimum turnaround time is 72 hours for identification and antibiotic susceptibility testing of the organisms.[28]

Studies reported in the 1970s, 1980s, and early 1990s suggested that two to three blood cultures from adults obtained at appropriate time during a 24-h period could detect 99% of all bloodstream infections.[29] However it is cautioned despite the high isolation rate, which may include contaminants and it could be difficult to determine the true

pathogen. It is recommended that a minimum of two sets of blood cultures should be taken at separate times from two separate sites to help rule out potentially contaminated samples.[27] According to the literature, the volume of blood per culture is the single most important variable in recovering microorganisms from patients with sepsis. The higher the blood volume cultured, the higher the rate of detection of bloodstream infections.[30]

COMMON ORGANISMS ISOLATED FROM BLOOD: [31]

The most common organisms isolated from blood among Gram positive organisms, Gram negative organisms and fungal organisms are listed in the table.

Table 1: Common organisms isolated from blood

GRAM POSITIVE BACTERIA	**GRAM NEGATIVE BACTERIA(GNB)**
• *Staphylococcus aureus* • *Coagulase negative Staphylococci* • *Streptococci* • *Streptococcus pneumoniae* • *Enterococcus species* • *Listeria monocytogenes*	• *Escherichia coli* • *Klebsiella pneumoniae* • *Citrobacter species* • *Proteus species* • *Enterobacter species* • *Salmonella species* • *Haemophilus influenzae*
NON-FERMENTING GNB	**FUNGAL ORGANISMS**
• *Psuedomonas aeruginosa* • *Acinetobacter species*	• *Candida species*

DEFINITIONS: [76]

Table 2: Definitions

Bacteremia	Presence of bacteria in blood, as evidenced by positive blood cultures
Septicemia	Presence of microbes or their toxins in blood
Systemic inflammatory response syndrome (SIRS)	Two or more of the following conditions: 1) Fever (oral temperature >38°C) or hypothermia (<36°C) 2) Tachypnea (>24 breaths/min) 3) Tachycardia (heart rate >90 beats/min) 4) Leukocytosis (>12,000/μL), leucopenia (<4,000/μL), or >10% bands; may have a noninfectious etiology.
Sepsis	SIRS that has a proven or suspected microbial etiology.
Severe sepsis (similar to "sepsis syndrome")	Sepsis with one or more signs of organ dysfunction—for example: 1) Cardiovascular*:* Arterial systolic blood pressure ≤ 90 mmHg or mean arterial pressure ≤ 70 mmHg that responds to administration of intravenous fluid 2) Renal*:* Urine output <0.5 mL/kg per hour for 1 h adequate fluid resuscitation 3) Respiratory: PaO2/FIO2 ≤ 250 or, if the lung is the only dysfunctional organ, ≤ 200 4) Hematologic: Platelet count <80,000/μL or 50% decrease in platelet count from highest value recorded over previous 3 days

	5) Unexplained metabolic acidosis: A pH ≤ 7.30 or a base deficit ≥ 5.0 mEq/L and a plasma lactate level >1.5 times upper limit of normal for reporting lab 6) Adequate fluid resuscitation: Pulmonary artery wedge pressure ≥ 12 mmHg or central venous pressure ≥ 8 mmHg
Septic shock	Sepsis with hypotension (arterial blood pressure <90 mmHg systolic, or 40 mmHg less than patient's normal blood pressure) for at least 1 hour despite adequate fluid resuscitation; OR Need for vasopressors to maintain systolic blood pressure ≥ 90 mmHg *or* mean arterial pressure ≥ 70 mmHg
Multiple-organ dysfunction syndrome (MODS)	Dysfunction of more than one organ, requiring intervention to maintain homeostasis

DATA FROM OTHER STUDIES:

Table 3: Organisms isolated by different studies in India:

Sl.no	Author	Place & Year of publication	% of positivity	Most common organisms isolated
1	Roy et al[26]	Lucknow, 2002	48.1	*Klebsiella species*
2	Kumhar et al[31]	New delhi, 2002	42	*Klebsiella species*
3	Shrestha et al[32]	Nepal, 2007	20	*S.aureus*
4	Tarai et al[33]	New delhi, 2012	PBC-13.7 SBC-4.59	*S.typhi* *CONS*
5	Murthy et al[24]	Tirupathi, 2007	Paired culture-18.7	*Klebsiella species*
6	Zakariya et al[13]	Puducherry, 2011	41.6	*Klebsiella species*
7	Qureshi et al[34]	Lahore	16.6	*E.coli*
8	Pavani et al[35]	Hyderabad, 2012	22.9	*S.aureus*
9	Chaudry A et al[36]	Tirupathi, 1999	11.8	*CONS*
10	Rekha.S et al[37]	Karnataka, 2011	50.4	*K.pneumonia*e
11	Gosalia E et al[38]	Rajkot, 2013	62	*Klebsiella species*
12	Shah MN et al[39]	Gujarat, 2011	20.87	*E.coli*
13	Jain NK et al[40]	Nepal, 2003	28.3	*E.coli*
14	Arora et al[21]	Amristar, 2007	20.02	*S.aureus*
15	Latif S et al[41]	Lahore, 2009	27.9	*Klebsiella species*
16	Rani V et al[42]	Chennai, 2011	8.39	*S.aureus*
17	Sharma et al[43]	Rohtak, 2002	33.9	*Klebsiella species*
18	Dhawan et al[44]	Chandigarh, 1993	43.2	*Streptococci viridans*
19	Mitra et al[45]	Chandigarh, 1997	5 Anaerobic bacteremia	*Clostridium species*
20	Vishwanathan et al[46]	Kolkata, 2011	13.14	*K.pneumoniae*
21	Khanal et al[47]	Puducherry, 2002	44	*S.aureus*
22	Amatya NM[48]	Kathmandur	2007	*Salmonella species*
23	Sundaram et al[49]	Chandigarh, 2009	89	*S.aureus*

Table 4: Organisms isolated by different studies worldwide

Sl.no	Author	Place and Year of publication	% of BSI	Most common
1	Meremikwu et al[50]	Nigeria, 2005	48.9	*S.aureus*
2	Rahbar et al[51]	Iran, 2005	9.1	*CONS*
3	Iregbu et al[52]	Nigeria, 2006	22	*S.aureus*
4	Gomez et al[53]	Spain, 2010	2.2	*E.coli*
5	Starakis et al[54]	Greece, 2010	13.1	*CONS*
6	Jomb et al[55]	Nigeria, 2010	6.2	*Staphylococci*
7	Schaffner et al[56]	Georgia, 2009	20	*CONS*
8	Mehdinejud et al[57]	Iran, 2009	5.6	*K.pneumonia*
9	Edmond et al[4]	Virginia, 1999	27	*CONS*
10	Ayobola et al et al[58]	Nigeria, 2011	19.3	*S.aureus*
11	Nili F et al[59]	Iran, 2008	Aerobic-74.1 Anaerobic-25.9	*CONS* *Propionobacterium*
12	Lenz R et al[60]	Canada, 2012	HA-BSI – 28 HCA-BSI – 32 CA-BSI – 40	*E.coli*
13	Ameen et all[61]	AL-Kindi Hospital, 2012	13.8	*S.typhi*
14	Nielsen et al[62]	Germany-Ghana, 2012	19.9	*Non-typhoidal Salmonella*
15	Osazuwa F et al[63]	Nigeria, 2011	28.2	*Klebsiella pneumoniae*
16	Phetsouvanh R et al[64]	Vietiane, 2006	10.7 – Community acquired	*S.typhi*
17	Pourakbari B et al[65]	Iran, 2012	10.23	*Ps.aeruginosa*

Table 5: *Candida species* isolated in different studies

Sl no	AUTHOR	Place & year of publication	% of BSI	Most common
1	Roy et al[66]	Calcutta, 1993	16.4	*C.albicans*
2	Sharma et al[23]	Haryana, 2011	21.4	*C.albicans*
3	Giri et al[22]	Newdelhi, 2012	18	*C.albicans*
4	Kumar et al[67]	Chennai, 2005	5.7	*C.albicans*
5	Goel et al[68]	Rohtak, 2009	8.1	*C.albicans*
6	Xess et al[69]	AIIMS, Newdelhi, 2007	6	*C.albicans*
7	Sahni et al[70]	Maulana Azad, Newdelhi, 2005	6.9	*C.albicans*
8	Verma et al[71]	Lucknow, 2003	4.14	*C.tropicalis*
9	Kothari et al[72]	Newdelhi, 2009	18	*C.tropicalis*
10	Das et al[73]	Brimingham-UK, 2010	10.9 episodes/1000 bed days	*C.albicans*
11	Roy et al[26]	Lucknow, 2002	6.8	*Not specified*
12	Rahbar et al[25]	Iran, 2005	1	*C.albicans*
13	Kumhar et al[31]	Newdelhi,2002	2.43	*Not specified*
14	Starakis et al[54]	Greece, 2010	3	*C.albicans*
15	Sardana et al[74]	Uttarpradesh, 2012	26.4	*C.tropicalis*
16	Edmond et al[4]	Virginia, 1999	8.4	*C.albicans*
17	Pavani et al[35]	Hyderabad, 2012	0.8	*Not specified*
18	Viswanathan et al[46]	Kolkota, 2011	5.3	*C.albicans*
19	Prakash et al[75]	Gujarat, 2012	6.25	*C.tropicalis*

CLASSIFICATION OF BSI

A. Based on mechanisms by which bacteria enter the bloodstream:[77]

1) Transient bacteremia

Organisms, like members of the normal flora, when introduced into the blood through minimal trauma to membranes cause transient bacteremia.

Examples include brushing of teeth, vigorous chewing, straining during bowel movements, and minor medical procedures like dental extraction etc.[7]

2) Intermittent Bacteremia

It occurs when bacteria from an infected site are periodically released into the blood (spill-over effect) such as from extravascular abscesses, spreading cellulitis, or infections of the body cavities like empyema, peritonitis or septic arthritis. It may also be a phase in the natural course of some infections such as meningococcal, gonococcal, and pneumonia infections and others.[1]

3) Continuous bacteremia

It usually occurs when the infection is intravascular, such as infected endothelium (bacterial endocarditis or aneurysms) or infected hardware (arterio-venous fistulas, intra-arterial catheters, or indwelling cannulas) and also infections like typhoid fever, brucellosis, etc.[3]

B. Based on the site of origin

1) Primary BSI

Bacteria from an endovascular source such as an infected cardiac valve or intravenous (IV) catheter.[1]

2) Secondary BSI

A distant site other than an IV catheter can be established as the point of origin. The distant site is usually confirmed to be the focus if the same pathogen with the same resistance pattern is isolated from that site and the bloodstream.[19]

C. Based on place of acquisition:[60,78]

Traditionally was classified as Community acquired and Health care associated BSIs.[60]

1) Community acquired BSI (CA-BSI)

BSI detected within the first 48 h of admission, in patients without having been hospitalized within the past 30 days and without a history of undergoing an invasive procedure either just before or at the time of admission.

2) Health-care associated BSI (HCA-BSI)

1. BSI detected within the first 48 h of admission, in recently discharged patients (2-30 days prior to the recent episode of BSI)

2. Patients with recent history of invasive procedures (insertion of Foleys catheter, IV line, placement of long-term IV device, or dialysis). 10

3. BSI detected ≥48 hours after hospital admission or within 1 day of discharge from the hospital.

PATHOPHYSIOLOGY:

Pathway to sepsis:

The pathogenesis mainly depends on the invading pathogen, the portal of entry, and the immune status of the patient. The skin and mucous membrane of the gastrointestinal, genitourinary and respiratory tract, act as the natural physical barrier to the invading pathogens. However, indwelling catheters, intravenous cannulas and urinary catheters in hospitalized patients can cause disruption of normal skin or mucosal barriers leading to bacterial invasion and cause infection.[1,79]

Severe sepsis is triggered by bacteria or fungi that usually do not cause systemic disease in immunocompetent hosts, but can occur when there are deficiencies in innate host defences (e.g. phagocytes, complement, and natural antibodies) to survive within the body. However, some microorganisms are able to circumvent innate defenses by producing toxins or other virulence factors. In both immunocompetent and immunocompromised hosts, the body can fail to kill the invaders despite mounting a vigorous inflammatory reaction and can result in severe sepsis.[76]

Sepsis is a complex interaction of both pro-inflammatory and anti-inflammatory responses, as well as disturbed hemostasis and coagulation system.[80] The

pathophysiological hallmark of sepsis is the mismatch of the host response to the pathogenic stimuli, leading to organ injury or dysfunction, with or without hypotension. This results in a predominantly Systemic Inflammatory Response Syndrome (SIRS) or mixed antagonistic response syndrome or compensatory anti-inflammatory syndrome.[81]

Host mechanisms for sensing microbes:

A series of pathogenic events occur from the onset of infection to the transition from sepsis to severe sepsis/ septic shock. Immune system has sensitive mechanisms for recognizing and responding to microbial pathogens.[76]

The recognition of an invading pathogen by the host cell mainly depends on:[82]

1. Pathogen associated molecular patterns (PAMPs)
2. Pattern recognition receptors (PRRs)

The PRRs like CD14, Toll like receptors (TLR), expressed by the immune cells, recognizes and binds to PAMPs of pathogens. This in turn activates the intracellular signal pathway and produces pro and anti-inflammatory cytokines. Pro-inflammatory cytokines causes damage to the host tissues, whereas the anti-inflammatory response causes down-regulation of the macrophage and interferes with the phagocytic function. During this time, circulatory abnormalities (vasodilatation, edema) occurs which causes an imbalance between oxygen delivery and consumption.[80,82,83] Tissue hypoxia and cellular hypoxia develop, leading to multiple organ dysfunction and irreversible shock.[80,83] The septic response may also be induced by microbial exotoxins that act as superantigens.[76]

Local and systemic host responses to invading microbes:[76]

Local response begins with recognition of pathogens by tissue phagocytes and production and/or release of numerous host molecules (cytokines, chemokines, prostanoids, leukotrienes, and others) that increase blood flow to the infected tissue, enhance the permeability of local blood vessels, recruit neutrophils to the site of infection. Systemic responses are activated by neural and/or humoral communication with the hypothalamus and brainstem; these responses enhance local defenses by increasing blood flow.

1) Cytokines and other mediators :[84]

Cytokines can exert endocrine, paracrine, and autocrine effects. TNF-α stimulates leukocytes and vascular endothelial cells to release other cytokines, and to express cell-surface molecules. Thus, enhances neutrophil-endothelial adhesion at sites of infection, and to increase prostaglandin and leukotriene production. Blood levels of TNF-α are elevated in individuals with severe sepsis or septic shock, but not with localized infections. Chemokines, most prominently interleukins IL-8 and IL-17, IL-1β, interferon (IFN) γ, IL-12 are also involved.

2) Coagulation factors:

Intravascular thrombosis promoted by IL-6 and other mediators, a hallmark of the local inflammatory response, may help to wall off invading microbes, thus prevent infection and inflammation from spreading to other tissues. IL-6 and other mediators promote intravascular coagulation. Sepsis causes activation of contact system activation, leading to development of hypotension.[76]

Control mechanisms :[76]

Elaborate control mechanisms occur within both local sites of inflammation and the systemic compartment. The anti-inflammatory processes benefits the host in important ways.

1) Local control mechanisms:

Host recognizes the pathogen, and kills them rapidly and the anti-inflammatory forces neutralize the microbial signals. Among these molecules are lipopolysaccharide (LPS), intracellular factors (example: suppressor of cytokine signaling 3 and IL-1 receptor associated kinase) diminishes the production of pro-inflammatory mediators by neutrophils and macrophages; anti-inflammatory cytokines (IL-10, IL-4); and molecules promote tissue restoration.

2) Systemic control mechanisms:

Systemic responses to infection diminish cellular responses to the pathogens. Anti-inflammatory cytokines increases in all, even in patients with mild infections. The acute-phase response increases the blood concentrations of numerous molecules like IL-1 receptor antagonist, protease inhibitors or antioxidants and hepcidin that have anti-inflammatory actions.

Organ dysfunction and shock:[76,85]

The body's response to infection and pathologic mechanism in sepsis which leads to multi organ failure is a complex process. High concentrations of both pro- and anti-inflammatory molecules are found to be involved in the process. The two important

factors leading to organ failure are persisting vasodilation leading to hypotension, endothelial injury and organ dysfunction.

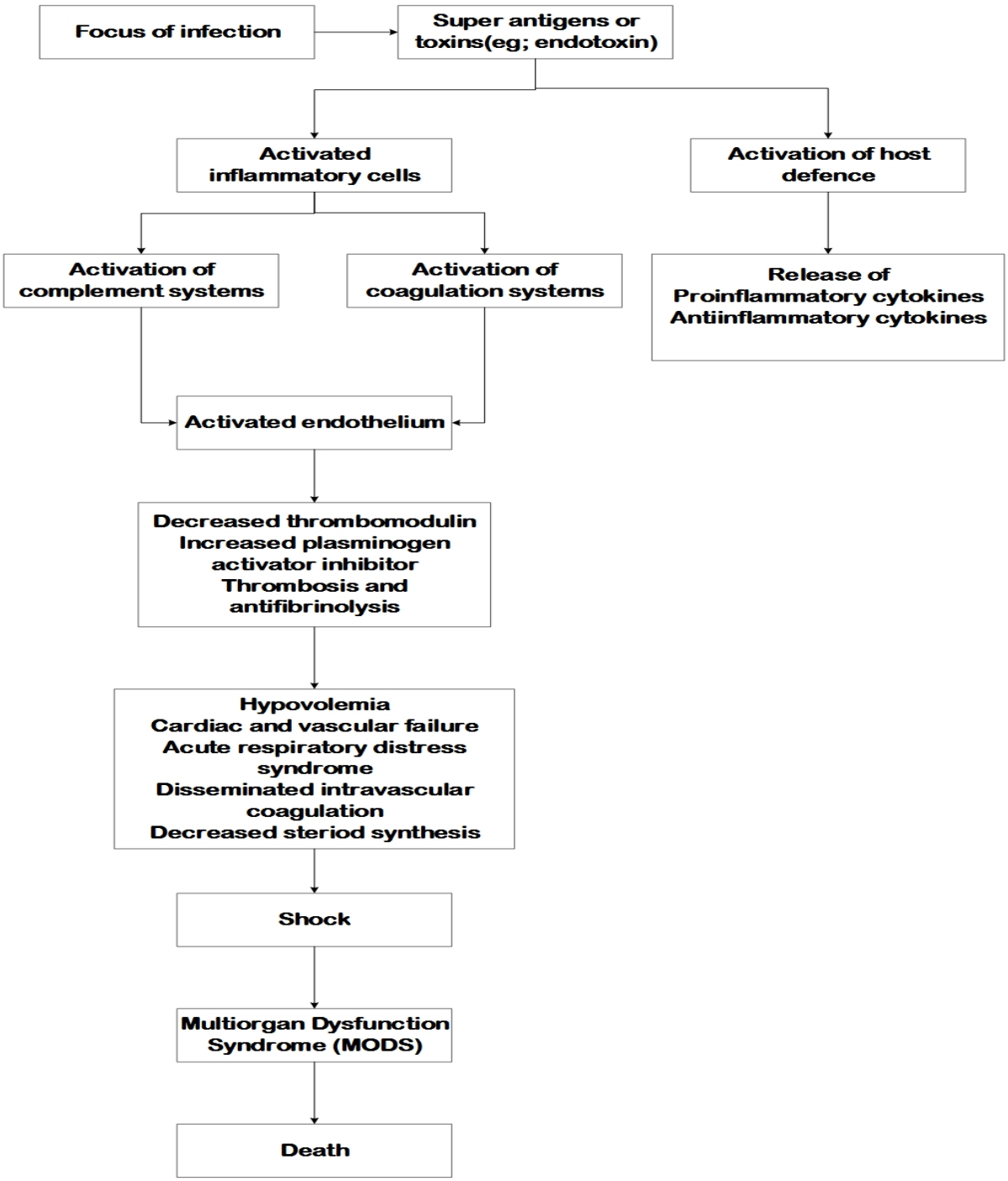
Table 6: Organ dysfunction and shock

Organ system involved	Effects on organ	Damaging effect on body
Vascular system	Vasodilatation	Hypotension, organ hypoperfusion
Heart	Myocardial depression	Decreased cardiac output and BP, hypoperfusion
Kidneys	Acute renal failure	Decreased urine output, accumulation of toxins
Lungs	ARDS	Hypoxia
Liver	Hepatic failure	Accumulation of toxins, hepatic encephalopathy
Brain	Encephalopathy	Altered mental status
Coagulation system	DIC	Bleeding and clotting

An understanding of the pathogenic mechanisms is vital as emerging key therapies to decrease mortality in severe sepsis are directed at reversing these mechanisms.

PATHOPHYSIOLOGY OF SEPTIC PROCESS: [87]

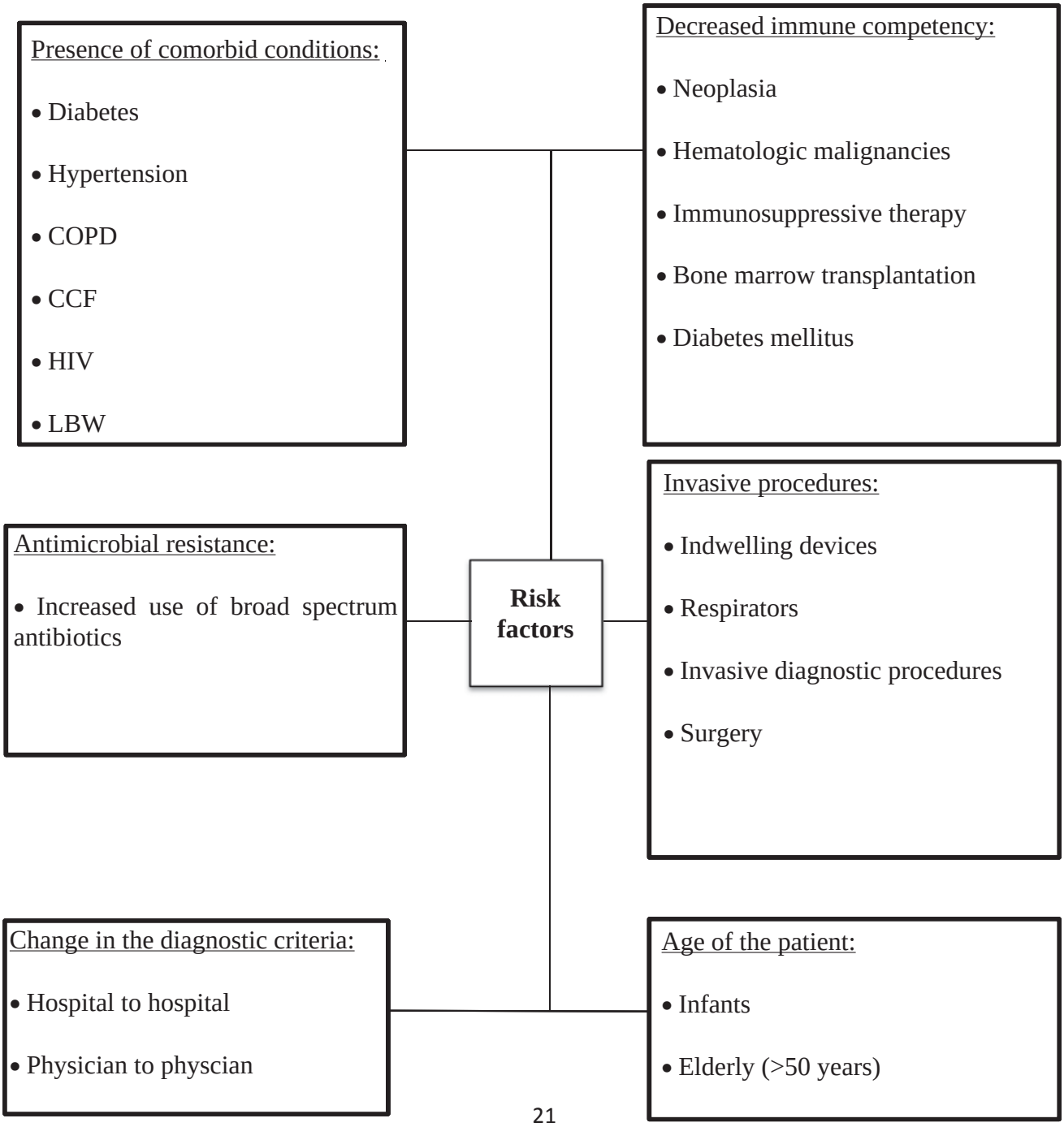
Figure 1: Pathophysiology of septic process



RISK FACTORS:

During the past 25 years, increase in the incidence of bacteremia has been observed due to the following conditions.

Figure 2: Risk factors for bacteremia



RISK FACTORS FOR NEONATAL SEPTICEMIA:

The pathogenesis of neonatal septicemia is **multifactorial**. These interact together in causing neonatal septicemia.

Table 7: Risk factors for Neonatal septicemia

PRENATAL/MATERNAL RISK FACTORS:	**NEONATAL FACTORS:**
- Prolonged rupture of membranes (PROM) - Chorioamnionitis - Foul smelling liquor - Unclean vaginal examination - Prolonged labor - Maternal colonization of Group B streptococci - Mode of presentation – abnormal presentation, difficult labor, instrument delivery - Type and place of delivery	- Prematurity - Low birth weight - Male sex - Twins - Congenital anomalies - Birth asphyxia - Difficult resuscitation - Skin wounds
NOSOCOMIAL RISK FACTORS:	**AGENT FACTORS:**
- Prolonged length of hospital stay - Degree of crowding	- Dose and virulence of microbes

- Invasive procedures - Lack of hand washing by hospital personnel - Indiscriminate use of prophylactic antibiotics	**OTHER FACTORS:** - Geographical factors - Socio economic status

RISK FACTORS IN ADULTS:

- Old age > 65 years
- Underlying diseases
- Association of comorbid conditions
- Malignancies
- Immunosuppressive chemotherapy
- Steroids
- Bone marrow transplantation
- Diabetes mellitus
- Cirrhosis
- Increased invasive procedures

RISK FACTORS FOR CANDIDEMIA:[22,67,70]

- Exposure to long term antibiotics
- Prolonged hospital stay
- Mechanical ventilation
- Intravascular catheters and central venous catheters
- Malignancies and cancer chemotherapy

- Neutropenia
- Steroid therapy
- Diabetes mellitus
- Ventricular assist devices
- Candida colonization – endogenous or exogenous
- Candiduria
- Prior surgery and risk of candidemia
- Total parentral nutrition
- Topical use of antifungals
- Low birth weight and prematurity in neonates

CLINICAL FEATURES:[1,76,87]

Severe BSIs lead to the clinical manifestations of sepsis as a consequence of the complex interplay among the immune, coagulation and neuroendocrine systems. The classic signs and symptoms include abrupt onset of chills, rigors, hyperthermia or hypothermia, tachycardia, tachypnea.

EARLY STAGES:

Alterations in temperature regulations, hyperthermia or hypothermia are seen. The absence of fever is most common in neonates, in elderly patients, and persons with uremia or alcoholism. Hyperventilation is often an early sign of the septic response.

LATE STAGES:

Hypotension is observed more commonly in adults. In children, it is a late finding, but not used as a criteria for diagnosis of shock. Delayed capillary refill, diminished peripheral and central pulses, cool extremities, decreased urine output, disorientation, confusion, lethargy anxiety, other manifestations of encephalopathy, and coma can also be noted due to poor cardiac output.

CUTANEOUS MANIFESTATIONS:

Petechiae, diffuse erythema, ecchymoses, ecthyma gangrenosum, and symmetric peripheral gangrene can be observed in septic patients.

GASTROINTESTINAL AND HEPATOBILIARY SYSTEM:

Gastrointestinal manifestations like nausea, vomiting, diarrhea, and ileus may suggest acute gastroenteritis. Stress ulceration can lead to upper gastrointestinal bleeding. Cholestatic jaundice, due to elevated levels of serum bilirubin (mostly conjugated) and alkaline phosphatase may precede other signs of sepsis. Prolonged or severe hypotension may induce acute hepatic injury or ischemic bowel necrosis.

CLINICAL SYNDROMES ASSOCIATED WITH SEPTICEMIA:[37]

Meningitis, Pneumonia, Urinary tract infection (UTI), Necrotising enterocolitis (NEC), Conjunctivitis, Otitis media and Osteomyelitis, etc.

LABORATORY DIAGNOSIS:

Identification of BSIs is crucial, because treatment has to be initiated early. Early and prompt diagnosis and the provision of appropriate antimicrobial therapy correlate with positive clinical outcome and reduced mortality.[5,11,24] There is no specific diagnostic test to detect septic response.[76] The diagnosis of BSIs is based on a combination of clinical, hematological, biochemical and microbiological criteria.

The different diagnostic modalities used in detecting BSI are described under different headings as follows:

I) Blood Culture

II) Hematology

III) Biochemistry

IV) Biomarker Assays

V) Arterial Blood Gas Analysis

VI) Imaging

VII) Molecular Techniques

I) BLOOD CULTURE :[1,3,8,77]

Blood culture remains the gold standard method for diagnosing BSIs. Detection of BSIs traditionally has been one of the most important functions of clinical microbiology laboratory. Culturing and identification of pathogenic microorganism from blood is a

highly specific indicator of BSI, and performing antimicrobial susceptibility testing and reporting plays an essential role in early, rapid and appropriate treatment, which reduces mortality. Thus, the clinical microbiology laboratory plays a significant role in the management of patients with BSI.

A "Blood Culture" is defined as culture of blood obtained from a single venipuncture, whether that blood is inoculated into 1/ multiple bottles. If >1 bottle is inoculated with each venipuncture, it is referred to as a "Blood Culture Set".[89]

To detect BSI, patient's blood must be obtained by aseptic venipuncture and then inoculated into culture media and incubated. Even though with antiseptic techniques used while collecting blood, the contamination rate of blood cultures is between 1-3%. Therefore it is important to prepare the skin properly before venipuncture.[1,77] The success of isolating bacterial pathogens from blood depends upon the quantity of blood, frequency of culture and duration of incubation. Bacterial growth can be detected using techniques ranging from manual to totally automated methods. Once growth is detected, the organism is isolated, identified by different biochemical reactions, and tested for its antimicrobial susceptibility.[3]

SPECIMEN COLLECTION:

a) PREPARATION OF THE SITE:[3,77]

Careful skin preparation before collecting the blood sample is of paramount importance to reduce the risk of introducing contaminating organisms from skin into the blood. The venipuncture site should ideally be prepared as follows –

A) Wash hands with soap

B) Rinse with sterile water and dry

C) Wear gloves

D) The vein from which the blood is to be drawn should be chosen by touching the skin before it is disinfected.

E) If the patient has an existing IV line, blood should be drawn below the existing line, not the above the line because it will be diluted with the fluid being infused.

b) ANTISEPSIS:

a) After selection of vein, the site should be cleansed with 70% isopropyl alcohol for 30 minutes. It is done in a circular manner approximately 5cm in diameter beginning at the center to the periphery and allowed to dry.

b) 2% tincture iodine/ povidine-iodine is applied starting from the center, in ever-widening circles saturating it with iodine and is allowed to air dry for 1 minute.

c) If the site has to be palpated again after preparation, palpate with the gloved finger. Then disinfect the gloved fingers used for palpation in identical fashion.

d) Sterile needle is inserted into the vein and the blood is drawn which is injected into the culture bottle.

e) The rubber stopper or septum of the container should be disinfected before inoculation of the blood.

f) After the needle is withdrawn, the site must be cleansed with 70% alcohol again. Blood cultures may be obtained either by using a needle and syringe or by a closed system, consisting of a vacuum bottle and double-needle collection tube.

VOLUME OF BLOOD AND BLOOD-BROTH RATIO:

Adequate volume is the most important factor in the detection of microbes, and there is a direct relation between the volume of blood and the yield. More the volume of the blood, there is greater chance of isolating the organism.[3] Blood to broth ratio is considered ideal if it is 1:5 to 1:10 dilutions to reduce the concentration of any therapeutically administered antibiotics and also to reduce the bactericidal and bacteriostatic constituents. This gives optimal yield of the organisms.[3,28]

Cockerill III FR et al have found 57.9% increase in pathogen recovery when 40 ml of blood was cultured as compared to 10 ml.[89]

Adults:

Bacteremia in adults has a low number of colony-forming units (CFU) per milliliter (ml) of blood. Because of direct relationship of blood for growth of the organism, it is strongly recommended 10-20 ml of blood per culture.[3]

Children:

Withdrawing large amounts of blood for culture in neonates is not only technically difficult but also not feasible because of the neonate's size and the necessity for treatment in many cases within hours after birth. Also neonates when infected usually have a high magnitude of bacteremia. Blood from septic children may yield fewer than 5

CFU/ml of the organisms, but less than 1 ml may not be adequate to detect the pathogens. But smaller volumes should still be cultured because of high levels of bacteremia (more than 1000 CFU/ml) in some infants. The most widely accepted sample size in neonates is 0.5 – 1 ml collected before starting antibiotics.[3,61]

NUMBER AND TIMING OF BLOOD CULTURES:

The number of blood culture sets that detects growth, especially when measured as a function of the total number obtained, has proved to be a useful tool in interpreting the significance of positive blood cultures.[90] If adequate volume is obtained, usually 2 or 3 blood cultures are sufficient. The timing of cultures is not as important as other factors, as the organisms are released into the bloodstream at a constant rate. But in case of intermittent bacteremia, the timing cannot be predicted, it is accepted that two or three blood cultures spaced an hour apart.[3]

Blood cultures should be drawn before the use of systemic antimicrobials. Fever is a delayed response to the bacteremia; it makes sense to collect the samples as soon as possible after a fever spike.[77]

Table 8: Volume of blood for blood culture[1]

Age	Amount
Younger than 10 years	1ml of blood for each year of life
10 years or older	20 ml
10 years or older with poor veins	Less than 20 ml

CULTURE MEDIA:[1,3,8,77]

Diverse and a large number of media are used in blood culture bottles which are nutritionally enriched and are multipurpose. These include:

A) Brain heart infusion broth

B) Brain heart infusion broth with cooked meat particles

C) Trypticase soy broth

D) Supplemented peptone

E) Thioglycollate broth

F) Casteneda biphasic medium

G) Bile broth

F) Glucose broth

ANTICOAGULANTS :

Bacteria may get entrapped in the blood clot and may go undetected. Thus most commercial blood culture media contain anticoagulants. Sodium Polyanethol Sulfonate (SPS, Liquoid) in concentrations of 0.025% to 0.05% is the best anticoagulant for blood culture. In addition to its anticoagulant properties, it is also anti-complementary and anti-phagocytic and inactivates certain antibiotics including aminoglycosides. However, it may also inhibit the growth of a few microorganisms, such as *Neisseria* spp, *Gardianella*

vaginalis, *Streptobacillus moniliformis* and *Peptostreptococcus anaerobius*. This effect can be neutralized by the addition of 1% gelatin to the medium.[3,77]

Culture media commonly used for subculture

i. Blood agar

ii. Mac Conkey agar

iii.Chocolate agar

CULTURE TECHNIQUES:[1,3,28,77]

I) CONVENTIONAL BLOOD CULTURES:

By this method, blood cultures normally need to be incubated for a minimum period of 7 days, which is substantially long enough to generate the growth of any significant bacteria. The blood culture bottles are incubated at 35^0 C, for 6 – 18 hours. In addition daily visualization of the bottles for growth (as indicated by hemolysis, turbidity or gas production) is required. Also, blind subculture is performed after 6 – 12 hours, onto blood agar, Mac Conkey's agar and Chocolate agar and the plates are incubated for 48hours. After 48 hours of incubation of the bottle, a second blind culture is repeated. The bottles are incubated for 5 – 7 days with serial blind cultures, before discarding as culture negative. Castaneda biphasic media is used to avoid contamination while subculturing.

II) SYSTEMS FOR PROCESSING BLOOD CULTURES:

1. MANUAL BLOOD CULTURE SYSTEMS:

a) OXOID SIGNAL SYSTEM:

A single bottle blood culture system that uses the production of CO2 to detect early bacterial growth. It has a main blood culture bottle that is connected by means of a long needle to a second plastic chamber (signal chamber) and it is positioned below the surface of the culture medium. The metabolization of the growing bacteria produce CO2, which results in an increase in pressure which forces liquid from the bottle into the signal chamber. This can be visualized and evaluated further by doing Gram's stain and subculture.

b) BBL SEPTI-CHEK BLOOD CULTURE SYSTEM:

It consists of standard blood culture broth bottle, connected to a second plastic chamber that contains a trisurface paddle with Chocolate, Mac Conkey and Malt agar surfaces. After the blood sample is inoculated, the plastic-contained slide is screwed on. To subculture, the bottle is inverted allowing broth to enter the slide's chamber, and flooding the agar surface. This does not require opening of the bottle, hence contamination will be less. The bottle is then placed upright for further incubation and it is followed at regular intervals. A large volume of broth is subcultured and hence the faster detection of organisms.

2. LYSIS-CENTRIFUGATION BLOOD CULTURE SYSTEM:

WAMPOLE ISOSTAT ISOLATOR MICROBIAL SYSTEM: It is a widely accepted alternative method for blood culture, useful for fastidious

organisms and slow growing organism. It is also used as a method of choice for filamentous fungi, dimorphic fungi, Malassezia fufur, and Legionella species. This system consists of a stoppered tube containing saponin to lyse blood cells, polypropylene glycol to decrease foaming, SPS as an anticoagulant, and EDTA to chelate calcium ions. Around 7.5 to 10 ml of blood is added to the tube and inverted several times to allow lysis, and it is then centrifuged at 3,000 rpm for 15 minutes to concentrate any microorganisms if present. Then the sediment is aspirated and subcultured to solid agar. The benefits of this system include the more rapid and greater recovery of fastidious organisms, the ability to quantify the CFU/ml present in the blood, rapid detection of polymicrobial bacteremia, possible greater recovery of intracellular organisms and ability to choose special media. However, contamination rates are quite high in this system.

3. AUTOMATED CONTINUOUS MONITORING BLOOD CULTURE SYSTEMS:

Automated system plays an important role in clinical microbiology practice by giving rapid results. These systems can rapidly and accurately detect organisms in the blood, can process many samples at one time, and also provide results cost effectively.

a) THE BACT/ALERT BLOOD CULTURE SYSTEM:

It is a fully automated system which measures CO2 with the help of CO2-sensitive chemical sensor which is present at the bottom of each bottle. The sensor is separated from the medium by a unidirectional membrane which is permeable

only to CO2. When the organisms grow in the blood-broth mixture, CO2 is liberated, which diffuses across the membrane, and dissolves in the water and liberates free hydrogen ions which cause a color change in the sensor (blue to light green to yellow as the pH decreases). This change is picked up by the computer and a visual/ audible 'alert' is generated. The positive bottle is indicted by flag on the screen, which can be removed for further processing.

b) BACTEC SYSTEMS:

BACTEC was the first automated detection system which is a semi-automated. Now BACTEC 9240 and BACTEC 9120 were introduced, which is a fully automated system. It detects the growth of microorganism using radiolabeled carbon (14C) in the broth medium. It is similar to BacT/Alert by appearance, but there is difference in the method of detecting CO2. This method detects by means of fluorescence to measure CO2. Organisms in the blood utilize the 14C-labelled substrates in the bottle, and releases CO2 which diffuses into the sensor and hydrogen (H+) ions are generated. This decreases the pH, which increases the fluorescent output of the sensor.

c) THE TREK ESP CULTURE SYSTEM II:

This system differs from the BacT/ ALERT and the BACTEC 9240/ 9120 systems in the following ways:

i) CO2 production is monitored manometrically.

ii) Both gas consumption and production by the organisms are monitored.

iii) Changes in the concentration of O2 and H2 along with CO2 are detected. These gases are detected by monitoring head space pressure changes.

The advantages of continuous monitoring blood culture system include a decrease in labor work, a decrease in number of contamination because of reduced handling of the bottles, and significant increase in rapid detection and also increase in recovery rates. Limited data base, limited media selection options, space constraint as the systems are large in size and its cost are the main disadvantages.

IDENTIFICATION OF THE ORGANISMS FROM AUTOMATED CONTINUOUS MONITORING SYSTEM:

The positively flagged bottle should be removed from the instrument. A small quantity of fluid is removed from the bottle and is used for Gram stain and subcultured onto Blood agar, Mac conkey agar and chocolate agar respectively. The Gram stained smear is examined and morphological description and initial identification as Gram positive or Gram negative cocci/bacilli is informed to the clinicians, for empirical antibiotic therapy. The growth in the subcultured plates may take 24 to 48 hours, and it is identified and antibiotic susceptibility testing is performed.

INTERPRETATION OF RESULTS:

There is an increasing incidence of BSIs caused by bacteria that are normally considered non-virulent or by commensal flora. It is difficult to identify the pathogens from contaminants. Reporting contaminants can lead to unnecessary antibiotic therapy, increased length of hospital stay and higher costs.

On the other hand ignoring of these presumed contaminants might lead to dire consequences.

However, few criteria to be followed while reporting:

1) PROBABLE TRUE BACTEREMIA :
    a. Growth of virulent organisms such as members of *Enterobacteriaceae*, *S. pneumoniae*, *S. pyogenes, S. aureus, P.aeruginosa, and Candida albicans* almost always indicate true infection.
    b. Growth of same organisms repeatedly at different times or from different sites.
    c. Isolation of commensal flora from patients with predisposing factors such as immunocompromized status, prosthesis, indwelling lines.
2) PROBABLE CONTAMINATION :
    a. Growth of organisms such as *Bacillus* species (other than *Bacillus anthracis*), *Corynebacterium* species, *CONS*, *Propionibacterium* acnes in only one of blood culture sets.
    b. Polymicrobial growth (usually uncommon).
    c. Lack of reproducibility in subsequent cultures.
    d. The organism isolated is not same at a primary site infection and from blood culture.
    e. Clinical presentation is not of sepsis.

Struthers et al have shown in their study that by doing 2 blood cultures, the number of cases misdiagnosed with *CONS* as cause of BSI was reduced and this resulted in 8.2% of unwarranted antibiotic usage 60.

LIMITATIONS OF BLOOD CULTURES:

a. Delayed reporting – positive results require hours to days of incubation.

b. No single medium or system has been shown to be best suited for recovery of all organisms.

c. Some pathogens grow poorly or not at all in conventional systems.

Relevant samples such as Pus, Cerebro-Spinal Fluid (CSF), Body fluids, Aspirates, Sputum, Urine, Genito-urinary secretions and others have to be cultured as indicated, to aid in the interpretation of results of blood cultures.

II) HEMATOLOGICAL PARAMETERS: [77,87]

1. Thrombocytopenia
2. Leukocytosis with a left shift/leukopenia
3. Elevated neutrophils with toxic granules, Dohle bodies, cytoplasmic vacuoles
4. Increased immature forms
5. Neutropenia (omnious sign of overwhelming sepsis)
6. Prolonged prothrombin and partial thromboplastin times
7. Decreased fibrinogen levels
8. Increase in ESR

III) BLOOD BIOCHEMISTRY:[77,87]

Deranged Liver and Renal function tests:

1. Hyperbilirubinemia
2. Increase levels of aminotransferases
3. Hyperglycemia/hypoglycemia
4. Hypocalcemia

5. Hypoalbuminemia
6. Protinuria

IV) BIOMARKER ASSAYS:

1. C-REACTIVE PROTEIN (CRP)

CRP is a rapidly responsive acute phase reactant, an abnormal β-globulin synthesized by the liver within 6-8 hours of an inflammatory stimulus. The levels may rise to more than 1000 fold during an acute-phase response. Elevation of CRP has been a useful marker for the early diagnosis of septicemia in 50-90% of cases although; sensitivity and negative predictive values are not high enough for CRP alone to be a definitive diagnostic test. CRP is a sensitive index that has diagnostic, therapeutic and prognostic value.[91]

2. PROCALCITONIN (PCT):

It is a precursor of calcitonin, used for detection and follow up of therapy. Procalcitonin is produced by many cells after pro-inflammatory stimulation like bacterial infection. In healthy individuals, levels of PCT are ≤0.05 ng/ml, but in case of severe sepsis and septic shock levels may increase upto 1000 ng/ml. Its level rises with increase in severity of infection and returns to normal level as the bacterial infection resolves. Thus, it acts as an early and highly specific marker for sepsis.[92,93]

V) ARTERIAL BLOOD GAS ANALYSIS:

1. During early sepsis, hyperventilation induced respiratory alkalosis occurs.
2. Later, lactic acidosis (metabolic acidosis with increased anion gap) typically supervenes.

3. Hypoxemia that is initially correctable but later becomes refractory to 100% oxygen, indicating right-to-left shunt.

VI) DIAGNOSTIC IMAGING :

1) Chest and abdominal radiography:
2) Ultrasonography
3) Computed tomography
4) Magnetic resonance imaging

These modalities help to assess for unrecognized sources of infection in the sinuses, lungs, liver, abdomen, central nervous system and other hidden foci.

VII) MOLECULAR TECHNIQUES:

Molecular amplification techniques have been developed to replace the incubation step in blood culture.

a. POLYMERASE CHAIN REACTION (PCR) :

It amplifies the DNA target by basic thermal cycles, 10^6 to 10^9 times, so that a single target molecule can be detected.

b. NUCLEIC ACID PROBES :

It is a hybridization method based on the ability of the nucleic acid strands that have complementary base sequences to specifically bind with each other and form a double stranded molecule or duplex hybrid. The hybrid is detected by standard methods.

Definitive etiological diagnosis requires the identification and isolation of organisms from blood. At the present time, other than 'Blood Culture', no laboratory test result is sufficiently reliable to warrant sepsis.

TREATMENT:[1,76,87]

Successful management of sepsis cases requires urgent measures in treating the infection, by providing hemodynamic and respiratory support, and also by eliminating the organisms. It is essential to start these measures within 1 hour of presentation of the patient with sepsis. Therefore rapid assessment and diagnosis is essential.

ANTIMICROBIAL THERAPY:

Although antibiotics remain the main stay of treatment in sepsis, it is administered empirically, as per the antibiotic policy of the hospital, before confirming it by positive blood culture. The initial choice of antibiotics is based on predisposing risk factors, clinical syndrome, immune status of the patient, recent environmental exposures (e.g., hospitalization that might have resulted in colonization by resistant organisms), and prior antimicrobial therapy. Preferably, it is started with broad-spectrum antibiotics and a combination of agents to cover the possible pathogens.

After identification of the organisms by culture along with their antimicrobial susceptibility, the initial therapy can be stepped down by selecting out the most appropriate antibiotics for the pathogen responsible. This will minimize the adverse effects and also emergence of resistance mechanism. Removal or drainage of a focal source of infection is essential. Indwelling IV catheters have to be removed, and the tip

sent for culture and new catheter should be inserted at a different site after institution of antimicrobial therapy along with supportive therapy.

PROGNOSIS:[76]

Around 20-35% of patients with severe sepsis and 40-60% with septic shock die within 30 days. Mortality in sepsis mainly depends on the initial site of infection, severity of bacteria, presence of multi organ dysfunction (MODS) and the host immune response.

PREVENTION:[76,87]

Prevention is the best method to reduce mortality and morbidity.

1. Immunization can be given for some infections like *H.influenzae* type b and S.pneumoniae for all infants.
2. Penicillin prophylaxis in pneumoccal infections.
3. Reducing the number of invasive procedures.
4. Limiting the use of indwelling vascular and bladder catheters.
5. Avoiding indiscriminate use of antibiotics and steroids.

MATERIALS AND METHODS:

STUDY DESIGN - Observational study.

SOURCE OF DATA - Blood samples collected from the patients admitted to R.L.Jalappa Hospital, Kolar with signs and symptoms of sepsis.

DURATION OF STUDY - February 2012- August 2013 (1 year 6 months)

METHOD OF COLLECTION:

Sample size: 300

Inclusion criteria:

1) Suspected cases of septicemia.

2) Fever of unknown origin.

CLINICAL SIGNS AND SYMPTOMS WHICH PREDICT SEPSIS:[76,87,94]

The patients were included in our study according to the signs and symptoms mentioned below

Table 9: Clinical signs and symptoms which predict sepsis

Pediatrics and Adults	Neonates
1) Oral temperature of >38^{0}C or <36^{0}C. 2) Respiratory rate >24/min or PaCo2 <32 mm Hg. 3) Heart rate >90/min. 4) Leukocyte >12000/mm2 or <4000/mm2 or >10% band forms. **Presence of any of the 2 signs**	1) Poor feeding and poor activity 2) Respiratory distress, grunting and Chest retractions 3) Seizure 4) Lethargy 5) Bulging anterior fontanel 6) Hyperthermia/Hypothermia

indicates sepsis.	7) Tachycardia 8) Tachypnea 9) Weak pulse 10) Vomiting or loose stools 11) Cyanosis **<u>Screening tests:</u>** 1) TLC <5000/mm3 2) Absolute neutrophil count <1800/mm3 3) Immature to total neutrophil ratio >0.2 4) Increased CRP 5) Increased ESR **Presence of one or more of the above signs along with one positive screening test indicates sepsis.**

Exclusion criteria: Patient already on antibiotics treatment.

SPECIMEN COLLECTION:

Standard aseptic precautions were followed before collection of blood, along with preparation of blood collection set.

SELECTION OF SITE:

Peripheral site was selected, preferably antecubital fossa. If only one peripheral site is available and if the patient had a central vein catheter in place, the second sample was obtained from it.

ANTISEPSIS:[1,3,77]

1) The site was disinfected with 70% isopropyl alcohol by cleansing in concentric circles approximately 5cm in diameter beginning at the center of site to periphery. Allowed to air dry.
2) Then the skin was disinfected with 10% povidine-iodine in ever-widening circles until the entire circle has been saturated with iodine and allowed to air dry for one minute before venesection.
3) The plastic caps of the BacT/Alert bottles were removed, and the rubber stopper of the bottle was cleansed using swab impregnated with 70% isopropyl alcohol and allowed to air dry.

VOLUME OF BLOOD COLLECTED;

The volume of blood collected was based on blood to broth ratio. We used BacT/Alert bottles of 20 ml and 30ml for pediatric and adult patients respectively. Blood was collected to suffice 1:5 ratio for adults and 1:10 ratio for pediatric patients. The

volume of 5-6 ml of blood from adults, 1-2 ml from infants and 2-3 ml from pediatric patients were obtained and inoculated into BacT/Alert bottles accordingly.

Figure 3: BacT/Alert 3D system

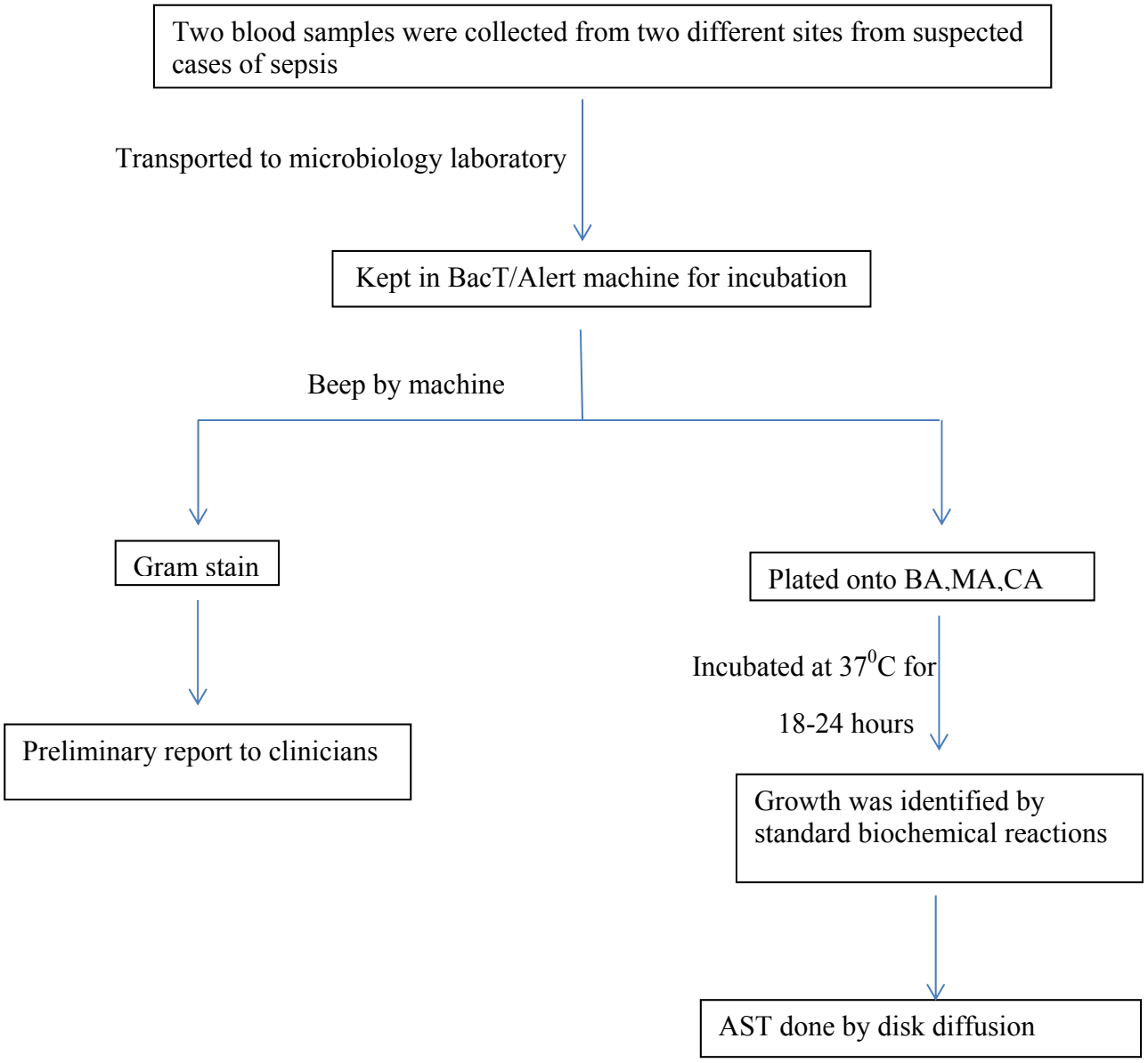
Figure 4: BacT/Alert Bottles

PROCESSING OF SAMPLE:

The inoculated BacT/Alert bottles, were then placed into the automated BacT/Alert 3D system (bioMerieux) which works on the principle of colorimetry. This system continuously monitors the bottle for growth in every 10 minutes. The microorganisms in the blood, metabolize the substrates present in the culture medium and liberates CO2. This causes color change of the gas-permeable sensor (blue green to yellow), which is present at the bottom of each bottle and machine gives a signal for growth. The positively flagged bottle was removed from the system and subjected for Gram staining and subcultured onto Blood agar, Mac-conkey agar and Chocolate agar respectively which is

further incubated at 37^0C for 18-24 hours for growth. The bottles which did not yield any growth for 7 days were reported as no growth.

Figure 5: Flow chart for sample processing



INTERPRETATION OF GRAM'S STAIN:[1]

The Gram stain smear was then examined to look for the morphology of the organisms, such as Gram positive cocci/Gram negative bacilli/Gram positive budding yeast cells. The gram stain report was conveyed immediately to the treating physicians for preliminary management of cases.

CULTURE AND IDENTIFICATION:

The plates which was kept for incubation for growth, was examined the next day (ie. After 18-24 hours of incubation). The colony morphology was noted and the smear was prepared from the colonies, Gram stain was performed and examined to confirm the preliminary report. Based upon the microscopy and the colony morphology, the organism was processed and identified by different biochemical reactions. If *Candida species* is grown, it is further subcultured onto SDA and Chrome agar plates and species was identified by Gram stain, germ tube test and growth on chrome agar.

DIFFERENT TESTS USED FOR IDENTIFICATION OF THE ORGANISMS:

GRAM POSITIVE COCCI IN CLUSTERS:

1. Catalase test
2. Coagulase test – Slide and tube coagulase
3. Mannitol fermentation
4. Urease test

GRAM POSITIVE COCCI IN PAIRS (*ENTEROCOCCI*) :

1. Bile esculin
2. Mannitol fermentation
3. Grouping - by latex agglutination test

GRAM POSITIVE COCCI IN CHAINS:

ALPHA HEMOLYTIC STREPTOCOCCI:

1. Bile solubility
2. Optochin sensitivity

BETA HEMOLYTIC STREPTOCOCCI:

1. Streptococcal grouping by latex agglutination test
2. CAMP test for Group B streptococci
3. Hippurate hydrolysis for Group B streptococci

FOR GRAM NEGATIVE ORGANISMS:

GRAM NEGATIVE BACILLI:

1. Catalase test
2. Oxidase test
3. Indole test
4. Mannitol motility test
5. Triple sugar iron agar test
6. Christensens Urease test
7. Simmons Citrate test
8. Lysine iron agar
9. Slide agglutination test for *Salmonella species*

H.INFLUENZAE:

1. Growth only on chocolate agar

2. Satellitism

CANDIDA SPECIES:

1. Germ tube test
2. Chrome agar

INTERPRETAION OF GRAM STAIN:

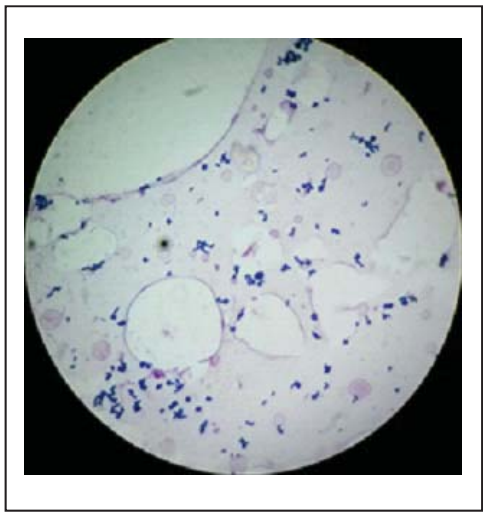


Figure 6: Gram positive cocci in clusters

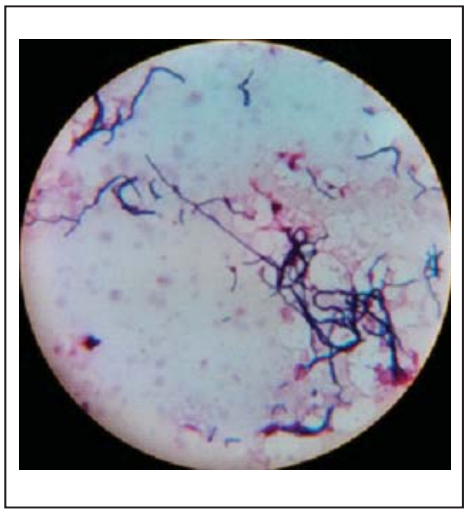


Figure 7: Gram positive cocci in chains

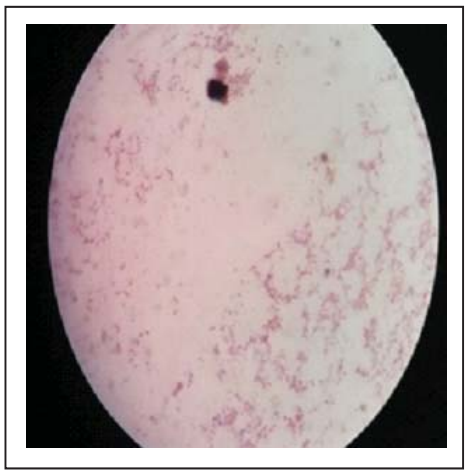


Figure 8: Gram negative bacilli

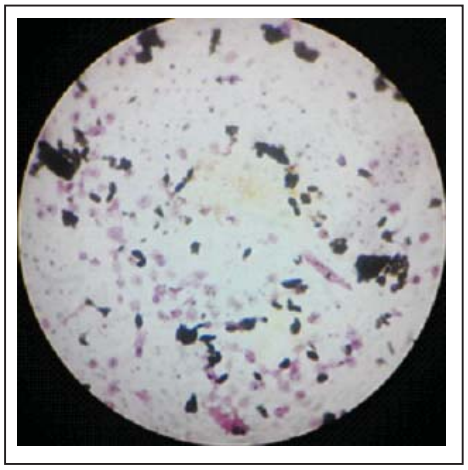


Figure 9: Gram positive budding yeast cells

ANTIBIOTIC SUSCEPTIBILITY TESTING:

Antibiotic susceptibility testing was done on Muller Hinton agar by Kirby-Bauer disk diffusion method as per annexure. Interpretation of the zone of inhibition and reporting were done according to CLSI guidelines 2012 as mentioned in the annexure.[96]

DETECTION OF MRSA AND MSSA:[96]

Susceptibility of S. aureus to Methicillin was determined using Cefoxitin (30µg) disc.

A zone diameter >22mm was considered as sensitive, and <22 mm was considered as resistant and interpreted as MSSA and MRSA respectively.

DETECTION OF ESBL:[96]

Double disc synergy test was done using ceftazidime disk alone and in combination with clavulanic acid, was performed for detection of extended spectrum beta-lactamase (ESBL) among the members of *Enterobacter*iaceae. >5mm increase in zone of inhibition for ceftazidime-clavulanic acid disc compared to ceftazidime disc was taken as ESBL producers.

DETECTION OF Amp C β LACTAMASE:[96]

Detection of Amp C β lactamase was done by placing Cefotaxime, Cefoxitin and Cefritaxone discs with a distance of 25mm from centre to centre of the disc. A flattening / indentation towards the cefoxitin disc and no zone around the cefoxitin disc was interpreted as AmpC β-lactamase producers.

DETECTION OF CARBAPENAMSES:[96]

Modified Hodge test was carried out for detection of carbapenemase only for the members of the family *Enterobacter*iaceae as per CLSI guidelines.

ANTIFUNGAL SUSCEPTIBILITY TESTING:[97]

Antifungal susceptibility testing done as per NCCLS guidelines and reported as mentioned in the annexure:

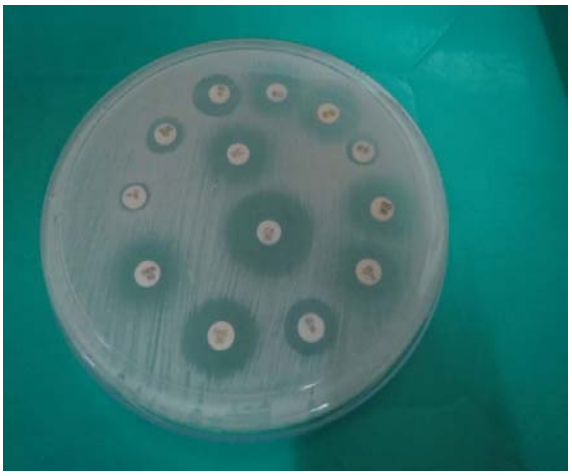


Figure 10: Detection of MSSA

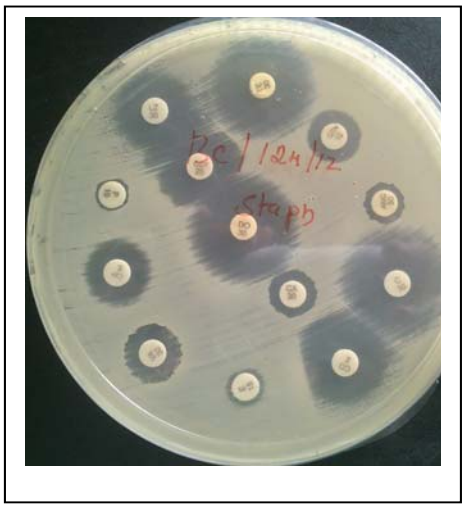


Figure 11: Detection of MRSA with inducible clindamycin resistance

Figure 12: ESBL production

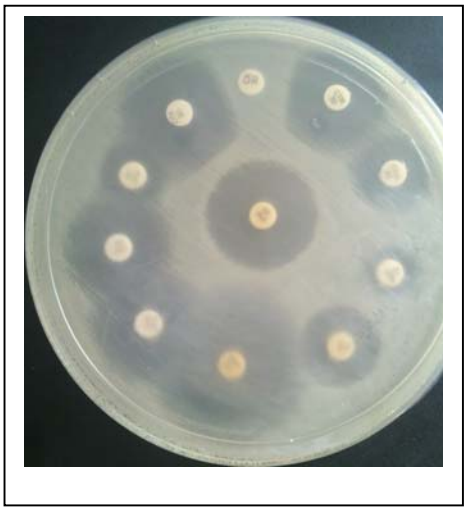


Figure 13: Inducible Amp C production

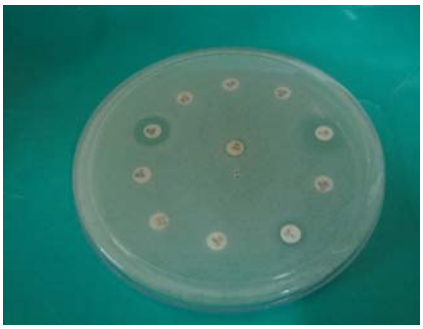


Figure 14: Detection of carbapenemase production

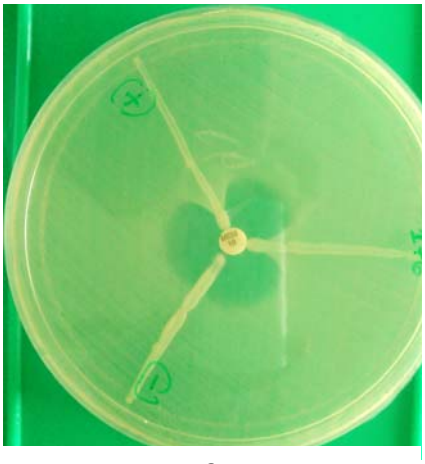


Figure 15: Modified hodge test

Figure 16: Different species of candida on SDA and chrome agar

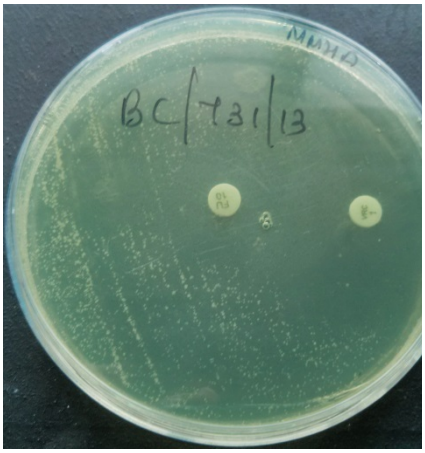


Figure 17: Antifungal susceptibility testing

RESULTS:

During the study period, a total of 300 paired blood culture samples were collected from the patients admitted to R.L. Jalappa hospital, Kolar as per the inclusion criteria.

Out of 300 paired blood culture, 179 (59.7%) samples yielded no growth, 115 (38.3%) were identified as pathogens and 6 (2%) were identified as contaminants.

Table 10: Blood culture results from processed samples:

Total (300)	Percentage
No growth (179)	59.7%
Growth (115)	38.3%
Contaminants (6)	2%

Chart 1 : Blood culture results from processed samples

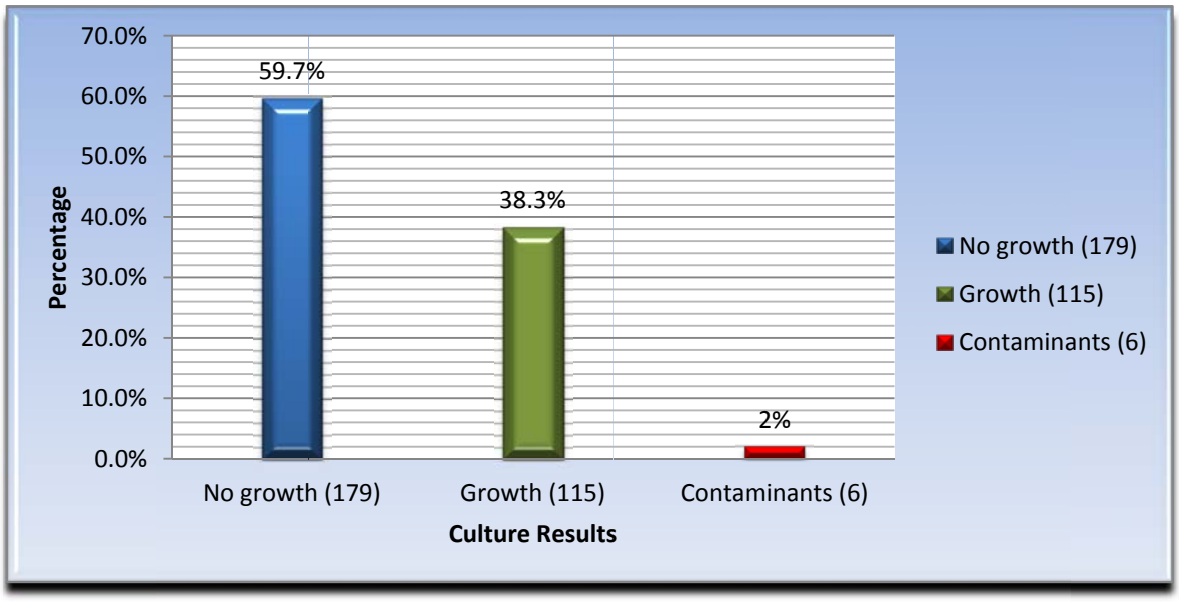


Table 11: Organisms isolated in paired samples

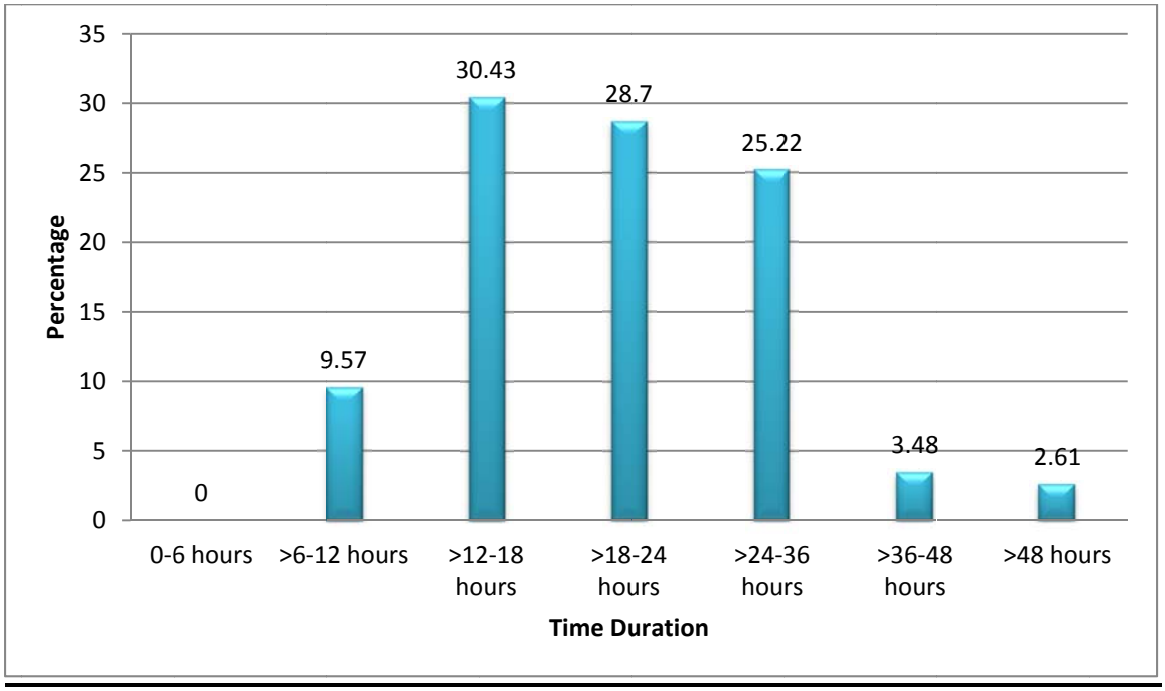
Isolates	1st sample	2nd sample
Candida species.	30	30
S.aureus	23	23
Klebsiella species.	11	11
E.coli	10	10
CONS	8	2
Acintobacter species	7	7
Enterococci	6	6
Enterobacter	5	5
Salmonella species.	5	5
Ps.aeruginosa	5	5
St.viridans	3	3
Gp-A Streptococci	3	3
Citrobacter species.	3	3
Gp-B Streptococci	1	1
H.influenzae	1	1
Total	121	115

We observed that out of 300 paired blood culture, 121 blood cultures yielded growth in the 1st sample, whereas only 115 blood culture yielded growth in the 2nd sample (paired sample). The samples which yielded the same organisms in paired sample were considered as pathogens, whereas the 6 samples which did not yield any growth in 2nd sample were considered as contaminants. The organisms which were identified as contaminants were *CONS*, 1 sample from pediatric patient and another 5 samples were from adult patients.

Table 12: Time duration for culture positivity

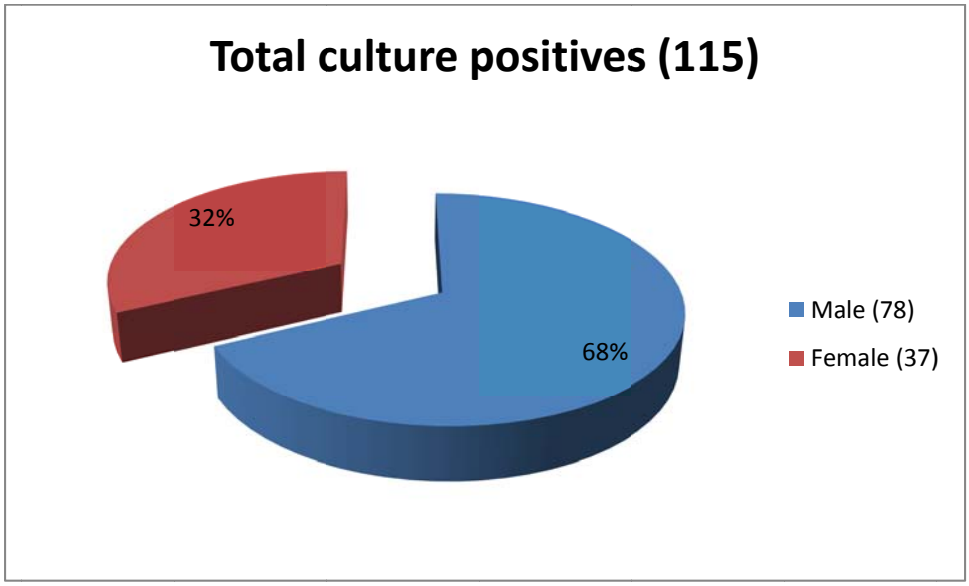
Time Duration for positivity	Total	Percentage
0-6 hours	0	0
>6-12 hours	11	9.57%
>12-18 hours	35	30.43%
>18-24 hours	33	28.7%
>24-36 hours	29	25.22%
>36-48 hours	4	3.48%
>48 hours	3	2.61%

Chart 2: Time duration for culture positivity



Majority of our blood cultures (68.7%) were detected by BacT/Alert within 24 hours, among which 9.57% were detected within 12 hours and 30.43% were detected within >12-18 hours and 28.7% in 18-24 hours. 25.22% growth were detected by >24-36 hours and 3.48% by >36-48 hours, whereas 2.61% were detected after 48 hours. The organisms which were detected after 48 hours were 2 *Salmonella species* and 1 *Candida species*.

Chart 3: Gender distribution among culture positive cases:

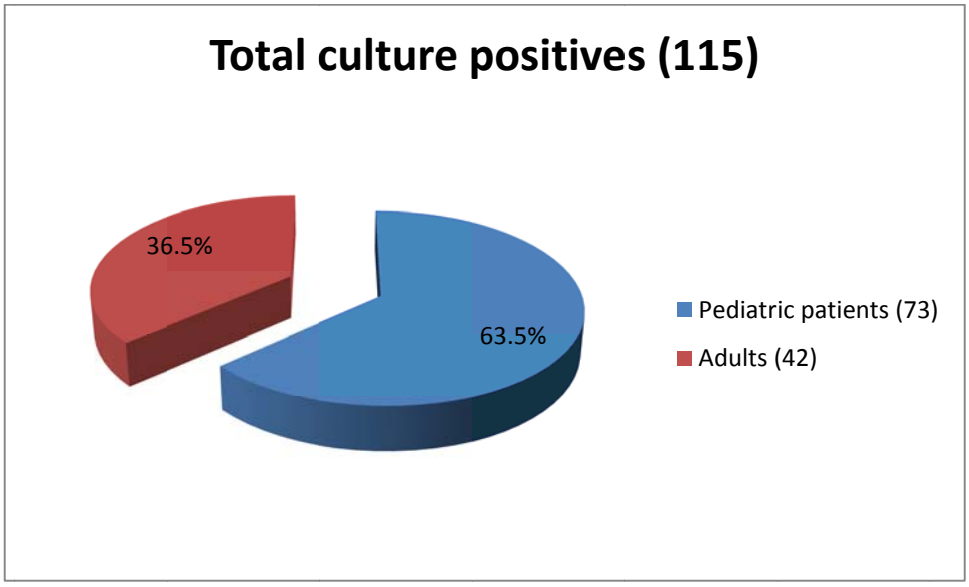


Of the 115 culture positives, 68% were from males and 32% were from females.

Table 13: Percentage of culture positivity among patients

Age group	Count	Percentage
Pediatric patients	73	63.50%
Adults	42	36.50%
Total	115	100%

Chart 4: Percentage of culture positivity among patients



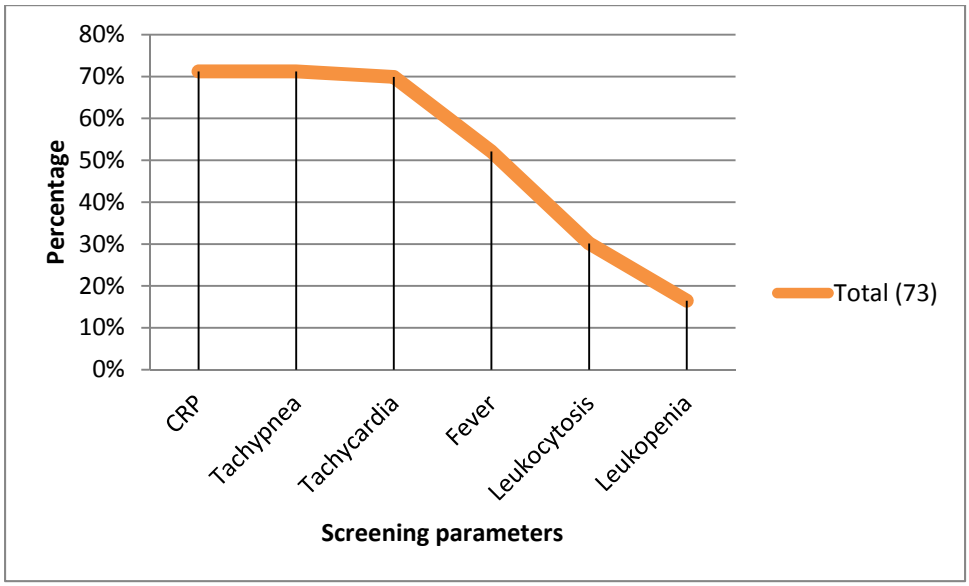
Among 115 blood culture positives, 2/3rd were from pediatric patients and 1/3rd from adults.

Table 14: Sepsis screening parameters in culture positive pediatric patients

Among 73 culture positive pediatric patients following screening parameters were observed.

Screening parameters	Percentage
CRP	71.2%
Tachypnea	71.2%
Tachycardia	69.9%
Fever	52.1%
Leukocytosis	30.14%
Leukopenia	16.44%

Chart 5: Sepsis screening parameters in culture positive pediatric patients



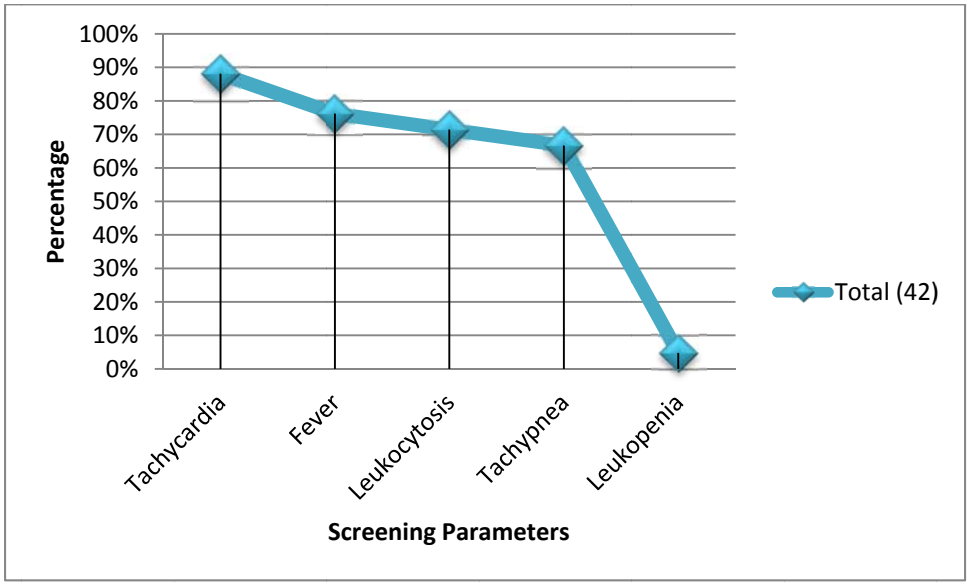
CRP and tachypnea were the predominant sepsis screening parameters, followed by tachycardia and fever.

Table 15: Sepsis screening parameters in culture positive adult patients:

Among 42 culture positive adult patients following screening parameters were observed.

Screening parameters	Percentage
Tachycardia	88%
Fever	76.20%
Leukocytosis	71.43%
Tachypnea	66.67%
Leukopenia	4.76%

Chart 6: Sepsis screening parameters in culture positive adult patients:

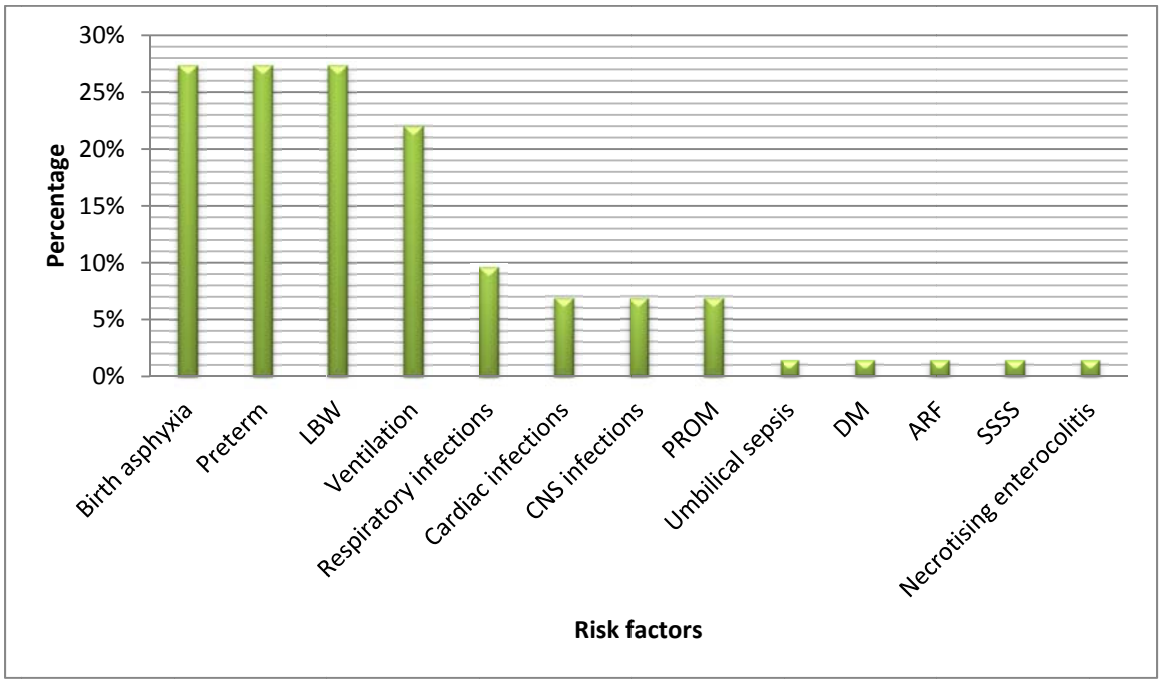


Majority of the adult patients with bacteremia had tachycardia, follwed by fever and leukocytosis.

Table 16: Risk factors observed among culture positive pediatric patients

Risk factors	Percentage
Birth asphyxia	27.4%
Preterm	27.4%
LBW	27.4%
Ventilation	22%
Respiratory infections	9.58%
Cardiac infections	6.85%
CNS infections	6.85%
PROM	6.85%
Umbilical sepsis, DM, ARF, SSSS, Necrotising enterocolitis	1.40%

Chart 7: Risk factors observed among culture positive pediatric patients:

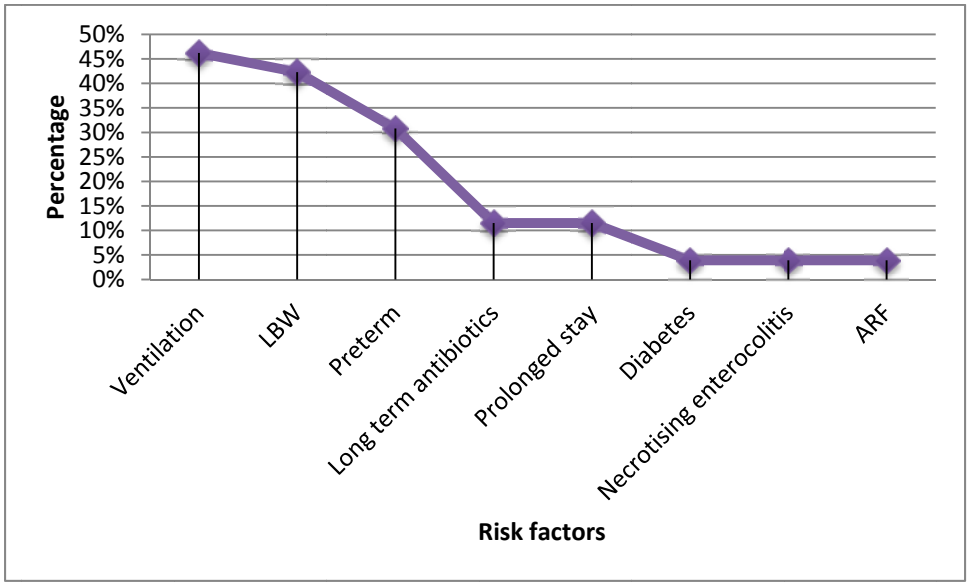


Among the 73 culture positives in pediatric patients, birth asphyxia, preterm babies, LBW were the common risk factors identified, followed by ventilated babies

Table 17: Risk factors for Candidemia in pediatric patients:

Risk factors	Percentage
Ventilation	46.20%
LBW	42.30%
Preterm	30.80%
Prolonged antibiotics	11.50%
Prolonged stay	11.50%
Diabetes	3.90%
Necrotising enterocolitis	3.90%
ARF	3.90%

Chart 8: Risk factors for Candidemia in pediatric patients:

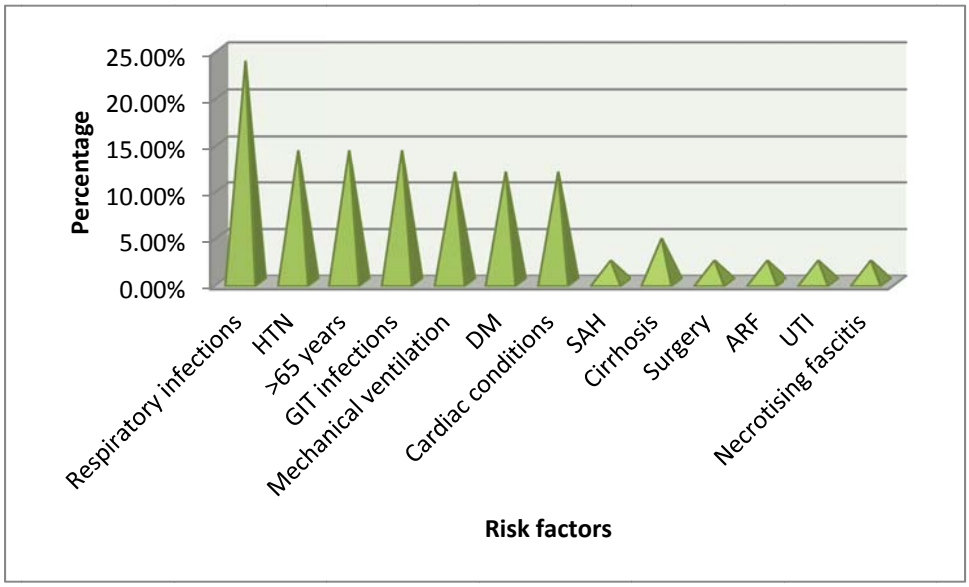


The risk factors among the pediatric patients with candidemia were evaluated and found that ventilation, LBW and preterm babies were associated with candidemia.

Table 18: Risk factors observed among culture positive adult patients

Risk factors	Percentage
Respiratory infections	23.80%
HTN	14.28%
>65 years	14.28%
GIT infections	14.28%
Mechanical ventilation	11.90%
DM	11.90%
Cardiac conditions	11.90%
SAH	2.38%
Cirrhosis	4.76%
Surgery	2.38%
ARF	2.38%
UTI	2.38%
Necrotising fascitis	2.38%

Chart 9: Risk factors observed among culture positive adult patients



Respiratory infections followed by hypertension, >65 years and GIT infections were the common risk factors associated with bacteremia among adults.

Table 19: Risk factors for candidemia in adult patients:

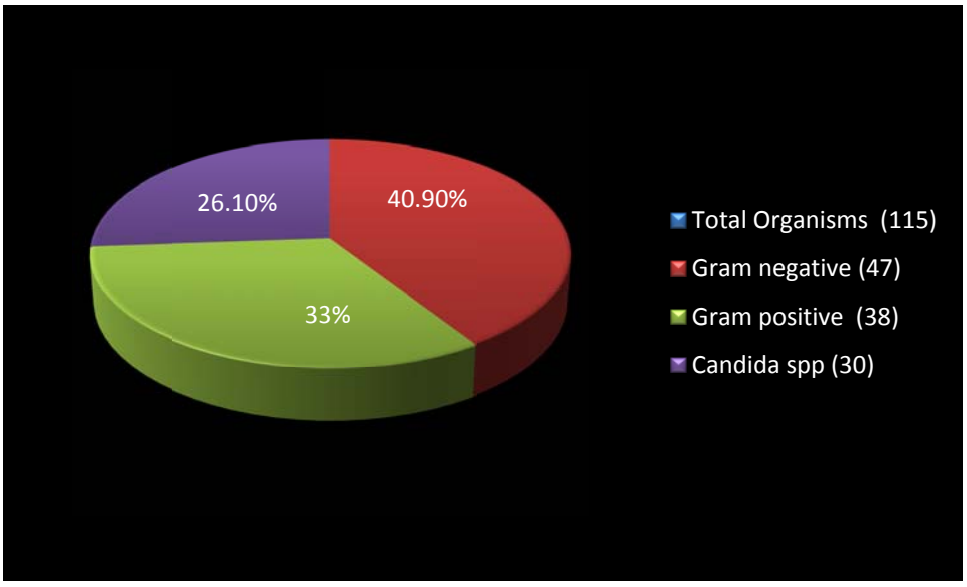
Risk factors	Percentage
Peritonitis	50%
Diabetes	25%
Ventilation	25%

The Risk factors for Candidemia in adults were observed as peritonitis, diabetes and ventilation.

Table 20: Type of organism isolated from blood culture:

Total Organisms (115)	Percentage
Gram negative organisms(47)	40.90%
Gram positive organisms (38)	33%
Candida species (30)	26.10%

Chart 10: Type of organism isolated from blood culture:



Out of 115 blood culture positives, gram negative organisms were 47 (40.9%), gram positive organisms were 38 (33%) and 30 (26.1%) were *Candida species*.

Table 21: Organisms isolated from pediatric patients:

Organisms	0-1 m	1m-1y	>1 - 5y	>5-18y	Total(73)
Gram positive organism N=22 (30.14%)					
S.aureus	9	3	0	1	13
CONS	0	2	0	0	2
St.viridans	0	2	0	1	3
Enterococci	2	1	1	0	4
Gram negative organisms N=25 (34.25%)					
E.coli	1	1	0	0	2
Klebsiella species.	6	1	0	0	7
Enterobacter	2	0	0	1	3
Citrobacter species.	1	0	0	0	1
H.influenzae	0	1	0	0	1
Salmonella species.	0	0	1	3	4
Ps.aeruginosa	4	0	0	0	4
Acintobacter species	3	0	0	0	3
***Candida species* N=26 (35.62%)**					
C.tropicalis	5	1	0	2	8
C.krusei	9	0	0	0	9
C.albicans	8	0	0	0	8
C.parapsilosis	1	0	0	0	1
Total (%)	**51 (69.9%)**	**12 (16.4%)**	**2 (2.7%)**	**8 (11%)**	**73(100%)**

The more culture positives (69.9%) were from 0-1 months of age with C.species being more common followed by Gram negative organism and gram positive organisms.

Table 22: Clinical outcome of culture positive pediatric patients

Outcome	Recovered	Expired	DAMA	Discharge at request	Referred to higher center
0-1 months	29	8	13	1	0
>1months-1 year	8	1	1	1	1
>1 -5 y	3	-	-	-	-
>5-18 years	5	1	1		1
Total (73)	45 (60.3%)	10 (13.7%)	15 (20.6%)	2 (2.74%)	2 (2.74%)

The mortality rate among pediatric patients was observed as 13.7%.

Adult patients:

Table 23: Organisms isolated from adult patients

Organisms	>18-45y	>45-65y	>65y	Total
Gram positive organisms N=16 (38.1%)				
S.aureus	6	3	1	10
Enterococci	2	0	0	2
Gp-A Streptococci	1	2	0	3
Gp-B Streptococci	0	1	0	1
Gram negative organisms N=22 (52.4%)				
E.coli	1	5	2	8
Klebsiella species	3	0	1	4
Enterobacter species	2	0	0	2
Citrobater species	1	1	0	2
Salmonella species	1	0	0	1
Acinetobacter species	*3*	*1*	*0*	*4*
Ps.aeruginosa	0	0	1	1
***Candida species* N=4 (9.5%)**				
C.tropicalis	2	0	0	2
C.krusei	0	0	1	1
C.albicans	0	1	0	1
Total (%)	22 (52.4%)	14 (33.3%)	6 (14.3%)	42 (100%)

Patients with 18-45 years of age yielded maximum growth with 52.4% showing *S.aureus* as the common organisms isolated.

Table 24: Clinical outcome of culture positive adult patients:

Outcome	Recovered	Expired	Discharged at request
18-45 years	10	12	-
45-65 years	6	8	-
>65 years	2	3	1
Total (42)	18 (42.9%)	23 (54.8%)	1 (2.38%)

>50% mortality was observed among adults, probably due to the underlying diseases.

Antibiotic sensitivity pattern:

Table 25: Resistance pattern of Gram positive organisms:

Organism	P (%)	AMP (%)	CX (%)	AMC (%)	E (%)	CD (%)	CIP (%)	COT (%)	TE,DO (%)	LZ (%)	C (%)	GEN (%)
S.aureus (n=23)	100	-	82.6	82.6	95.75	47.8	65.2	70	21.7	0	0	65.2
CONS (n=2)	100	-	100	100	100	50	0	50	50	0	0	50
Enterococci (n=6)	100	100	-	-	83.4	-	83.4	-	16.6	0	-	33.3
Streptococci viridans (n=3)	0	0	-	-	100	-	66.7	66.7	33.3	0	0	0
Gp-A Streptococci (n=3)	0	0	-	-	100	-	33.3	66.7	33.3	0	0	0
Gp-B Streptococci (n=1)	0	0	-	-	0	-	0	0	0	0	0	0

S.aureus showed higher resistance to penicillin (100%), followed by erythromycin (95.75%), amoxyclav (82.6%), cotrimoxazole (70%), ciprofloxacin (65.2%), and gentamycin (65.2%) and showed 100% sensitivity to chloramphenicol and linezolid.

Streptococci organisms were 100% sensitivity to penicillin, ampicillin, chloramphenicol, gentamicin and linezolid and shows resistance to erythromycin (66.7%), cotrimoxazole (44.5%), ciprofloxacin (33.33%), and tetracycline (22.2%).

Enterococci were 100% resistant to penicillin and ampicillin and 83.4% to ciprofloxacin and erythromycin, whereas it was sensitive to other antibiotics like linezolid (100%), tetracycline (83.4%) and gentamycin (66.7%).

Table 26 : Resistance pattern of Gram negative organism:

Organism	*E.coli* (n=10)	*Klebsiella species*p (n=11)	*Entero bacter* spp (n=5)	*Citro bacter species* (n=3)	*Salmo nella species* (n=5)	H.influ enza (n=1)	Ps.aeru ginosa (n=5)	Acineto bacter spp (n=7)
AMP(%)	90	100	100	100	80	0	-	-
CAZ(%)	80	90.9	100	100	-	0	100	85.7
CTX(%)	80	90.9	100	100	0	0	100	85.7
CX(%)	40	27.3	40	33.3	-	-	-	-
CTR(%)	80	90.9	100	100	0	0	-	85.7
CXM(%)	80	90.9	100	100	-	-	-	-
PI(%)	80	90.9	100	100	-	-	-	85.7
AMC,PIT(%)	40	27.3	40	33.3	-	0	0	14.3
CIP(%)	100	45.5	60	33.3	0	0	60	85.7
LE(%)	80	36.4	20	33.3	0	0	20	28.6
COT(%)	80	45.5	60	66.7	0	0	100	14.3
TE(%)	70	27.3	60	66.7	0	0	60	0
C(%)	0	9.1	20	66.7	0	0	-	-
AK(%)	10	9.1	0	33.3	-	-	0	14.3
GEN(%)	50	63.6	100	100	-	-	40	57.1
TB(%)	50	63.6	100	100	-	-	40	57.1
IPM,MRP ,ETP(%)	10	9.1	0	0	-	0	0	0

E.coli were highly resistance to ciprofloxacin (100%), followed by ampicillin (90%), cephalosporins, pipericillin, levofloxacin and cotrimoxazole with 80% each.

Klebsiella species showed high resistance to ampicillin(100%) and cephalosporins (90.1%), but were sensitive to other antibiotics.

Enterobacter species showed 100% resistance to ampicillin, pipericillin, cephalosporins, gentamycin and tobramycin, 60% resistance to ciprofloxacin, cotrimoxazole and tetracycline, but percentage of resistance was less with other antibiotics.

Citrobacter species 100% resistant to ampicillin, cephalosporins, gentamicin and tobramycin. but were 100% sensitive to carbapenems, 66.7% sensitive to amikacin, fluroquinolones, amoxyclav and pipericillin tazobactam.

The *Salmonella* and *H.influenza* organism showed 100% sensitivity to most of the antibiotics, but *Salmonella species* was resistant to ampicillin (80%).

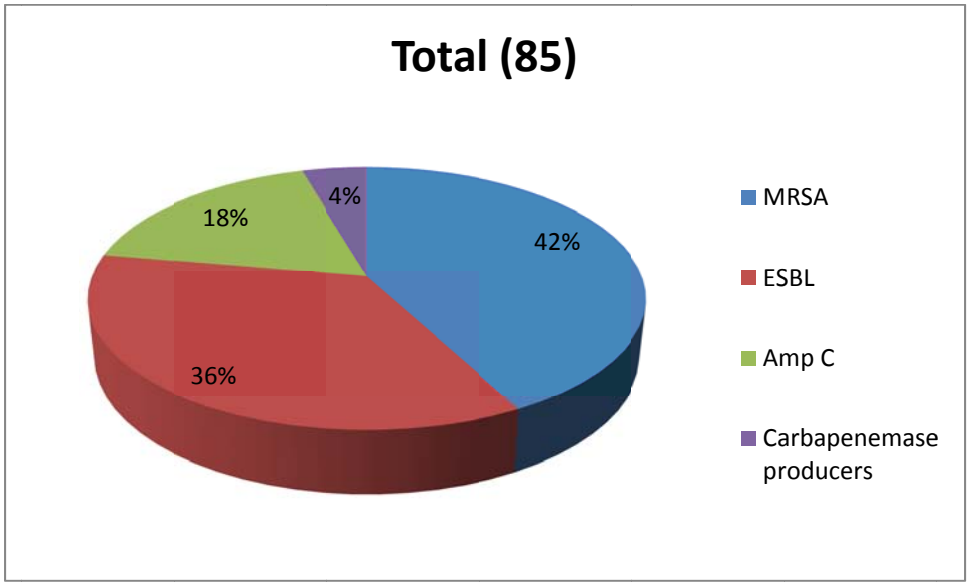
Ps.aeruginosa showed high resistance (100%) to ceftazidime, cefotaxime, pipericillin and cotrimoxazole followed by ciprofloxacin (60%) and tetracycline (60%), but showed sensitivity to other antibiotics.

Acinetobacter species showed resistance to many commonly used antibiotics like cephalosporins, pipericillin, ciprofloxacin, gentamicin and tobramycin.

Table 27: Distribution of MDR strains among bacterial isolates

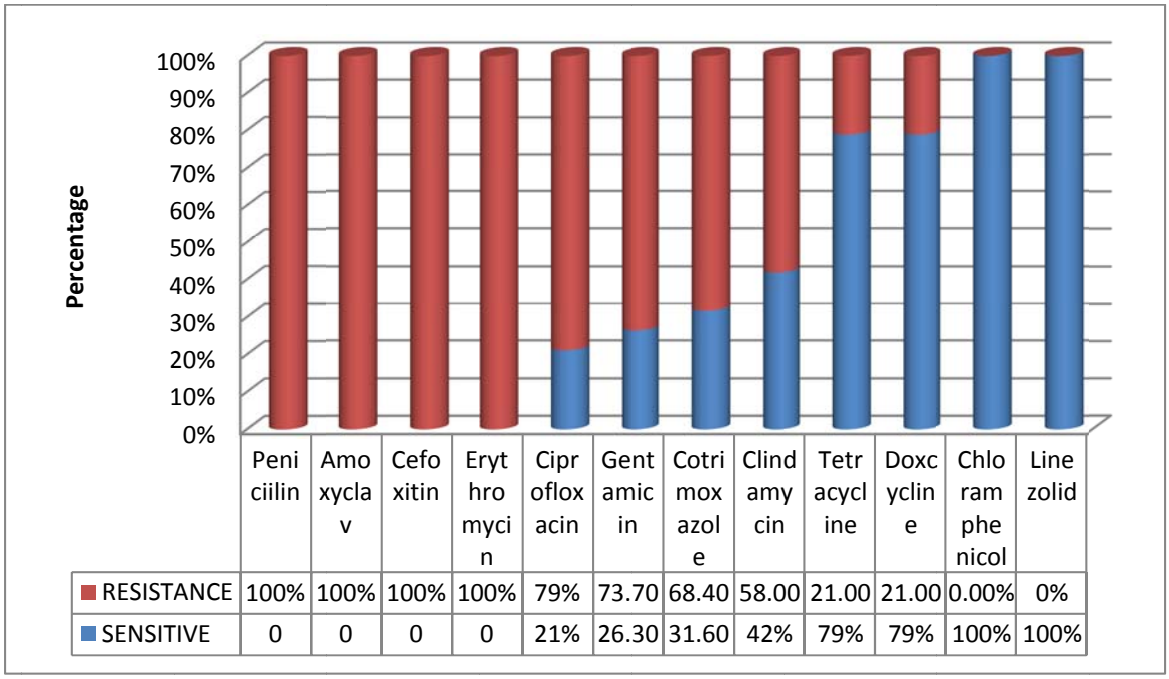
Resistance pattern	Total (85)	Percentage
MRSA	19	22.35%
ESBL	16	18.82%
Amp C	8	9.41%
Carbapenemase producers	2	2.40%

Chart 11: Distribution of MDR strains among bacterial isolates



52.9% of our bacterial isolates showed multidrug resistance strains, of which 22.35% were MRSA, 18.82% were ESBL producers, 9.41% of AMP C producers and 2.4% of carbapenemase producers.

Chart 12: Antibiotic Susceptibility pattern among MRSA

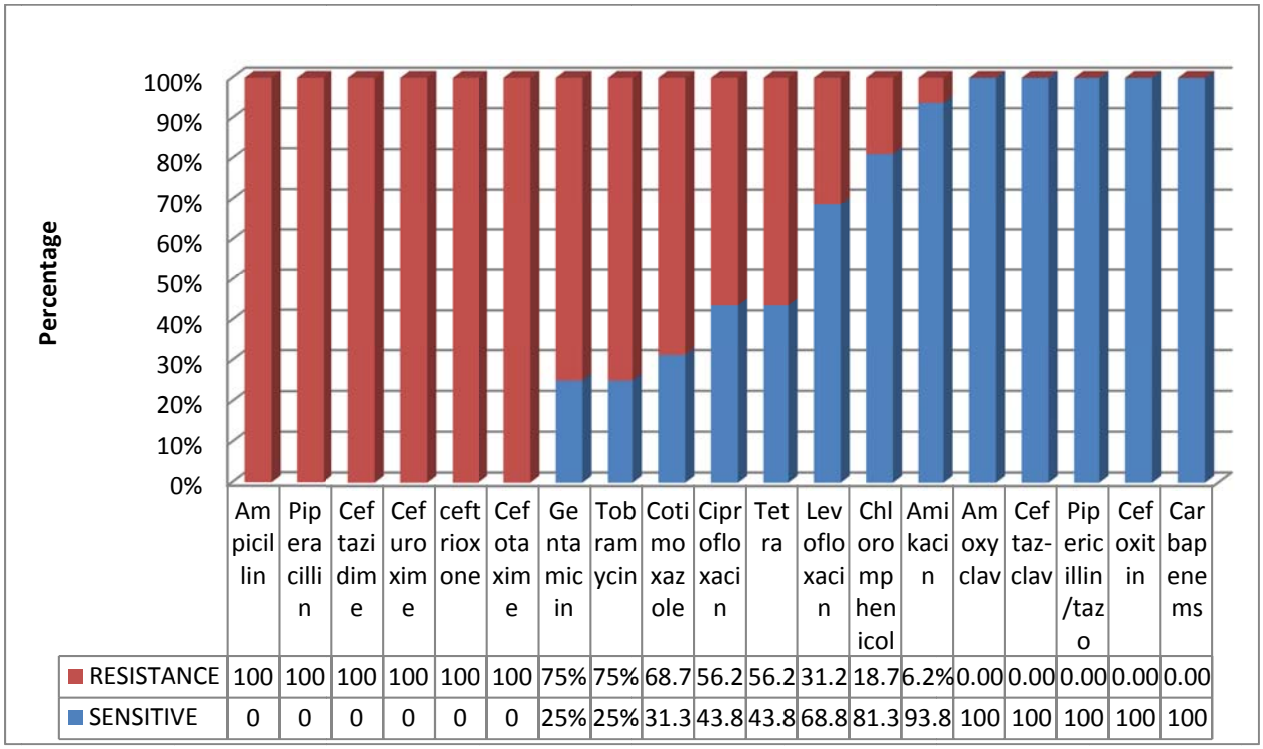


	Penicillin	Amoxyclav	Cefoxitin	Erythromycin	Ciprofloxacin	Gentamicin	Cotrimoxazole	Clindamycin	Tetracycline	Doxcycline	Chloramphenicol	Linezolid
RESISTANCE	100%	100%	100%	100%	79%	73.70	68.40	58.00	21.00	21.00	0.00%	0%
SENSITIVE	0	0	0	0	21%	26.30	31.60	42%	79%	79%	100%	100%

MRSA isolates were showing 100% sensitivity to chloramphenicol, linezolid. 79% sensitivity were observed with tetracycline and doxycycline, whereas other drugs were found to be less sensitive.

Around 47.3% of MRSA isolates showed inducible clindamycin resistance.

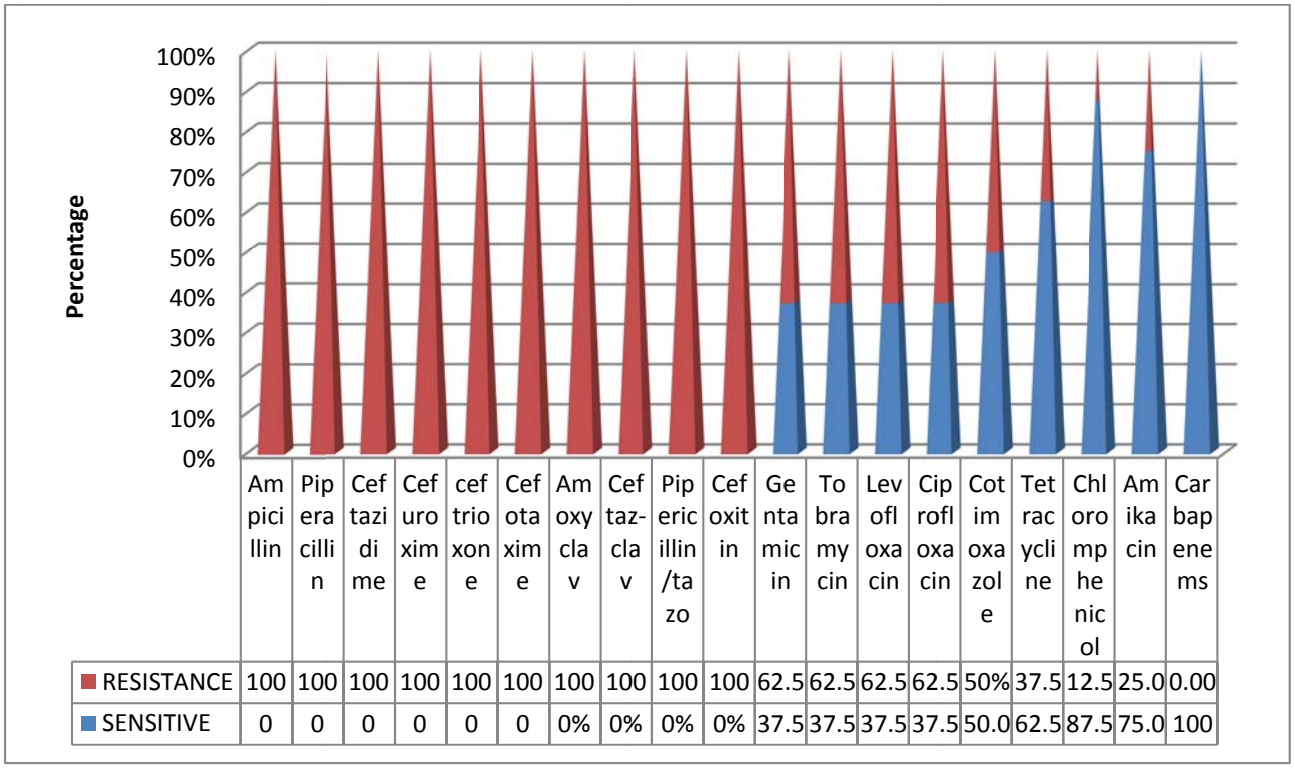
Chart 13: Antibiotic Susceptibility pattern among ESBL producers:



	Ampicillin	Piperacillin	Ceftazidime	Cefuroxime	ceftrioxone	Cefotaxime	Gentamicin	Tobramycin	Cotimoxazole	Ciproflo xacin	Tetra	Levoflo xacin	Chloro mphen icol	Amikacin	Amoxy clav	Ceftaz-clav	Piperic illin /tazo	Cefoxitin	Carbap enems
RESISTANCE	100	100	100	100	100	100	75%	75%	68.7	56.2	56.2	31.2	18.7	6.2%	0.00	0.00	0.00	0.00	0.00
SENSITIVE	0	0	0	0	0	0	25%	25%	31.3	43.8	43.8	68.8	81.3	93.8	100	100	100	100	100

Among the ESBL producing *Enterobacteriacea* isolates, were showing resistance to most of the drugs, with least resistance to chloramphenicol, amikacin, amoxyclav, pipericillin-tazobactam and carbapenems.

Chart 14: Antibiotic susceptibility pattern among AMP C producers



	Ampicillin	Piperacillin	Ceftazidime	Ceftriaxone	cefotriaxone	Cefotaxime	Amoxyclav	Ceftaz-clav	Piperacillin/tazo	Cefoxitin	Gentamicin	Tobramycin	Levofloxacin	Ciprofloxacin	Cotimoxazole	Tetracycline	Chloromphenicol	Amikacin	Carbapenems
RESISTANCE	100	100	100	100	100	100	100	100	100	100	62.5	62.5	62.5	62.5	50%	37.5	12.5	25.0	0.00
SENSITIVE	0	0	0	0	0	0	0%	0%	0%	0%	37.5	37.5	37.5	37.5	50.0	62.5	87.5	75.0	100

AMP C producing strains showed least resistance to chloramphenicol, amikacin and carbapenems.

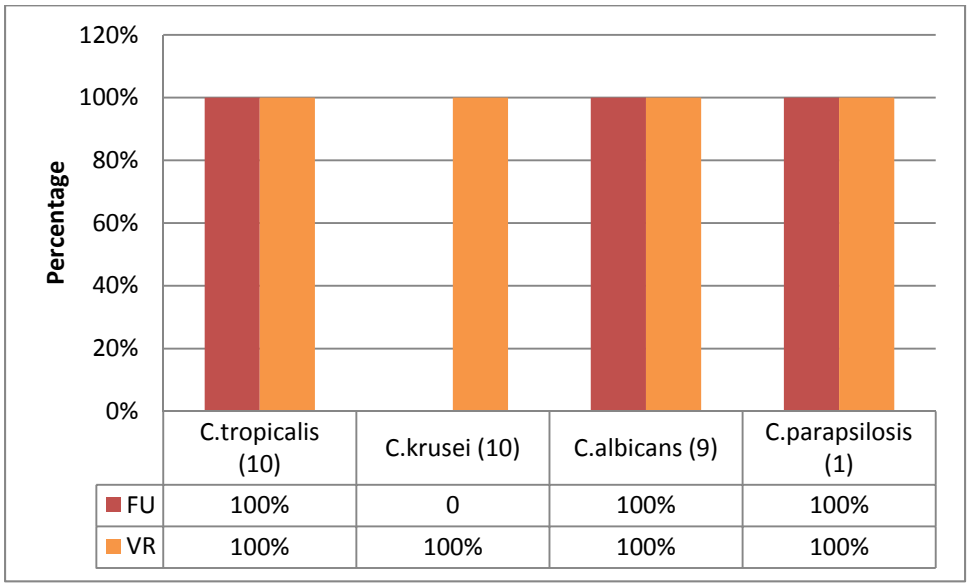
Antifungal susceptibility testing:

Table 28: Sensitivity pattern of *Candida species*:

Species	FU	VR
C.tropicalis (10)	100%	100%
*C.krusei (10)**	-	100%
C.albicans (9)	100%	100%
C.parapsilosis (1)	100%	100%

* ***C.krusei*** is intrinsically resistance to fluconazole.

Chart 15: Sensitivity pattern of *Candida species*



Candida species were 100% sensitive to fluconazole and voriconazole.

DISCUSSION:

Blood culture remains the most valuable tool in the diagnosis of blood stream infections (BSI). Timely detection of bacteremia has profound influence on the outcome of the patients.[24] CLSI guidelines states that paired blood culture should be followed to maximize yield of organism.[98] Using a paired blood culture sample will not only yield a maximum growth but also helps to identify and differentiate pathogens from contaminants.[24] The volume of blood drawn is also important in the speed of recovery of organism.[98] As physicians rely greatly on blood culture reports, it is the duty of the microbiologists to differentiate between the pathogens and contaminants.[24] However, practicing a paired blood cultures as a routine is still not followed in many centers, possibly because of requirement of additional blood sample along with additional cost for the culture.[98]

In our study we used the automated blood culture system (BacT/Alert 3D) for the detection of BSI. We have also made an attempt to find out the use of BacT/Alert 3D system in early detection of BSI and helping the clinicians with the preliminary report.

INCIDENCE OF BSI:

A total of 300 paired blood culture samples were processed from patients admitted with clinical signs and symptoms of sepsis. The percentage of culture positivity obtained in our study was 38.3%. This higher positivity rate is comparable with the other studies by Meremikwu et al[50] (48.9%), Roy et al[26] (47.5%), Kumhar et al[31] had(42%). The low positivity rate was observed in many other studies like Jombo et al[55] (6.2%), Starakis et al[54] (13.1%), Iregbu et al[52] (22%), Shrestha et al[32] (20%), and Mehdinejad et

al[57] (5.2%). In a study done by Tarai et al[98] who compared single blood culture (SBC) with the paired blood culture (PBC) and found high positivity rate (13.7%) in PBC than SBC (4.59%).

The variation in the blood culture positivity is related to different factors like number and volume of blood taken as reported by Lee et al.[29] The system and type of blood culture medium used are the other factors affecting the final bacterial yields.[57] The lower level of bacteremia can also be explained due to collecting blood samples after administration of antibiotics[32, 52,57], and over dilution of the small quantity of blood in the broth.32

In our study, 2% yielded skin contaminants. The organisms which were identified as contaminants in our study was *CONS*. The contamination rates in other studies showed 14.3% by Rahbar et al [51], 12.5% by Kumher at al[31], 3.25% by Tarai et al[98], 10.7% by by Starakis et al[54]. Ideally contamination rate should not surpass 2-3% in a hospital.[54] Rahbar et al[51] stated that one important source of contamination of blood culture is the insufficient asepsis during blood sampling. We have followed strict aseptic precautions which could possibly be the reason for lower contamination rate.

TIME DURATION FOR CULTURE POSITIVITY:

Our blood cultures were processed by BacT/Alert 3D system (bioMerieux). We found that majority of our blood cultures (68.7%) were detected within 24 hours, among which 9.57% were detected within 12 hours and 30.43% were detected within 12-18 hours and 28.7% in 18-24 hours. 25.22% growth were detected by 24-36 hours and 3.48% by 36-48 hours, whereas 2.61% were detected after 48 hours. The organisms which were detected after 48 hours were *Salmonella species* (2) and *Candida species* (1).

Similar study by Tarai et al[98] who used BacT/Alert 3D system, showed most of their blood cultures (99.27%) grew within 72 hours, among which 95.8% were isolated within 48 hours and 75.81% in 24-36 hours. They also showed *Salmonella* and *Candida* were isolated after 48 hours which is similar to our study.

GENDER ASSOCIATED TREND:

Out of 115 paired culture positives in our study, males were 78 (68%) and females were 37 (32%). The maximum number of positivity was observed among males patients than females patients. This was similar to the findings observed by Rekha S (60.3%), Tallur et al[98] (63.6%), Schaffner et al[56] (60%), Karki S et al[99] (63.3%) who showed male preponderance among the culture positives.

Age associated trend:

In our study, among the 115 culture positives, 2/3rd (63.5%) were from pediatrics patients and 1/3rd (36.5%) were from adults, showing more culture positives among pediatric patients. Similar findings were observed by Arora et al[21], who found 76.55% were from pediatric age group.

The most common age group among pediatric patients with bacteremia was observed in 0-1 months of age with 69.9% of blood culture positivity rate. The vulnerability to infection in this age group may be due their weak immunological barrier. This was similar to the other study done by Meremikwu et al[50], who found 50.78% in <1 month and 44.8% in 1month-1 year. The study by Murthy et al[24] and Rahbar et al[51] observed higher culture positivity rates among neonates with 52.63% and 54.5%

respectively. Karki S, Rai GK and Manandhar R in their study observed that the maximum number of culture positives (98.8%) was among the 0 – 10 years age group.[99]

Among the adults, 52.4% blood culture positivity was observed in 18-45 years of age group, followed by 33.3% in 45-65 years and 14.3% in >65 years. Elderly individuals have an increased risk of developing sepsis, due to comorbidities, institutionalization, declining performance status, and altered immune function.[80] Girard et al[80] mentioned in their study that >60% of patients who develop sepsis are >65 years.

Sepsis screening parameters identified among cultures positives in pediatrics and adults:

Rapid tests like C-Reactive Proteins (CRP) assays is a long-established marker of sepsis which may help in the preliminary diagnostic assessment of suspected septicaemia.[50,56] CRP and tachypnea with 71.2% respectively, followed by tachycardia (69.9%) and fever(52.1%) were the predominant sepsis screening parameters among pediatric patients observed in our study. This is similar to the study done by Rekha S[37] where CRP was positive in 91.4% of cases. In the other study done by Khinchi et al[100] majority of the neonates presented with refusal of feeds (74%), followed by tachypnea (75%) and fever (69%).

Among adults, Tachycardia (88%) was the most common parameter observed in our study, followed by fever (76.2%), and leukocytosis (71.43%). In a study by Groeneveld et al[101] found 19% and Chirouze et al[102] found 33.85% were associated fever. Coburn et al[103] stated that elevated temperature alone do not predict bacteremia, nor isolated leukocytosis.

Risk factors among pediatrics patients and adult patients:

Clinical assessment using a combination of symptoms and signs are useful in identifying infants and children with septicemia but these have limited specificity.[50] Among the 73 culture positives pediatric patients, birth asphyxia, preterm babies, low birth weight were the common risk factors identified with 27.4% respectively. This was followed by ventilated babies (22%), respiratory infections (9.58%), cardiac infections, CNS infections and PROM with 6.85% each. Reza et al[104] reported respiratory distress (80%), prematurity (71.4%) as common presentations. In other studies by Ahsan et al[105] fever (46%) followed by respiratory difficulties (39%) were reported as the common risk factors and Roy et al reported LBW (63.8%), perinatal asphyxia (37.5%) and prematurity (32.8%) as the most common risk factors.

We evaluated risk factors for candidemia and found that mechanical ventilation (46.2%), LBW (42.3%) and preterm (30.8%), prolonged antibiotics (11.5) and prolonged stay (11.5%) were common among pediatric patients. A study by Femitha et al[108] found VLBW had significantly higher risk of developing fungal infection. Another study by Prakash et al[75] evaluated that broad spectrum antibiotics, LBW were the most common risk factors associated with candidemia.

Among adults, the common risk factors associated with bacteremia were evaluated. We found respiratory infection (23.8%), hypertension (14.3%), elderly patients >65 years (14.3%), GIT infections (14.3%), patients on mechanical ventilation (11.9%), DM (11.9%) and cardiac disease (11.9%) were the common risk factors. A study by Starakis et al[54] observed lower respiratory tract infection (39.1%) as the most common risk factor, followed by urinary tract infections (25.7%), surgical site infections (7.2%),

GIT infections (4.2%). In a study done by Igra et al[78] found UTI (39%) was the common source of bacteremia in their hospital, followed by vascular device (14%), respiratory tract (10%), skin and soft tissue infections (9%). A study by Bates et al[107] found that 48% had suspected focal infection, 29% had PUO, 5% had suspected IE.

In our study, *Candida species* was found to be associated with peritonitis, diabetes and mechanical ventilation among adults. Risk factors have been evaluated in many studies and found to be most commonly associated with prolonged hospital stay, use of broad spectrum antibiotics, mechanical ventilation.[22]

Identification of culture positives:

Out of 115 paired blood culture positives in our study, 47 (40.9%) were gram negative organisms, 38 (33%) were gram positive organisms and *Candida species* were 30 (26.1%). The study done by Kumhar et al[31] also found predominance of gram negative organisms (59.8%), followed by gram positive organisms (37.5%) and *Candida species* (2.43%). Another study by Schaffner et al[56] showed 55% of the isolates were gram negative organisms and 45% were gram positive cocci, Qureshi et al[10] found 60% of gram negative bacilli and 40% of gram positive cocci. These studies along with Roy et al[26] (67.71%) showed the overall predominance of gram negative organisms as seen in our study.

However, a study by Starakis et al[54] showed predominance of gram positive organism (58.5%), followed by gram negative organism (38.5%) and fungi (3%), showing discordance with our study. Interestingly in a study by Iregbu et al[52] almost equal proportions of gram negative (50.5%) and gram positive (49.5 %) organisms were

observed and Rahbar et al[51] also showed equal proportion of gram negative and gram positive organisms with 42.3% each and 1% of candida along with 14.3% contaminants.

Organisms isolated from pediatric patients:

Among the 73 blood culture positives from pediatrics patients, 25 (34.35%) were Gram negative organisms, 22 (30.14%) Gram positive organisms and 26 (35.62%) *Candida species*.

Among the Gram negative organisms, *Klebsiella species* (28%) was the most common organism isolated followed by *Ps.aeruginosa* (16%), *Salmonella species* (16%), *Enterobacter* species (12%), *Acinetobacter species* (12%), *E.coli* (8%), *Citrobacter species* (4%) and *H.influenzae* (4%). Studies by Roy et al[26], Ayobola et al[58] and Vishwanathan et al[46] also observed *Klebsiella species* as a predominant organisms showing 24.5%, 15.4% and 36.6% respectively, which is in concordance with our study. A study by Schaffner et al[56], observed *Klebsiella species* (6%), *Pseudomonas species* (3.3%), *Salmonella species* (0.7%) as the common organisms isolated among gram negative organisms which is in accordance with our study. Among the gram negative organisms in pediatric patients, we found 24% of them were ESBL producers, 12% were AMP C producers and 4% were Carbapenemase producers. This is in accordance with the study by Osazuwa et al[63] who found 26.2% of ESBL producers among pediatric patients.

S.aureus (59.1%) was the predominant organisms isolated among the gram positive organisms, followed by *Enterococci* (18.2%), *Streptococci viridans* (13.6%) and *CONS* (9.1%). A study by Iregbu et al[52] also showed *S.aureus* as the most common organism among gram positive organism (81%), followed by *Enterococcus* (9.5%) which is in concordance with our study. We found 50% of our gram positive isolates were from

neonates with *S.aureus* being the predominant one. Similarly, a study by Shrestha et al[32] observed *S.aureus* as the common organism isolated among neonates. In our study, the incidence of MRSA among the *S.aureus* isolates was 69.2%. In a study done by Osazuwa et al[63] 83.9% of MRSA was observed in pediatric patients

Majority of the *Candida species* isolated in pediatric patients were among the neonates (88.5%). In the present study, an outbreak of *Candida species* was detected in SNICU during the study period, which was brought under control by strict aseptic precautions. Most of our *Candida species* were *non-albicans Candida* (NAC) with 69.2%. Similarly studies by Sardana et al[74] and Agarwal et al[108] showed predominance of NAC with 86.4% and 84.4% respectively. Among the *Candida species* isolated, *C.krusei* (34.6%) was most common, followed by *C.tropicalis* (30.8%), *C.albicans* (30.8%) and *C.parapsilosis* (3.8%). In a similar study by Prakash et al[75] observed *C.tropicalis* (36%) was common, followed *by C.albicans* (24%), *C.parapsilosis* (12%) and *C.krusei* (8%). A study done by Sardana et al[74] 30.1% of *Candida species* were isolated among neonates, which is similar to our study showing high percentage of candidemia among neonates. Another study by Sharma et al[23] observed only 4.27% of candidemia in neonates.

CLINICAL OUTCOME OF PEDIATRIC PATIENTS WITH CULTURE POSITIVE:

Among the culture positives in the pediatric patients 60.3% of them recovered with appropriate antibiotic therapy as per the culture report. The mortality rate in our study was 13.7%. Similar mortality rate was observed in other studies by Karthikeyen et al[109] (13.53%), Mehmat et al[110] (16%).

ORGANISMS ISOLATED AMONG ADULTS:

Among the 42 blood culture positives from adults, 22 (52.4%) were gram negative organisms, 16 (38.1%) gram positive organisms and 4 (9.5%) *Candida species*.

Among the gram negative organisms, we found *E.coli* as the common organism (19%), followed by 9.5% each of *Klebsiella* and *Acinetobacter species*, 4.8% each of *Enterobacter* and *Citrobacter species*, and 2.4% each of *Ps.aeruginosa* and *Salmonella species*. In a study done by Qureshi et al[10], *E.coli* (16%) was found to be the most common organism among gram negative organisms, followed by *Klebsiella* (13.3%), *Ps.aeruginosa* (10.7%), *Salmonella species* (8%), *Acinetobacter* (6.6%), *Citrobacter* (5.4%) and *Enterobacter* species (5.4%). Another study by Ayobola et al[58] observed *Ps.aeruginosa* (33.33%), *E.coli* (11.1%), *Enterobacter* (11.1%) were the common organisms isolated and did not isolate *Klebsiella species* and *Citrobacter species*. Among the gram negative organisms in adults, we found 45.5% of them were ESBL producers, 22.7% were AMP C producers and 4.5% of Carbapenemase producers.

Among the 16 (38.1%) gram positive organisms, *S.aureus* (62.5) was the common organism isolated among adults followed by *Group-A streptococci* (18.8%), *Enterococci* (12.5%) and *Group-B Streptococci* (6.3%). A study by Meremikwu et al[50] showed *S.aureus* (48.7%) as the most frequent isolate, followed by *Streptococci species* (4.7%) which is in concordance with our study. Studies by Arora et al[21] and Pavani et al[35] *S.aureus* was observed as the predominant organisms followed by *CONS*. Another study by Edmond et al[4] observed *CONS* (32%), *S.aureus* (16%) and *Enterococci* (11%) as the common organisms isolated among gram positive organisms. All the *S.aureus* isolates

among adults in our study were found to be MRSA (100%). However, Edmond et al[4] and Pavani et al[35] found that the incidence of MRSA as 29% and 37.1% respectively.

OUTCOME AMONG ADULTS:

42.9% of adults with positive blood cultures were recovered successfully following antibiotic therapy as per the culture report. We observed 54.8% mortality rate among adults. The mortality rate observed by other studies was 44.4% by Femitha et al[106] , 33.2% by Kang et al[111], 30% by Schaffner et al[56] and 11.9% by Asgher A H[112].

ANTIBIOTIC SUSCEPTIBILITY OF THE ISOLATES:

GRAM POSITIVE COCCI:

S.aureus showed higher resistance to penicillin (100%), followed by erythromycin (95.75%), amoxyclav (82.6%), cotrimoxazole (70%), ciprofloxacin (65.2%), and gentamycin (65.2%). However, lower resistance was observed to clindamycin (47.8%) and tetracycline (21.7%), and the organisms showed 100% sensitivity to chloramphenicol and linezolid. In a study by Roy et al[26] *S.aureus* showed high resistant to penicillin (95.9%), and low resistance was observed for cotrimoxazole (57.1%), Erythromycin (48.9%), gentamycin (44.8%) and tetracycline (42.88%).

Among the bacterial isolates in our study MRSA was seen in 22.35%. The MRSA isolates showed 100% sensitivity to chloramphenicol, linezolid and 79% sensitivity to tetracycline, with high degree of resistance to other commonly used antibiotics. Studies by Edmond et al[4], Pavani et al[35], Wisplinghoff et al[5], Kaistha et al[113] reported MRSA with 29%, 37.1%, 41% and 11.11% respectively. In our study, 47.3% of MRSA isolates showed inducible clindamycin resistance.

In our study, *Streptococci* organisms showed 100% sensitivity to penicillin, ampicillin, chloramphenicol, gentamicin and linezolid and showed resistance to erythromycin (66.7%), cotrimoxazole (44.5%), ciprofloxacin (33.33%), and tetracycline (22.2%). In a study by Pavani et al[35], *Streptococcal* isolates showed 100% sensitivity to penicillin and erythromycin, but least resistance was observed with amoxicillin.

We observed *Enterococci* were 100% resistant to penicillin and ampicillin and 83.4% to ciprofloxacin and erythromycin, whereas it was sensitive to other antibiotics like linezolid (100%), tetracycline (83.4%) and gentamycin (66.7%). A study by Pavani et al[35] found least resistance to penicillin among *Enterococci* with 100% sensitivity to amoxicillin, erythromycin, ciprofloxacin, and tetracycline. In our study, *CONS* showed 100% resistance to penicillin, amoxyclav, erythromycin and 50% resistance to clindamycin, cotrimoxazole, tetracycline and gentamycin. However, we did not observe inducible clindamycin resistance among the isolates of *CONS*.

A study by Mehdinejud et al[57] observed gram positive organisms with high degree of resistance to penicillin (100%), ampicillin (100%), and cefotaxime (95.2%). Another study by Arora et al[21] showed maximum resistance amongst the gram positive organisms was with ampicillin (74.61%) and erythromycin (69.67%). A study by Ayobola et al[58] showed gram positive cocci were highly resistant to tetracycline (100%), augmentin (100%), amoxicillin (97.3%), cotrimoxazole (81.1%).

GRAM NEGATIVE ORGANISMS:

In our study, *E.coli* showed high resistance to ciprofloxacin (100%), followed by ampicillin (90%), cephalosporins, pipericillin, levofloxacin and cotrimoxazole with 80% each. Sensitivity was limited to antibiotics like chloramphenicol (100%), amikacin

(90%), carbapenems (90%), amoxyclav (60%) and pipericillin-tazobactam (60%). Edmond et al[4] in their study observed *E.coli* was resistance to ampicillin and pipericillin with 41% and displayed good activity with other antibiotics. Another study by Asghar et al[112] observed *E.coli* with high resistance to ampicillin (89%), and sensitive to other antibiotics.

Klebsiella species showed high resistance to ampicillin (100%) and cephalosporins (90.1%), but were sensitive to other antibiotics. We observed 90.9% sensitivity to amikacin, chloramophenicol and carbapenems and 72.7% sensitivity to amoxyclav and pipericillin-tazobactam and tetracycline, 54.5% to ciprofloxacin. Similarly studies by Edmond et al[4] and Asghar et al[112] also observed high level ampicillin resistance among *Klebsiella species* with 98% and 96% respectively, but good sensitivity to most of the other antibiotics.

Enterobacter species showed 100% resistance to ampicillin, pipericillin, cephalosporins, gentamycin and tobramycin, 60% resistance to ciprofloxacin, cotrimoxazole and tetracycline, but showed 100% sensitivity to carbapenems and amikacin, 80% to chloramphenicol and levofloxacin and 60% sensitive to amoxyclav and pipericillin-tazobactam. A study by Edmond et al[4] observed high level resistance among *Enterobacter* species to ampicillin (98%), followed by 1st and 2nd generation cephalosporins with 96% and 66% respectively, but good sensitivity was found with other antibiotics.

Citrobacter species were 100% sensitive to carbapenems, 66.7% sensitive to amikacin, fluroquinolones, amoxyclav and pipericillin tazobactam, but highly resistant to ampicillin, cephalosporins, gentamicin and tobramycin.

Mehdinehud et al[57] in their study, observed gram negative bacteria with high degree of resistance to commonly used antibiotics with highest to ampicillin (98.5%), cephalexin (71.6%) and cotrimoxazole (69.7%) but low resistance with ciprofloxacin (22.4%) and amikacin (28.4%). Another study by Pavani et al[35] showed gram negative organisms were more sensitive to amoxyclav and pipericillin tazabactam. The studies by Roy et al[26] showed >95% of *Enterobacter*iacea were resistant to penicillin and >40% were resistant to extended spectrum cephalosporins and by Ayobola et al[58] gram negative organisms showed a high degree of resistance to tetracycline(100%) and augmentin (100%) followed by amoxycillin (97.3%), cortimoxazole (81.1%).

ESBL producing strains (18.82%), showed 100% sensitivity to amoxyclav, pipericillin-tazobactam, and carbapenems, followed by amikacin (93.8%), chloramphenicol (81.3%), and levofloxacin (68.8%), and were least sensitive to other antibiotics. Studies by Pavani et al [35], Arora et al[21], and Kavitha et al[114] observed 32.6%, 34.4% and 32% of ESBL producers.

We found, AMP C producers (9.41%) were resistant to most of the common antibiotics, showing sensitivity only to carbapenems (100%), chloramphenicol (87.5%), amikacin (75%), tetracycline (62.5%) and cotimoxazole (50%). Among the carbapenemase producing strains (2.4%) 100% sensitivity was observed with chloramphenicol and amikacin, whereas it showed resistance to other antibiotics.

The *Salmonella* and *H.influenzae* organism showed 100% sensitivity to most of the antibiotics, but *Salmonella species* was resistant to ampicillin (80%).

Ps.aeruginosa showed high resistance (100%) to ceftazidime, cefotaxime, pipericillin and cotrimoxazole followed by ciprofloxacin (60%) and tetracycline (60%),

but showed sensitivity to other antibiotics. A study by Rahbar et al[51] showed *Ps.aeruginosa* was resistant to the aminoglycosides, but were sensitive to ciprofloxacin unlike our study. Another study by Edmond et al[4] found *Ps.aeruginosa* was highly resistant to ceftazidime, cefotaxime, ampicillin and cotrimoxazole and showing sensitivity to other antibiotics which is in concordance with our study.

Acinetobacter species showed resistance to many commonly used antibiotics like cephalosporins, pipericillin, ciprofloxacin, gentamicin and tobramycin, but showed sensitivity to carbapenems, amikacin, cotrimoxazole, levofloxacin and pipercillin tazobactam. In a study by Pavani et al[35] who observed least resistance to amoxicillin and cefotaxime, but no resistance was observed to amikacin ciprofloxacin, ceftazidime, gentamicin.

CANDIDA SPECIES:

All our *Candida species* were susceptible to fluconazole and itraconazole. Fluconazole susceptibility was not tested for C.krusei as it is intrinsically resistant to this drug. Non albicans candida tend to be less susceptible to azoles, particularly fluconazole.[74] We did not observe resistance to azoles in our study, but resistance was observed by other studies done in India. Studies by Kumar et al[67], Goel et al[68], Xess et al[69] observed resistance to fluconazole with 17.2%, 4.5%, and 11.7% respectively. A study by Kothari et al[72], observed resistance to fluconazole, itraconazole and voriconazole with 36%, 24% and 56% respectively.

CONCLUSION:

- In our study, incidence of blood culture positivity was 38.3%. Clinical selection of cases with specific signs and symptoms of sepsis and collection of paired blood sample before starting the antibiotic therapy helped in high positive cultures.
- BacT/Alert system helped in early detection thus reduced the turnaround time, which helped our clinicians with the early gram stain report.
- Blood stream infections was found to be more common among males and also observed to be more among neonates.
- Gram negative organisms showed overall predominance among the organisms isolated, followed by Gram positive organisms and *Candida species*.
- The incidence of Candidemia was observed to be more among the neonates may be due to the neonatal risk factors and also because of the outbreak of *Candida species* in SNICU.
- The most common organisms isolated were *S.aureus* among gram positive organisms, *Klebsiella species* and *E.coli* among gram negative organisms, *C.tropicalis* and *C.krusei* among *Candida species*.
- Gram positive organisms showed high resistance to penicillin, erythromycin, ciprofloxacin, cotrimoxazole, gentamicin and amoxyclav, except *Streptococcus* species and *Enterococci* which showed high sensitivity to pencillin and ampicillin. All the gram positive organisms showed 100% sensitivity to chloramphenicol and linezolid.

- Gram negative organisms were highly resistance to most of the commonly used antibiotics, with high sensitivity only to carbapenems, amikacin and chloramphenicol.
- We observed high prevalence of MRSA (22.35%) and ESBL producers (18.82%) in our bacterial isolates.
- *Candida isolates* were 100% sensitive to fluconazole and itraconazole, except *C.krusei* isolates which is intrinsically resistant to fluconazole.
- The mortality rate observed in pediatric patients was 13.7% and 54.8% in adults.
- Our study highlighted the pattern of isolates in blood culture among different age group and the correlation with the risk factor and the sepsis parameters associated with them.

SUMMARY:

- It is a observational study conducted between Febraury 2012 - August 2013 in R.L.Jalappa Hospital, Kolar. A total of 300 paired blood samples were collected from the patients with signs and symptoms of sepsis and pyrexia of unknown origin. All the samples were collected under strict aseptic precautions before administration of antibiotics.

- The blood samples were incubated in automated BacT/Alert 3d system. Once the growth signal was given by the machine, the samples were processed according to the standard laboratory techniques.
- The initial identification report based on the gram stain was conveyed immediately to the clinicians for the preliminary management of cases.
- The organisms grown were identified and their antibiotic susceptibility testing were done according to the CLSI guidelines. Most of our blood culture yielded growth within 24 hours (68.76%).
- Out of 300 paired samples processed, 115 (38.3%) were identified as pathogens and 6 (2%) as contaminants. Among the 115 culture positives, bacteremia was common among males (68%) than females (32%) and in pediatric patients (63.5%) than adults (36.5%). The positive cultures were more among neonates (71.2%).
- Evaluation of sepsis screening parameters showed, CRP (71.2%), tachypnea (71.2%), tachycardia (69.9%) and fever (52.1%) were common among culture

positive pediatric patients. Tachycardia (88%), fever (76.2%), and leukocytosis (71.43%) were observed commonly among adults with culture positives.

- Risk factors evaluated among the culture positive patients, birth asphyxia, preterm, Low birth weight each with 27.4% among pediatrics patients and respiratory infection (23.8%), hypertension (14.3%), elderly patients >65 years 14.3%), GIT infections 14.3%) among adults were commonly observed.
- The risk factors for Candidemia among pediatric patients were associated with mechanical ventilation (46.2%), LBW (42.3% and preterm (30.8%). Among adults, candidemia was found to be associated with peritonitis, DM, mechanical ventilation.
- Among the culture positives, gram negative organisms accounted for 47%, gram positive organisms were 33% and *Candida species* were 26.1%. *S.aureus* was the predominant organism isolated among the pediatric patients, followed by *C. krusei, C.tropicalis, C.albicans and Klebsiella species*. Among adults, *S.aureus*, *E.coli*, *Klebsiella species*, *Acinetobacter species* were the common organisms isolated.
- Gram positive organisms especially *S.aureus* was highly resistant to commonly used antibiotics and showed 100% sensitivity to chloramphenicol, linezolid and tetracycline. Similarly gram negative organisms showed resistance to commonly used antibiotics, with variable percentage of sensitivity to cabapenems, amikacin, chloramphenicol, levofloxacin, amoxyclav and piericillin-tazobactam. *Candida species* were sensitive to fluconazole and itraconazole.

- We observed multidrug resistance strains among the bacterial isolates, of which 22.35% were MRSA, 18.82% were ESBL producers, 9.41% of AMP C producers and 2.4% of carbapenemase producers.
- The mortality rate observed in pediatric patients was 13.7% and 54.8% in adults.

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ANNEXURES

ANNEXURE: I

PROFORMA FOR ADULTS AND PEDIATRICS

Name: Age: Sex:

Address: Hospital no:

Lab no:

DOA:

Chief complaints:

Diagnosis:

Is history suggestive of any new infection?

Signs and symptoms:

☐ Hyperthermia(>380 C) ☐ Hypothermia(<360 C)

☐ Tachycardia(>90/min) ☐ Tachypnea(>24/m

☐ Paco2 <32 mmHg ☐ Leucocyte >12000/mm2

☐ Leucocyte <4000/mm2 ☐ Band forms >10%

Any other test:

ANNEXURE: II

PROFORMA FOR NEONATES

Name: Age: Sex:

Address: Hospital no:

Lab no:

DOA:

Chief complaints:

Diagnosis:

Is history suggestive of any new infection?

Signs and symptoms:

- ☐ Lethargy
- ☐ Poor feeding
- ☐ Poor activity
- ☐ Hyperthermia
- ☐ Hyphothermia
- ☐ Tachycardia
- ☐ Bradycardia
- ☐ Respiratory distress
- ☐ Grunting
- ☐ Chest retractions
- ☐ Seizure
- ☐ Cyanosis
- ☐ Vomiting
- ☐ Loose stools
- ☐ Bulging anterior fontanale

Any other test:

ANNEXURE: III

GRAM STAINING TECHNIQUE:

PREPARATION OF THE SMEAR:

A drop of broth which was drawn from the BacT/Alert bottle, were placed on a clean labeled glass slide, and the smear was prepared. It was air dried, heat fixed, and kept on the staining rack.

STAINING METHOD:

1. Smear was flodded with crystal violet dye and allowed to stand for 1 minute and washed with water.
2. Then, Gram's iodine was added for 1 minute and washed with water.
3. Decolourization done by using acetone by Flush and wash technique.
4. Saffranine was added and allowed to stand for 30 seconds and washed with water.
5. The slide was blotted dry, and examined under oil immersion objective.

ANNEXURE: IV

ANTIBIOTIC SUSCEPTIBILITY TESTING (AST) : [95]

The antibiotic sensitivity testing was done on Muller Hinton agar by Kirby Bauer disc diffusion method [42]. The antibiotic discs were procured from Hi Media.

TECHNIQUE:

1. Using a straight wire the 3-4 similar looking colonies of the test strain were picked from the plate.
2. It was inoculated into a test tube containing peptone water and incubated at 37^0C for 2 hours.
3. The turbidity of the test strain was matched with 0.5 Mc-Farland turbidity standards.
4. A sterile swab was dipped into the inoculum and excess was removed by pressing and rotating the swab firmly against the side of the tube.
5. A lawn culture was made on the media by streaking the swab thrice onto the surface of the medium, rotating the plate through an angle of 60 degree after each application.
6. The antibiotic discs were placed on the inoculated plates using sterile forceps with a distance of 25mm from the center of the disc.
7. The plates were incubated overnight at 37^0C.

ANNEXURE: V

LIST OF ANTIBIOTICS TESTED: [96]

Antimicrobial agent	Disk content (µg)	STAPH. AUREUS (mm)		*CONS* (mm)	
		Sensitive	Resistant	Sensitive	Resistant
Penicillin(P)	10 UNITS	≥29	≤28	≥29	≤28
Amoxyclav(Amc)	20/10	≥20	≤19	≥20	≤19
Cefoxitin(Cx)	30	≥22	≤21	≥25 (except S.lugdunensis ≥22)	≤24 (except S.lugdunensis ≥21)
Erythromycin(E)	15	≥23	≤13	≥23	≤13
Clindamycin(Cd)	2	≥21	≤14	≥21	≤14
Chloramphenicol(C)	30	≥18	≤12	≥18	≤12
Tetracycline(Te)	30	≥19	≤14	≥19	≤14
Doxycycline(Do)	30	≥16	≤12	≥16	≤12
Ciprofloxacin(Cip)	5	≥21	≤15	≥21	≤15
Gentamycin(Gen)	10	≥15	≤12	≥15	≤12
Cotrimoxazole(Cot)	1.25/23.75	≥16	≤10	≥16	≤10
Linezolid(Lz)	30	≥21	≤20	≥21	≤20

Antimicrobial agent	Disk content (µg)	ENTEROCOCCI (mm)		STREPTOCOCCI (mm)	
		Sensitive	Resistant	Sensitive	Resistant
Penicillin(P)	10 UNITS	15	14	24	-
Ampicillin (amp)	10	17	16	24	-
Erythromycin(E)	15	23	13	21	15
Chloramphenicol(C)	30	-	-	21	17
Tetracycline(Te)	30	19	14	19	14
Doxycycline(Do)	30	16	12	16	12
Ciprofloxacin(Cip)	5	21	15	21	15
Gentamycin(Gen)	10	10	6	15	12
Cotrimoxazole(Cot)	1.25/23.75	-	-	16	10
Linezolid(Lz)	30	23	20	30	21
Vancomycin (Va)	30	17	-	17	-

Antibiotics	Disc content (µg)	*Enterobacter*iaceae (mm)		Acinetobacter species (mm)		Pseudomonas aeruginosa(mm)	
		Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Ampicillin (Amp)	10	≥17	≤13	-	-	-	-
Pipericillin (Pi)	100	≥21	≤17	≥21	≤17	≥21	≤14
Ceftazidime (Caz)	30	≥21	≤17	≥18	≤14	≥18	≤14
Cefotaxime (Ctx)	30	≥26	≤22	≥23	≤14	-	-
Ceftriaxone (Ctr)	30	≥23	≤19	≥21	≤13	-	-
Cefoxitin (Cx)	30	≥18	≤14	-	-	-	-
Cefuroxime (Cxm)	30	≥23	≤14	-	-	-	-

Amoxyclav (Amc)	20/10	≥18	≤13	-	-	-	-
Pipericillin-Tazobactam (Pit)	100/10	≥21	≤17	≥21	≤17	≥21	≤14
Ceftazidime - clavulinic acid (Cac)		≥	≤	-	-	-	-
Ciprofloxacin (Cip)	5	≥21	≤15	≥21	≤15	≥21	≤15
Levofloxacin (Le)	5	≥17	≤13	≥17	≤13	≥17	≤13
Amikacin (Ak)	30	≥17	≤14	≥17	≤14	≥17	≤14
Gentamicin (Gen)	10	≥15	≤12	≥15	≤12	≥15	≤12
Tobramycin (Tb)	10	≥15	≤12	≥15	≤12	≥15	≤12
Tetracycline (Te)	30	≥15	≤11	≥15	≤11	-	-
Cotrimoxazole (Cot)	1.25/ 23.75	≥16	≤10	≥16	≤9	-	-
Chloramphenicol (C)	30	≥18	≤12	-	-	-	-
Imipenem (Ipm)	10	≥23	≤19	≥16	≤13	≥19	≤15
Meropenem (Mrp)	10	≥23	≤19	≥16	≤13	≥19	≤15
Ertapenem (Etp)	10	≥22	≤18	-	-	-	-
Colostin (CL)	10	-	-	-	-	≥11	≤10
Polymyxin B (PB)	300 U	-	-	-	-	≥12	≤11

Antibiotics	Disc content (µg)	*H.influenzae* (mm)		*Salmonella species* (mm)	
		Sensitive	Resistant	Sensitive	Resistant
Ampicillin (Amp)	10	22	18	17	13
Ceftazidime (Caz)	30	26	-	-	-
Cefotaxime (Ctx)	30	26	-	26	22
Ceftriaxone (Ctr)	30	26	-	23	19

Amoxyclav (Amc)	20/10	20	19	-	-
Pipericillin-Tazobactam (Pit)	100/10	21	-	-	-
Ciprofloxacin (Cip)	5	21	-	21	15
Levofloxacin (Le)	5	17	-	17	13
Tetracycline (Te)	30	29	25	15	11
Cotrimoxazole (Cot)	1.25/23.75	16	10	16	10
Chloramphenicol (C)	30	29	25	18	12
Imipenem (Ipm)	10	16	-	-	-
Meropenem (Mrp)	10	20	-	-	-
Ertapenem (Etp)	10	19	-	-	-

ANNEXURE: VI

DETECTION OF CARBAPENEMASE RESISTANCE BY MODIFIED HODGE TEST: [96]

Procedure:

- A lawn of ATCC *E.coli* 25922 was made on a Muller hinton agar.
- With a Meropenem disc placed at the centre, The test strain was streaked using a sterile loop from the edge of the disc placed in the centre of the plate to the periphery
- A Positive control (*Klebsiella pneumoniae ATCC 1705*) and negative control (*Klebsiella pneumonia ATCC 1706*) was streaked on the same plate at 45 degree angulations from the edge of the meropenem disk to periphery.
- The plates were incubated overnight at 37^0C.
- The presence of a cloverleaf-shaped zone of inhibition due to carbapenemase production by the test strain was considered positive

ANNEXURE: VII

ANTIFUNGAL SUSCEPTIBILITY TESTING: [97]

Preparation of Modified Mueller-HintonAgar:

1. Mueller-Hinton agar prepared from a commercially available dehydrated Mueller-Hinton agar base according to the manufacturer's (Hi-media) instructions.
2. Add 500µg of methylene blue and 20 g of glucose per liter of agar suspension.
3. The pH of the medium should be 7.2-7.4.
4. Autoclave as directed by manufacturer's instructions.
5. After autoclaving, allow the agar solution to cool to 45 ^{o} to 50^{o} C.
6. Pour the medium into the plastic flat-bottomed petri dishes to give a uniform depth of 4mm approximately. This corresponds to 20-25 ml per plate of 90 mm.
7. The agar medium should be allowed to cool to room temperature.
8. It can be stored at refrigerator temperature (2 ^{o} to 8 ^{o} C).
9. Plates should be used within seven days after preparation.
10. Plates also should be checked for sterility control and also for quality control.

Preparation of inoculum:

Direct colony suspension method:

1. Fungal growth was subcultured onto Sabouraud dextrose agar to ensure purity and viability.
2. Inoculum is prepared by picking up of five distinct colonies, approximately 1 mm in diameter from a 24 hour old culture of *Candida species*.
3. Colonies are suspended in 5 ml of sterile saline (0.85% NaCl).
4. The suspension is vortexed for 15 seconds and its turbidity is matched visually with a 0.5 McFarland standard, by adding sterile saline.
5. This procedure will yield a yeast stock suspension of 1 x 10^{6} to 5 x 10^{6} per ml, and produces semi-confluent growth with most *Candida species* isolates.

Inoculation of test plates:

1. A cotton swab is dipped into the suspension.
2. The swab should be rotated several times and pressed firmly against the inside wall of the tube above the fluid level, to remove excess fluid from the swab.
3. The modified Mueller-Hinton agar plate is inoculated by evenly streaking the swab over the entire agar surface. This is repeated twice by rotating the plate approximately 60^{o} C each time for even distribution of inoculum.
4. This should be done within 15 minutes after adjusting the turbidity of the inoculum.
5. Antifungal disks are placed with 24 mm distance between each discs.
6. The plates are then incubated within 15 minutes after placing the disks, for 20-24 hours of incubation at 35^{o} C (±2^{o} C)
7. Interpretation done according to annexure:

Recommended Zone diameter:

Antifungal agent	Disk content (µg)	C.albicans	C.parapsilosis	C.tropicalis	C.krusei
Fluconazole	25	28-39	22-33	26-37	-
Voriconazole	1	31-42	28-37	-	16-25

ANNEXURE: VIII

Key to master chart

Pediatric Master Chart:

1. SEX
   - F = FEMALE
   - M = MALE

2. FEVER
   - 1 = ABSENT
   - 2 = PRESENT

3. TACHYCARDIA
   - 1 = ABSENT
   - 2 = PRESENT

4. TACHYPNEA
   - 1 = ABSENT
   - 2 = PRESENT

5. C – REACTIVE PROTEIN (CRP)
   - 1 = ABSENT
   - 2 = PRESENT

6. BIRTH WEIGHT
   - 1 = LOW BIRTH WEIGHT
   - 2 = APPROPRIATE BIRTH WEIGHT

7. PROM
   - 1 = ABSENT
   - 2 = PRESENT

8. BIRTH ASPHYXIA
   - 1 = ABSENT
   - 2 = PRESENT

9. RESPIRATORY DISTRESS
   - 1 = ABSENT
   - 2 = PRESENT

10. VENTILLATION
    - 1 = ABSENT
    - 2 = PRESENT

11. ANTIBIOTIC SENSITIVITY
    S = SENSITIVE
    R = RESISTENT

Adult Master Chart:

1. SEX
    F = FEMALE
    M = MALE

2. FEVER
    1 = ABSENT
    2 = PRESENT

3. TACHYCARDIA
    1 = ABSENT
    2 = PRESENT

4. TACHYPNEA
    1 = ABSENT
    2 = PRESENT

5. VENTILATION
    1 = ABSENT
    2 = PRESENT

6. ANTIBIOTIC SENSITIVITY TESTING
    S = SENSITIVE
    R = RESISTENT

S.NO	AGE	SEX	H.NO	DIAGNOSIS	FEVER	TLC	TACHYCARDIA	TACHYPNEA	CRP	B.WT	PROM	BA	RD	ENTILATION	1STSAMPLE	2ND SAMPLE	DURATION	P	AMP	CAZ	CTX	CX	CTR	CXM	PI	AMC,PIT,CAC	E	CD	CIP	LE	COT	TE,DO	LZ	C	AK	GEN	TB	TPM,MRP,ETP	CL	PB	FU	VR	OUTCOME	RESISTANT TYPE
1	5M	F	724305	PUO	2	7800	2	1	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
2	14	M	736418	RHD	2	11600	2	2	1	1	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
3	4M	M	737446	CHD	1	13200	2	2	1	1	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
4	1D	F	745780	LOS	1	11000	1	2	2	1	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
5	1D	M	747523	PNEUMONIA	2	17000	2	2	2	1	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
6	8M	M	750221	PUO	2	10200	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
7	1	M	752208	PNEUMONIA	1	12800	2	2	2	2	1	2	2	1	KL.PNEUMONIAE	KL.PNEUMONIAE	14 HOURS		R	R	R	S	R	R	R	S			S	S	R	R		R	S	R	R	S				EXPIRED	ESBL
8	10	M	753104	PNEUMONIA	2	13400	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
9	10	F	756486	PUO WITH SEIZURES	2	11800	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
10	5D	F	756781	PUO	2	6500	2	1	1	1	1	1	1	1	S.AUREUS	S.AUREUS	13 HOURS	R				S				S	S	S	S		R	S	S	S		R					RECOVERED	MSSA
11	3	M	756862	PUO	2	13000	1	2	2	2	1	1	1	1	CONS	CONS	18 HOURS	R				R				R	R	S	S		S	S	S	S		R					RECOVERED	MRSE
12	16	M	757101	PUO	2	3900	1	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
13	1	M	757135	PUO WITH SEIZURES	2	13400	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
14	5	F	757490	PUO	2	4600	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
15	12	M	673959	PUO	2	10600	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
16	3D	M	759210	EOS	2	12000	1	1	2	1	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
17	5D	F	759429	EOS	1	5300	1	2	2	1	1	1	2	1	C.PARAPSILOSIS	C.PARAPSILOSIS	32 HOURS																									S	S	RECOVERED	
18	1	M	760060	PUO	2	13000	2	2	2	2	1	1	1	1	S.AUREUS	S.AUREUS	14 HOURS	R				R				R	R	S	R		R	S	S	S		S					RECOVERED	MRSA
19	1D	F	760161	EOS	1	8600	1	1	2	2	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
20	1M	M	760481	LOS	2	13000	1	2	1	2	2	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
21	4D	M	761122	LOS	2	2000	2	1	2	2	1	1	2	2	PS.AERUGINOSA	PS.AERUGINOSA	26 HOURS			R					R	S			S	S	R	R			S	S	S	S	S	S			RECOVERED	
22	2D	M	761130	EOS	1	5700	2	2	1	1	1	1	1	1	C.TROPICALIS	C.TROPICALIS	34 HOURS																									S	S	DAMA	
23	1D	M	761147	EOS	1	46400	1	1	2	2	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
24	1D	M	761154	EOS	1	13600	1	1	1	1	2	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
25	3D	M	761323	LOS	1	6100	2	1	2	1	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
26	4M	F	761410	PNEUMONIA	2	17900	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
27	7	M	761452	PUO	2	12000	2	2	1	2	1	1	1	1	S.AUREUS	S.AUREUS	20 HOURS	R				S				S	R	S	S		R	S	S	S		S					RECOVERED	MSSA
28	8	M	762244	PNEUMONIA	2	11300	1	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
29	3D	M	762433	LOS	1	15100	1	1	2	1	1	1	2	1	NO GROWTH	NO GROWTH																											EXPIRED	
30	16	F	762636	PUO	2	7400	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
31	2D	F	762646	LOS	1	11300	2	2	2	2	1	1	1	1	S.AUREUS	S.AUREUS	12 HOURS	R				S				S	R	S	S		R	R	S	S		S					RECOVERED	MSSA
32	6D	M	762875	LOS	1	14800	2	2	2	2	1	1	1	1	S.AUREUS	S.AUREUS	16 HOURS	R				R				R	R	R	S		S	R	S	S		R					RECOVERED	MRSA
33	1	M	763100	PNEUMONIA	2	5800	2	2	1	1	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
34	4	M	763443	PNEUMONIA	2	8700	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
35	7M	F	763601	PUO	2	12600	1	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
36	4D	M	763690	EOS	1	6700	1	1	1	1	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
37	1M	M	763959	PNEUMONIA	2	7000	2	2	1	2	1	1	2	1	S.AUREUS	S.AUREUS	19 HOURS	R				R				R	R	R	R		R	S	S	S		S					RECOVERED	MRSA
38	15	M	764290	PUO	2	18000	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
39	3	M	764995	PUO	2	13000	1	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
40	3	M	765238	PUO WITH SEIZURES	2	13300	1	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											EXPIRED	
41	5D	F	765578	LOS	2	12800	1	1	2	2	1	1	1	1	C.KRUSEI	C.KRUSEI	28 HOURS																									-	S	RECOVERED	
42	10D	M	765608	PNEUMONIA	1	14000	1	2	2	2	1	1	1	1	PS.AERUGINOSA	PS.AERUGINOSA	22 HOURS			R					R	S			R	S	R	S			S	S	S	S	S	S			EXPIRED	
43	14	M	765722	PYOGENIC MENINGITIS	2	9900	1	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
44	3D	M	765875	LOS	1	15000	1	1	2	2	2	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
45	3D	M	765985	EOS	2	11900	1	2	1	2	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
46	18	F	766068	PERITONITIS	2	16000	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
47	3	F	766433	PUO	2	9900	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
48	3	F	766443	PUO	2	28300	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
49	2M	F	766583	LOS	2	17700	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
50	5	F	766598	PYOGENIC MENINGITIS	2	10600	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
51	3M	M	766806	PUO	2	14700	2	1	2	2	1	1	1	1	S.AUREUS	S.AUREUS	19 HOURS	R				S				S	R	S	S		S	S	S	S		S					RECOVERED	MSSA
52	6	F	766829	PYOGENIC MENINGITIS	2	11200	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	

53	8M	M	766858	PUO	2	12400	2	1	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
54	15D	F	766981	LOS	1	12300	2	2	1	2	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
55	6	M	767145	PUO	2	10500	1	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
56	10D	F	767196	PNEUMONIA	1	12200	1	1	2	2	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
57	12	M	767232	RHEUMATIC FEVER	2	17400	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
58	12	M	767733	PYOGENIC MENINGITIS	2	11500	1	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
59	7	F	767834	PUO	2	3300	2	2	1	2	1	1	1	1	S.TYPHI	S.TYPHI	54 HOURS		R		S		S						S	S	R	S		S									RECOVERED	
60	7	F	768079	PUO	2	2500	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
61	1D	F	768407	EOS	2	16000	2	2	1	2	2	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
62	2D	M	769155	EOS	1	16300	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
63	8	M	770300	PUO	2	11300	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
64	3D	M	770694	LOS	1	15400	2	1	2	2	2	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
65	9M	M	770696	PUO	2	17000	2	1	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
66	3M	M	770940	PYOGENIC MENINGITIS	1	12000	1	2	2	2	1	1	2	1	NO GROWTH	NO GROWTH																											DAMA	
67	20D	M	771362	LOS	1	13400	1	1	1	2	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
68	5	M	772021	PNEUMONIA	2	13000	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
69	5D	M	773223	PYOGENIC MENINGITIS	1	9100	1	1	2	1	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
70	3D	M	773256	LOS	1	7900	2	1	2	1	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
71	5	F	773269	PYOGENIC MENINGITIS	2	13100	2	2	2	1	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
72	1M	M	773557	PNEUMONIA	2	7800	1	2	1	2	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
73	4M	M	773872	BRONCHITIS	2	1400	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
74	11	F	773881	PNEUMONIA	2	10300	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
75	8	F	777078	PUO	2	12000	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
76	2D	M	780460	RDS	1	13300	2	2	2	2	1	2	2	1	ENTEROCOCCI	ENTEROCOCCI	18 HOURS	R	R														S			S							DAMA	
77	4D	F	781737	EOS	1	13000	1	2	1	2	2	2	2	1	PS.AERUGINOSA	PS.AERUGINOSA	29 HOURS			R					R	S			R	S	R	R			S	R	R	S	S	S			RECOVERED	
78	5M	M	781784	PNEUMONIA	2	10600	2	2	2	2	1	1	1	1	H.INFLUENZA	H.INFLUENZA	27 HOURS		R	S	S		S	S		S			S	S	S	S		S				S					RECOVERED	
79	6	F	782440	SSSS	2	3700	2	2	2	2	1	1	1	1	ENTEROBACTER	ENTEROBACTER	12 HOURS		R	R	R	S	R	R	R	S			S	S	R	R		R	S	R	R	S					REFFERED TO HIGHER CENTRE	ESBL
80	2D	M	783116	PYOGENIC MENINGITIS	1	16700	1	2	2	1	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
81	1D	M	783125	PYOGENIC MENINGITIS	2	22800	2	2	1	2	2	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
82	3M	M	783458	CHD	2	13600	2	2	2	2	1	1	1	1	ENTEROCOCCI	ENTEROCOCCI	16 HOURS	R	R														S			R							RECOVERED	
83	1D	M	783650	PYOGENIC MENINGITIS	2	20900	2	2	2	2	2	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
84	1M	M	784066	PNEUMONIA	2	16000	2	2	2	2	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
85	4M	M	784895	PUO	2	11700	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
86	15	M	785562	RHD	2	13100	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
87	15	M	785799	QUADRIPLEGIA	2	2600	2	2	2	2	1	1	1	1	C.TROPICALIS	C.TROPICALIS	23 HOURS																								S	S	RECOVERED	
88	1D	F	787537	EOS	2	14000	2	1	1	2	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
89	10D	F	788376	RDS	1	13000	1	2	2	1	2	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
90	2D	M	789786	EOS	2	17200	2	1	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
91	4	M	790295	PYOGENIC MENINGITIS	2	5400	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
92	6D	F	793785	LOS	2	12700	2	1	2	2	1	1	1	1	ENTEROCOCCI	ENTEROCOCCI	13 HOURS	R	R														S			S							DAMA	
93	1D	M	794079	EOS	1	13000	1	2	2	1	1	1	1	1	ACINETOBACTER	ACINETOBACTER	12 HOURS			R	R		R		R	R			R	S	S	S			S	R	R	S					RECOVERED	
94	1M	M	795777	PYOGENIC MENINGITIS	2	12500	2	1	2	2	1	1	1	1	KL.OXYTOCA	KL.OXYTOCA	16 HOURS		R	R	R	S	R	R	R	S			R	R	R	R		S	S	S	S	S					RECOVERED	ESBL
95	4	M	796745	PUO	2	8000	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
96	2M	M	797514	PNEUMONIA	2	13300	1	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
97	11M	M	798060	PYOGENIC MENINGITIS	2	10400	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
98	1M	F	798946	PUO	2	14200	2	1	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
99	1	M	801156	APLASTIC ANAEMIA	2	18800	2	1	1	2	1	1	1	1	E.COLI	E.COLI	15 HOURS		R	R	R	R	R	R	R	R			R	R	R	R		S	S	R	R	R					REFERRED TO HIGHER CENTRE	CARBAPENAMASE
100	4D	M	801161	EOS	2	11700	1	1	2	2	1	1	1	1	C.TROPICALIS	C.TROPICALIS	26 HOURS																								S	S	RECOVERED	
101	D43	M	802676	LOS	2	11200	2	2	2	1	1	1	1	1	C.TROPICALIS	C.TROPICALIS	22 HOURS																								S	S	RECOVERED	
102	1D	M	803781	RDS	1	11900	1	2	1	1	1	2	2	2	PS.AERUGINOSA	PS.AERUGINOSA	28 HOURS			R					R	S			R	R	R	S			S	R	R	S	S	S			EXPIRED	
103	8	M	804669	ENTERIC FEVER	2	6500	2	2	1	2	1	1	1	1	S.TYPHI	S.TYPHI	49 HOURS		R		S		S						S	S	R	S		S									RECOVERED	
104	16	F	807072	IE	2	3400	2	2	1	1	1	1	1	1	STREPTOCOCCI VIRIDA	STREPTOCOCCI VIRIDA	17 HOURS	S									R		S		R	S	S	S									DAMA	
105	1	M	808606	PUO	2	12600	2	1	2	2	1	1	1	1	CONS	CONS	22 HOURS	R				R				R	R	R	S		R	R	S	S		S							RECOVERED	MRSE
106	1D	F	809307	RDS	1	12900	1	2	2	1	1	2	2	1	ACINETOBACTER	ACINETOBACTER	10 HOURS			R	R		R		R	S			R	S	R	S			S	S	S	S					RECOVERED	
107	3M	M	809639	CHD	2	16000	2	1	2	2	1	1	1	1	STREPTOCOCCI VIRIDA	STREPTOCOCCI VIRIDA	20 HOURS	S									R		R		S	S	S	S		S							DAMA	
108	1M	M	809647	LOS	2	13000	2	1	2	2	1	1	1	1	ACINETOBACTER	ACINETOBACTER	30 HOURS			R	R		R		R	S			R	R	S	S			S	S	S	S					EXPIRED	
109	18	M	810456	PNEUMONIA	2	11900	2	1	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
110	6	M	812110	PUO	2	10000	2	1	1	2	1	1	1	1	S.TYPHI	S.TYPHI	26 HOURS		R		S		S						S	S	R	S		S									RECOVERED	

111	6D	M	814596	RDS	1	11900	1	2	1	1	2	2	2	1	KL.PNEUMONIAE	KL.PNEUMONIAE	26 HOURS		R	R	R	S	R	R	R	S			S	S	S	S		S	S	R	R	S					RECOVERED	ESBL
112	2	M	815304	PUO	2	14500	2	1	2	2	1	1	1	1	ENTEROCOCCI	ENTEROCOCCI	18 HOURS	R	R														S			R							RECOVERED	
113	10D	M	818111	LOS	2	12600	2	1	2	2	1	1	1	1	KL.PNEUMONIAE	KL.PNEUMONIAE	36 HOURS		R	R	R	S	R	R	R	S			S	S	S	S		S	S	S	S	S					RECOVERED	ESBL
114	1D	F	820681	EOS	1	9900	1	2	2	1	1	1	1	1	S.AUREUS	S.AUREUS	18 HOURS	R				R				R	R	R	R		R	S	S	S		S							DAMA	MRSA
115	D8	M	820726	BIRTH ASPHYXIA	1	14200	1	2	2	1	1	2	2	2	S.AUREUS	S.AUREUS	13 HOURS	R				R				R	R	S	S		R	R	S	S		R							DAMA	MRSA
116	D3	M	823099	MSAF/BA/HIE	1	13100	1	2	2	1	1	2	2	1	C.TROPICALIS	C.TROPICALIS	19 HOURS																								S	S	RECOVERED	
117	2D	M	823133	RDS	2	12000	1	2	2	2	1	2	2	1	S.AUREUS	S.AUREUS	10 HOURS	R				R				R	R	R	R		S	S	S	S		R							RECOVERED	MRSA
118	D18	F	823534	MSAF	1	4000	1	2	2	1	2	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
119	2	F	823560	PYOGENIC MENINGITIS	2	12700	2	1	2	2	1	1	1	1	S.SPECIES	S.SPECIES	23 HOURS		S		S		S						S	S	S	S		S									RECOVERED	
120	13	M	824900	RHEUMATIC FEVER	2	14300	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
121	9	M	825236	ENTERIC FEVER	2	10800	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
122	D1	F	829293	EOS	1	12400	2	2	2	1	2	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
123	8M	M	830959	CHD	2	16900	2	2	2	2	1	1	1	1	STREPTOCOCCI VIRIDA	STREPTOCOCCI VIRIDA	18 HOURS	R									R		R		R	R	S	S		S							DISCHARGE AT REQUEST	
124	5M	M	834066	PUO	2	13600	2	1	2	2	1	1	1	1	S.AUREUS	S.AUREUS	13 HOURS	R				R				R	R	R	R		R	S	S	S		S							RECOVERED	MRSA
125	D10	M	835570	LOS	2	7300	2	1	1	2	1	1	1	1	S.AUREUS	S.AUREUS	16 HOURS	R				R				R	R	R	R		R	S	S	S		R							RECOVERED	MRSA
126	18	F	837412	UTI	2	14200	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
127	D10	M	861773	MSAF	1	13000	2	2	2	2	1	1	2	1	C.KRUSEI	C.KRUSEI	48 HOURS																								-	S	RECOVERED	
128	12	F	862425	BRONCHOPNEUMONA	2	11300	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																												
129	D2	F	863969	MSAF, HIE	1	13000	2	2	2	1	2	2	2	2	KL.PNEUMONIAE	KL.PNEUMONIAE	13 HOURS		R	R	R	R	R	R	R	R			S	S	S	S		S	S	S	S	S					RECOVERED	AMP C
130	1M	M	864092	ASPIRATION PNEUMONIA	1	11800	1	2	2	2	1	1	2	2	C.KRUSEI	C.KRUSEI	22 HOURS																								-	S	EXPIRED	
131	D1	F	864323	SEIZURE	2	9700	1	1	2	1	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
132	D1	M	868919	EOS	2	10300	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
133	1M	M	869029	ASPIRATION PNEUMONIA	2	14100	2	2	1	2	1	1	2	2	S.AUREUS	S.AUREUS	15 HOURS	R				R				R	R	R	S		R	S	S	S		R							RECOVERED	MRSA
134	D12	F	869255	LOS	2	15700	1	1	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
135	D1	M	869864	MSAF	1	10300	1	2	1	1	2	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
136	D1	M	870921	EOS	2	16900	2	1	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
137	D1	M	872308	RDS	1	10800	1	2	1	1	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
138	D1	F	875874	BIRTH ASPHYXIA	1	11600	1	2	2	2	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
139	D1	M	877566	EOS	2	12800	2	1	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
140	D17	M	880376	PYOGENIC MENINGITIS	1	12000	2	2	2	2	1	1	2	1	C.KRUSEI	C.KRUSEI	29 HOURS																								-	S	DAMA	
141	D1	M	883253	MSAF	1	8900	2	1	2	2	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
142	16	M	886291	CELLULITIS	2	12100	2	1	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
143	D18	M	886756	RDS,BA	1	3200	2	2	2	1	1	2	2	2	C.ALBICANS	C.ALBICANS	39 HOURS																								S	S	DISCHARGE AT REQUEST	
144	D1	M	889566	PROM, EOS	2	11800	2	1	1	1	2	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
145	3	F	890801	BRONCHOPNEUMONIA	2	10900	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
146	D15	F	891137	IUGR, LOS	2	3300	2	2	2	1	1	1	2	2	C.ALBICANS	C.ALBICANS	42 HOURS																								S	S	EXPIRED	
147	D2	M	891704	RDS	1	14000	2	2	2	1	1	1	1	1	ENTEROBACTER	ENTEROBACTER	9 HOURS		R	R	R	R	R	R	R	R			R	R	S	S		S	S	R	R	S					RECOVERED	AMP C
148	D5	M	893561	PYOGENIC MENINGITIS	2	7000	2	1	2	2	1	1	1	1	E.COLI	E.COLI	10 HOURS		R	R	R	R	R	R	R	R			R	R	R	S		S	S	R	R	S					DAMA	AMP C
149	D2	M	893582	RDS	1	10200	1	2	2	1	1	2	2	1	CONS	NO GROWTH																											RECOVERED	
150	D1	F	895560	EOS	2	13100	2	2	2	2	1	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
151	D1	M	895872	HIE	1	21000	1	2	1	2	2	2	2	1	KL.PNEUMONIAE	KL.PNEUMONIAE	16 HOURS		R	R	R	R	R	R	R	R			S	S	S	S		S	R	R	R	S					RECOVERED	AMP C
152	D1	F	895909	MSAF WITH RDS	1	15600	1	2	2	2	1	2	2	1	KL.PNEUMONIAE	KL.PNEUMONIAE	18 HOURS		R	S	S	S	S	S	S	S			R	R	R	R		S	S	S	S	S					DAMA	
153	D15	M	895925	RDS	1	13100	2	2	2	2	1	2	2	2	C.ALBICANS	C.ALBICANS	23 HOURS																								S	S	RECOVERED	
154	D20	M	895951	LOS	2	14400	2	2	2	2	1	1	2	2	C.ALBICANS	C.ALBICANS	23 HOURS																								S	S	EXPIRED	
155	D1	F	895958	RDS, BA	1	13000	1	2	1	1	1	2	2	1	ENTEROBACTER	ENTEROBACTER	15 HOURS		R	R	R	R	R	R	R	R			S	S	S	S		S	S	R	R	S					RECOVERED	AMP C
156	D5	M	895969	LOS	2	7700	2	2	2	2	1	1	2	1	C.KRUSEI	C.KRUSEI	29 HOURS																								-	S	RECOVERED	
157	15	F	896801	RHD	2	6700	2	2	1	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
158	14	M	896970	PNEUMONIA	2	4700	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
159	D2	M	897284	LIPOIDAL PNEUMONIA WITH NECROTISING ENTEROCOLITIS	1	3300	2	2	2	1	1	1	2	2	C.ALBICANS	C.ALBICANS	21 HOURS																								S	S	EXPIRED	
160	D1	M	897602	CHD	1	14500	2	2	1	1	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
161	D1	M	898620	RDS	1	2900	2	2	2	1	1	1	1	1	C.ALBICANS	C.ALBICANS	22 HOURS																								S	S	RECOVERED	
162	D12	M	898630	EOS	1	13100	2	2	1	1	1	1	1	1	C.ALBICANS	C.ALBICANS	35 HOURS																								S	S	DAMA	
163	D1	F	899243	RDS	1	13300	1	2	2	2	1	2	2	1	C.FREUNDI	C.FREUNDI	27 HOURS		R	R	R	S	R	R	R	S			S	S	R	R		R	S	R	R	S					RECOVERED	ESBL
164	D7	F	899528	PRE RENAL ARF	1	2400	2	2	2	1	1	1	1	2	C.ALBICANS	C.ALBICANS	24 HOURS																								S	S	EXPIRED	
165	D3	M	899861	IDM/CHD	2	7000	1	1	2	2	1	1	1	2	C.TROPICALIS	C.TROPICALIS	30 HOURS																								S	S	RECOVERED	
166	D1	F	901458	MSAF WITH HIE	1	12800	1	1	1	2	1	2	2	2	C.TROPICALIS	C.TROPICALIS	28 HOURS																								S	S	DAMA	
167	D3	F	901998	MSAF WITH HIE	1	12300	2	2	1	2	2	2	2	2	C.KRUSEI	C.KRUSEI	33 HOURS																								-	S	DAMA	

168	D1	F	902018	BA WITH HIE	1	56300	2	2	2	2	1	2	2	1	C.KRUSEI	C.KRUSEI	27 HOURS																								-	S	DAMA	
169	D12	F	902332	LOS	1	12100	2	2	1	2	1	1	1	1	C.KRUSEI	C.KRUSEI	23 HOURS																								-	S	DAMA	
170	D1	F	903611	MSAF	1	17100	1	2	2	1	1	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
171	6M	M	905270	RHD	2	11600	2	2	2	2	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
172	D1	M	907651	EOS	2	7900	2	2	1	1	2	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
173	D10	M	907657	EOS	2	5450	2	2	2	1	2	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
174	1M	M	910548	SEPSIS	2	4000	2	2	2	1	1	1	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
175	D1	M	910670	UMBILICAL SEPSIS	2	13400	2	1	2	2	1	1	1	1	C.KRUSEI	C.KRUSEI	29 HOURS																								-	S	RECOVERED	
176	11	F	932390	PLEURAL EFFUSION	1	19300	1	2	1	2	1	1	1	2	C.TROPICALIS	C.TROPICALIS	26 HOURS																								S	S	EXPIRED	

S.NO	H.NO	AGE	SEX	DIAGNOSIS	FEVER	TLC	TACHYCARDIA	TACHYPNEA	VENTILATION	1ST SAMPLE	2ND SAMPLE	DURATION	P	AMP	CAZ	CTX	CX	CTR	CXM	PI	AMC, PIT, CAC	E	CD	CIP	LE	COT	TE, DO	LZ	C	AK	GEN	TB	IPM, MRP, ETP	CL	PB	FU	VR	OUTCOME	RESISTANT TYPE
1	750967	59	M	RHD	2	10300	2	1	1	C.ALBICANS	C.ALBICANS	56 HOURS																								S	S	EXPIRED	
2	753486	27	M	SSI	1	8800	2	2	1	NO GROWTH	NO GROWTH																												
3	755741	50	M	COPD	1	24,400	2	1	1	C.FREUNDI	C.FREUNDI	36 HOURS		R	R	R	R	R	R	R	R			R	R	R	R		R	R	R	R	S					RECOVERED	AMP C
4	760881	70	F	PERITONITIS	1	12,300	2	2	1	C.KRUSEI	C.KRUSEI	24 HOURS																								-	S	EXPIRED	
5	761151	56	M	PYOGENIC MENINGITIS	2	13000	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
6	761744	55	M	PUO	2	11300	2	2	2	NO GROWTH	NO GROWTH																											RECOVERED	
7	762451	70	F	COPD	2	26500	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
8	762789	45	F	PERITONITIS	2	12300	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
9	763407	26	M	BURNS	2	25500	2	1	1	KL.PNEUMONIAE	KL.PNEUMONIAE	26 HOURS		R	R	R	S	R	R	R	S			R	R	R	S		S	S	R	R	S					EXPIRED	ESBL
10	763648	50	F	PNEUMONIA	2	22800	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
11	764287	55	M	INTESTINAL OBSTRUCTION	2	10500	2	2	1	E.COLI	E.COLI	15 HOURS		R	R	R	S	R	R	R	S			R	S	R	S		S	S	S	S	S					EXPIRED	ESBL
12	766223	70	M	COPD	2	14900	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
13	766459	26	F	PNEUMONIA	1	19,700	2	2	1	ACINETOBACTER	ACINETOBACTER	19 HOURS			S	S		S		S	S			R	R	S	S			S	R	R	S					EXPIRED	
14	766485	24	M	UTI	2	12200	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
15	766860	53	M	PNEUMONIA	2	6,700	1	2	1	S.AUREUS	S.AUREUS	18 HOURS	R				R				R	R	S	R		R	S	S	S		R						EXPIRED	MRSA
16	766904	25	M	RTA	2	43600	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
17	767658	67	F	UTI	2	13400	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
18	769740	70	M	PNEUMONIA	1	24100	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
19	770260	60	M	PNEUMONIA WITH DM	2	11800	2	2	1	S.AUREUS	S.AUREUS	18 HOURS	R				R				R	R	R	R		S	S	S	S		R						RECOVERED	MRSA
20	770867	80	F	CCF, DM	2	12300	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
21	772685	19	M	RTA	2	10900	2	2	1	S.AUREUS	S.AUREUS	15 HOURS	R				R				R	R	S	R		R	R	S	S		R						EXPIRED	MRSA
22	774038	35	M	PYOGENIC MENINGITIS	1	11900	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
23	774439	30	M	PUO	2	11800	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
24	778820	67	M	COPD	2	12800	1	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
25	779838	26	F	PERITONITIS	2	12,500	2	1	1	GP-A STREPTOCOCCI	GP-A STREPTOCOCCI	11 HOURS	S									R		R		S	S	S	S		S						EXPIRED	
26	783781	58	M	COPD	2	12600	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
27	784697	19	M	BRONCHOPNEUMONIA	2	14200	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
28	785538	45	M	RTA	1	13300	2	2	2	NO GROWTH	NO GROWTH																											EXPIRED	
29	785950	50	F	ARF	2	10800	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
30	786241	25	M	UTI	2	11800	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
31	786544	50	F	PUO	2	13300	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
32	787546	20	F	UTI	2	10800	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
33	787874	30	F	PERITONITIS	1	13000	2	2	1	C.TROPICALIS	C.TROPICALIS	40 HOURS																								S	S	EXPIRED	
34	787892	23	F	ORBITAL CELLULITIS	2	16000	2	2	1	NO GROWTH	NO GROWTH																											RECOVERED	
35	788179	20	M	RTA	2	11500	2	1	1	ACINETOBACTER	ACINETOBACTER	28 HOURS			R	R		R		R	S			S	S	S	S			S	R	R	S					RECOVERED	
36	788562	20	M	PUO	2	14000	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	
37	789562	35	F	PERITONITIS	2	15300	2	1	1	NO GROWTH	NO GROWTH																											EXPIRED	
38	789628	63	M	CRF	2	9400	2	1	1	NO GROWTH	NO GROWTH																											RECOVERED	

39	789908	50	M	IHD	2	12700	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
40	791154	28	M	RTA	1	9900	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
41	791667	22	F	PERITONITIS	2	16600	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
42	792452	20	M	RTA	2	19200	2	2	2	ENTEROBACTER	ENTEROBACTER	20 HOURS		R	R	R	S	R	R	R	S			R	S	R	R		S	S	R	R	S				RECOVERED	ESBL
43	793148	40	F	PNEUMONIA	2	13000	1	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
44	793440	27	M	RTA	1	12300	2	1	1	NO GROWTH	NO GROWTH																												EXPIRED	
45	793580	40	F	PERITONITIS	2	12800	2	2	1	S.AUREUS	S.AUREUS	20 HOURS	R				R				R	R	S	R		R	R	S	S		R							RECOVERED	MRSA
46	794135	25	M	UTI	2	14200	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
47	794155	85	M	COPD	2	11700	1	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
48	796141	60	M	ARF	2	14300	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
49	796727	22	M	PUO	2	12100	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
50	797395	70	M	DKA	1	8800	2	2	1	NO GROWTH	NO GROWTH																												EXPIRED	
51	797483	60	M	COPD	1	11800	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
52	797772	35	M	PNEUMONIA	2	13600	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
53	798056	45	F	PUO	2	10300	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
54	798352	38	M	CHRONIC OSTEOMYELITIS	2	13400	2	1	1	S.AUREUS	S.AUREUS	14 HOURS	R				R				R	R	S	R		S	S	S	S		R							RECOVERED	MRSA
55	798359	34	M	SSI	2	11900	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
56	798606	45	M	CELLULITIS, DM	2	16700	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
57	798963	35	M	RTA	2	12900	2	1	1	ENTEROBACTER	ENTEROBACTER	16 HOURS		R	R	R	S	R	R	R	S			R	S	R	R		S	S	R	R	S				RECOVERED	ESBL
58	799545	55	M	RTA	2	7500	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
59	801486	40	F	PNEUMONIA	2	12800	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
60	801546	60	M	ARF	2	10600	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
61	802422	21	M	PUO	2	11400	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
62	803177	75	M	SEPTICEMIC SHOCK	2	18000	2	2	1	E.COLI	E.COLI	15 HOURS		R	R	R	S	R	R	R	S			R	R	S	R		S	R	R	R	R	S				EXPIRED	ESBL
63	803788	50	F	RHD	2	9700	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
64	804432	75	M	RTA	2	11800	2	1	1	KL.PNEUMONIAE	KL.PNEUMONIAE	20 HOURS		R	R	R	S	R	R	R	S			S	S	S	S		S	S	R	R	S				RECOVERED	ESBL
65	807129	21	F	CYSTIC FIBROSIS	2	14200	2	2	1	ACINETOBACTER	ACINETOBACTER	23 HOURS			R	R		R		R	S			R	S	S	S		S	R	R	R	S				EXPIRED	
66	808285	70	F	UTI	2	10600	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
67	808541	70	M	PNEUMONIA, DM	2	12100	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
68	808573	30	M	INFECTIVE ENDOCARDITIS	1	5200	2	2	1	CONS	NO GROWTH																												RECOVERED	
69	808993	31	M	RTA	2	6400	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
70	809059	63	M	SEPSIS	2	13800	2	2	1	ACINETOBACTER	ACINETOBACTER	28 HOURS			R	R		R		R	S			R	S	S	S		S	S	S	S	S				RECOVERED	
71	809169	40	F	PUO	2	10400	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
72	809322	48	F	COPD	2	13600	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
73	810500	38	F	FEVER WITH UTI	2	14900	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
74	812156	43	M	CYSTITIS WITH BPH	2	18600	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
75	812877	22	F	OP POISONING	1	12100	2	2	2	NO GROWTH	NO GROWTH																												RECOVERED	
76	815821	43	M	PNEUMONIA	2	13600	1	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
77	816138	50	M	ALCOHOLIC CIRRHOSIS, DM,HTN	1	19100	2	1	1	NO GROWTH	NO GROWTH																												EXPIRED	
78	816868	48	F	DKA	2	11400	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
79	817506	32	M	RTA	2	8800	2	1	1	KL.PNEUMONIAE	KL.PNEUMONIAE	20 HOURS		R	R	R	R	R	R	R	R			R	R	S	S		S	S	R	R	R				EXPIRED	CARBAPENAMASE
80	817543	68	M	SAH	1	12200	2	2	1	S.AUREUS	S.AUREUS	19 HOURS	R				R				R	R	S	R		S	S	S	S		R							EXPIRED	MRSA
81	818521	50	M	SEPTIC SHOCK	2	2800	2	2	2	NO GROWTH	NO GROWTH																												RECOVERED	

82	830541	55	M	NECROTISING FASCITIS	1	14800	2	1	1	GP-A STREPTOCOCCI	GP-A STREPTOCOCCI	23 HOURS	S								R		S		R	S	S	S		S							EXPIRED	
83	835121	50	F	PERITONITIS	2	12300	2	1	1	S.AUREUS	S.AUREUS	19 HOURS	R			R				R	R	R	R		R	S	S	S		R							RECOVERED	MRSA
84	837199	45	F	PNEUMONIA	2	13200	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
85	838185	20	F	POST LSCS	2	12600	2	1	2	ENTEROCOCCI	ENTEROCOCCI	13 HOURS	R	R													S			S							EXPIRED	
86	862830	50	F	LOBAR PNEUMONIA WITH SEPSIS	2	18100	2	1	1	E.COLI	E.COLI	13 HOURS		R	R	R	S	R	R	R	S			R	R	R	R		S	S	R	R	S					RECOVERED	ESBL
87	863671	26	M	RHD	2	11600	2	2	1	S.AUREUS	S.AUREUS	20 HOURS	R				R				R	R	S	S		R	S	S	S			R						RECOVERED	MRSA
88	865049	20	M	GTCS	2	7800	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
89	867980	50	M	UTI, SEPTIC SHOCK	2	2600	1	2	1	E.COLI	E.COLI	9 HOURS		R	R	R	R	R	R	R	R			R	R	R	R		S	S	S	S	S					EXPIRED	AMP C
90	871564	20	F	SEPTICEMIC SHOCK	2	3100	2	2	2	C.FREUNDI	C.FREUNDI	14 HOURS		R	R	R	S	R	R	R	S			S	S	S	S		S	S	R	R	S					EXPIRED	ESBL
91	872250	45	M	PUO WITH DM	2	11600	2	1	1	CONS	NO GROWTH																												RECOVERED	
92	873134	22	F	PUO	2	10800	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
93	876158	35	M	INFECTIVE ENDOCARDITIS	2	13900	2	2	1	ENTEROCOCCI	ENTEROCOCCI	19 HOURS	R	R													S			S							RECOVERED	
94	878899	22	M	RTA	2	14100	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
95	880986	37	F	ENTERIC FEVER	2	10900	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
96	881272	27	F	BRONCIAL ASTHMA	2	11300	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
97	883873	21	M	RTA	2	16100	2	1	1	S.AUREUS	S.AUREUS	19 HOURS	R				R				R	R	R	R		S	S	S	S		R						RECOVERED	MRSA
98	883921	22	M	RHD	2	1300	2	2	1	CONS	NO GROWTH																												RECOVERED	
99	884656	50	F	PNEUMONIA	2	9900	2	2	1	NO GROWTH	NO GROWTH																												EXPIRED	
100	886020	75	F	CCF	1	39500	2	2	1	PS.AERUGINOSA	PS.AERUGINOSA	28 HOURS			R					R	R			S	S	R	R			S	S	S	S					DISCHARGE AT REQUEST	
101	888608	45	F	PUO	2	18200	2	1	1	E.COLI	E.COLI	10 HOURS		R	R	R	S	R	R	R	S			R	R	R	R		S	S	S	S	S					RECOVERED	ESBL
102	889346	45	F	CCF	2	10600	1	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
103	889731	26	F	PUO	2	11200	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
104	891456	45	M	CCF	2	10200	2	1	2	NO GROWTH	NO GROWTH																												RECOVERED	
105	892093	55	M	COPD	2	8500	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
106	895920	40	M	PUO	2	10200	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
107	897280	59	M	IHD	1	12700	2	2	1	E.COLI	E.COLI	20 HOURS		R	S	S	S	S	S	S	S			R	R	R	R		S	S	S	S	S					RECOVERED	
108	898635	37	M	RTA	1	13800	2	2	1	S.AUREUS	S.AUREUS	16 HOURS	R				R				R	R	R	R		S	S	S	S		S						EXPIRED	MRSA
109	899808	56	M	CELLULITIS WITH PNEUMONIA	2	18100	2	2	2	GP-A STREPTOCOCCI	GP-A STREPTOCOCCI	11 HOURS	S								R		S		R	R	S	S		S							EXPIRED	
110	900258	70	F	PLEURAL EFFUSION	2	11300	2	2	1	CONS	NO GROWTH																												RECOVERED	
111	901649	55	F	IHD	2	11700	1	2	1	NO GROWTH	NO GROWTH																												RECOVERED	
112	908045	20	F	GTCS	2	10400	1	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
113	908131	32	M	RTA	2	6800	2	1	1	CONS	NO GROWTH																												RECOVERED	
114	908462	58	F	PLEURAL EFFUSION	2	24000	2	2	1	E.COLI	E.COLI	16 HOURS		S	S	S	S	S	S	S	S			R	S	S	S		S	S	S	S	S					EXPIRED	
115	908752	75	F	ARF	2	31000	2	1	1	E.COLI	E.COLI	20 HOURS		R	R	R	R	R	R	R	R			R	R	R	R		S	S	S	S	S					RECOVERED	AMP C
116	909451	40	M	CVA	1	31700	2	1	1	NO GROWTH	NO GROWTH																												RECOVERED	
117	909481	60	M	PLEURAL EFFUSION WITH ASCITIS	2	15600	2	1	2	NO GROWTH	NO GROWTH																												EXPIRED	
118	910337	35	F	PERITONITIS	2	18600	2	2	2	NO GROWTH	NO GROWTH																												DISCHARGE ON REQUEST	
119	912361	35	M	SEPSIS	2	16200	2	2	2	C.TROPICALIS	C.TROPICALIS	30 HOURS																										S	S	EXPIRED	
120	913573	53	M	COPD	1	12000	2	2	1	NO GROWTH	NO GROWTH																												RECOVERED	

121	916225	58	M	PLEURAL EFFUSION WITH CELLULITIS	1	14300	2	2	1	GP B STREPTOCOCCI	GP B STREPTOCOCCI	15 HOURS	S									S		S		S	S	S	S		S							EXPIRED		
122	911087	38	M	PNEUMONIA, ALCOHOLIC CIRRHOSIS	2	8700	1	2	1	KL.PNEUMONIAE	KL.PNEUMONIAE	20 HOURS		R	R	R	S	R	R	R	S			R	S	R	S		S	S	R	R	S					RECOVERED	ESBL	
123	923885	40	M	PLEURAL EFFUSION	2	12400	2	2	1	S.SPECIES	S.SPECIES	36 HOURS		R		S		S						S	S	S	S		S									EXPIRED		
124	864805	50	M	CELLULITIS, DM	2	13600	2	1	1	NO GROWTH	NO GROWTH																													