VENTILATOR ASSOCIATED PNEUMONIA, MICROBIAL SPECTRUM, QUANTITATIVE PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN AT R.L.JALAPPA HOSPITALAND RESEARCH CENTRE, KOLAR.

BY

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DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION & RESEARCH

TAMAKA, KOLAR, KARNATAKA

IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE

IN

MICROBIOLOGY

UNDER THE GUIDANCE OF

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APRIL 2013

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'VENTILATOR ASSOCIATED PNEUMONIA, MICROBIAL SPECTRUM, QUANTITATIVE PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN' AT R.L.JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR

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ACKNOWLEDGEMENT

I take this opportunity to express my humble and sincere gratitude and indebtedness to my teachers Dr Beena PM, Professor & Head and Dr Gokul BN, Professor, Department of Microbiology, SDUMC, Kolar, whose guidance and constant encouragement helped me in successful completion of the study. I wish to express my humble and sincere gratitude to ***Dr Gokul BN*** *Professor, Department Microbiology, SDUMC, Kolar, who encouraged me to take this study and for his constant support and guidance.*

I wish to express my sincere thanks to ***Dr Dinesh K****, Professor, Department of Anesthesiology, for his constant support and clinical guidance during the period of study.*

*I wish to express my deepest gratitude to **Dr Prasad SR**, Professor, Department of Microbiology and PG Director, SDUMC, Kolar for his guidance and expert advice.*

*I wish to express my sincere thanks to **Dr Rajini M, Dr Jeevan Shetty, Dr Tanveer, Dr Rekha and Dr Parimala, Department of Microbiology** for their advice and co- operation..*

*I am grateful to **Dr Kiran, Dr Suresh, Dr Krishna, Dr Rajesh and Dr Priya** Department of Anaesthesiology, for their invaluable help, guidance, kind support and co-operation.*

*I render my hearty thanks to **Dr. Deepthi Kiran**, Assistant Professor, Department of Community Medicine, SDUMC for helping me in statistical analysis.*

*My sincere thanks to my colleagues **Dr Savitha N, Dr Savitha P, Dr Archana, Dr Vidhya, Dr Anitha, Dr Trupthi, Dr Bipin and Dr Sudha** for their kind co-operation.*

I am extremely greatful to my Anaesthesia PG's for helping me in procuring the samples.

*I express my sincere gratitude to all the **subjects** for giving me an opportunity to carry out this study.*

*I would like to acknowledge the help rendered by all the **technicians** in the Department of Microbiology during my study.*

*I also thank all **non teaching staff** for their kind co-operation.*

I thank my family for their constant support.

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LIST OF ABBREVIATIONS

VAP - VENTILATOR ASSOCIATED PNEUMONIA

HAP - HOSPITAL ACQUIRED INFECTION

ICU - INTENSIVE CARE UNIT

CDC - CENTRE FOR DISEASE CONTROL

CFU - COLONY FORMING UNIT

CPIS - CLINICAL PULMONARY INFECTION SCORE

BAL - BRONCHO ALVEOLAR LAVAGE

PSB - PROTECTED SPECIMEN BRUSH

BBS - BLIND BRONCHIAL SAMPLING

MDR - MULTI DRUG RESISTANT

MRSA - METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

MSSA - METHICILLIN SENSITIVE STAPHYLOCOCCUS AUREUS

ESBL	-	EXTENDED SPECTRUM BETA LACTAMASES
SDD	-	SELECTIVE DECONTAMINATION OF THE DIGESTIVE TRACT
ATS	-	AMERICAN THORACIC SOCIETY
IMV	-	INVASIVE MECHANICAL VENTILATION
MV	-	MECHANICAL VENTILATION
GNB	-	GRAM NEGATIVE BACILLI
HCAI	-	HEALTH CARE ASSOCIATED INFECTION

ABSTRACT

TITLE OF THE STUDY: VENTILATOR ASSOCIATED PNEUMONIA , MICROBIAL SPECTRUM, QUANTITATIVE PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN AT R. L. JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR.

BACKGROUND: Ventilator associated pneumonia (VAP) is one of the most important form of Hospital acquired infections which is associated with increased mortality and morbidity. VAP occurs in about 9 to 27% of all intubated patients. Intubation is

associated with 3 to 10 fold increase in the incidence of VAP among all patients receiving mechanical ventilation. In contrast to other nosocomial infections the crude mortality rate occurring due to VAP ranges from 24% to 76%. ICU patients with VAP have a 2- to 10-fold higher risk of death when compared with patients without pneumonia

OBJECTIVES:

1) To find out the frequency of occurrence of VAP in clinically suspected patients who are mechanically ventilated for more than 48 hours at **R. L. Jalappa Hospital and Research Centre, Kolar.**
2) To evaluate the quantitative bacterial and fungal isolates from Endotracheal aspirates (ETA) or other respiratory secretions.
3) To study the major pathogens and their antibiotic sensitivity pattern.

MATERIALS AND METHODS

A total of hundred patients who are mechanically ventilated for more than 48 hours with clinical suspicion of VAP were included in the study. Endotracheal aspirate was collected and subjected to Grams stain and culture. Culture was performed by quantitative culture technique .Growth on the culture plate was identified by standard biochemical reactions and subjected to antibiotic sensitivity testing by Kirby Bauer disc diffusion method.

RESULTS

The incidence of VAP in our ICU set up was 25.5/1000 patient ventilated days. The most common age group affected was 31- 40 years with male preponderance. The mean duration of ventilation was 5.38 days. 26.76% of the infections were categorized as early onset VAP while 73.3% as late onset VAP. Acinetobacter was the most common organism causing both early onset and late onset VAP. Most of the Multi Drug Resistance (MDR) strains were associated with late onset VAP. Mortality rate was more in the late onset group (48.07%) while it was 26.31% in the early onset group.

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INTRODUCTION

Ventilator associated pneumonia (VAP) is defined as pneumonia occurring in patients who have been intubated and received mechanical ventilation for more than 48 hours. It is the second most common nosocomial infection accounting to 15% of all

hospital acquired infections. Approximately 10-28 % of critical care patients develop VAP[2]. Prevalence ranges from 10-65 % in tertiary care hospitals and a case fatality rate of more than 20%[3]. Incriminating pathogens vary among hospitals and also in same centre over time. Therefore the frequency of occurrence of VAP and its etiological agents needs to be studied in each setting so as to guide the clinician in choosing the most effective and appropriate treatment.

It is classified as either early onset (occurring within 96 hours of start of mechanical ventilation) or late onset (>96 hours after start of mechanical ventilation[1]). The commonest organisms causing early onset VAP are Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus (methicillin sensitive), Escherichia coli and Klebseilla pneumoniae[1]. The organisms causing late onset VAP are Pseudomonas aeruginosa, Acinetobacter baumanii and Staphylococcus aureus (methicillin resistant) [1]. Most strains responsible for early onset VAP are antibiotic sensitive. Those responsible for late onset VAP are usually multiple drug resistant.

The pathogenesis of ventilator-associated pneumonia is due to[8]:

- Bacterial colonization of the aero digestive tract and
- The aspiration of contaminated secretions into the lower airway.

There is no gold standard test for the diagnosis of VAP. Diagnosing VAP requires a high degree of clinical suspicion combined with bedside examination, radiographic picture, and microbiologic analysis of respiratory secretions. Detection of causative organism is done by collecting respiratory secretions either by invasive [protected sample brush (PSB) or broncho-alveolar lavage (BAL)] or noninvasive [endotracheal aspirate (ETA)] techniques and, culturing quantitatively and semi-quantitatively.

The diagnosis and management of VAP remains one of the most challenging and controversial topics in management of critically ill patients. Aggressive surveillance is vital in understanding local factors leading to VAP and the microbiologic milieu of a given unit. Judicious antibiotic usage is essential to improve outcome and decrease mortality, as resistant organisms continue to plague intensive care units and critically ill patients.

As there was no data on the incidence, bacterial spectrum and their antibiotic sensitivity pattern at our hospital, this study was undertaken. Knowledge of the microbial spectrum of the ICU may help in the management of the critically ill patients thereby decreasing the mortality.

AIMS AND OBJECTIVES

1) **TO FIND OUT THE FREQUENCY OF OCCURANCE OF VAP IN CLINICALLY SUSPECTED PATIENTS WHO ARE MECHANICALLY VENTILATED FOR MORE THAN 48 HOURS AT R L JALAPPA HOSPITAL AND RESEARCH CENTRE, KOLAR.**

2) **TO EVALUATE THE QUANTITATIVE BACTERIAL AND FUNGAL ISOLATES FROM ENDO-TRACHEAL ASPIRATES (ETA) OR OTHER RESPIRATORY SECRETIONS.**

3) **TO STUDY THE MAJOR PATHOGENS AND THEIR ANTIBIOTIC SENSITIVITY PATTERN.**

REVIEW OF LITERATURE

Ventilator associated pneumonia is one of the most important form of Hospital acquired infections which is associated with increased mortality and morbidity. It is defined as pneumonia occurring in patients who are intubated and received mechanical ventilation for more than 48 hours.

CLASSIFICATION

It is classified as either early onset (occurring within 96 hours of start of mechanical ventilation) or late onset (occurring > 96 hours after start of mechanical ventilation[1]).

EARLY ONSET VAP: It is commonly associated with antibiotic sensitive strains. The common organisms causing early onset VAP are Haemophilus influenzae, Streptococcus pneumoniae, Staphylococcus aureus (Methicillin sensitive), Escherichia coli and Klebseilla pneumoniae.

LATE ONSET VAP: It is most commonly associated with antibiotic resistant strains. The common organisms causing late onset VAP are Pseudomonas aeruginosa, Acinetobacter species, Enterobacter species and Methicillin resistant Staphylococcus aureus.

EPIDEMIOLOGY

VAP is the second most common nosocomial infection accounting to 15% of all hospital acquired infections[2]. Approximately 10-28 % of critical care patients develop VAP[3]. Prevalence ranges from 10-65 % in tertiary care hospitals and a case fatality rate of more than 20%[4]. VAP occurs in about 9 to 27% of all intubated patients[4]. Intubation is

associated with 3 to 10 fold increase in the incidence of VAP among all patients receiving mechanical ventilation[8]. In contrast to other nosocomial infections the crude mortality rate occurring due to VAP ranges from 24% to 76%[5].

ICU patients with VAP have a 2 to 10 fold higher risk of death when compared with patients without pneumonia. Development of VAP is associated with increased duration of mechanical ventilation, prolonged stay in the ICU and increase in costs.

PATHOGENESIS

Pneumonia is an inflammatory response by the host due to the invasion of the microbes into the lung parenchyma. The magnitude of the inflammatory response depends on the type and load of the inoculum, the virulence of the organism and the competence of the host immune response[5].

The normal respiratory tract possesses a variety of defence mechanisms that protect the lung from infection. The anatomical barriers like glottis and larynx, muco-ciliary and mechanical clearance in the upper airways are the important defense mechanism against infection. The alveolar macrophages and polymorphonuclear leukocytes interact and eliminate the invading pathogens by mounting an humoral and cell mediated immune response[6]. All these components have to function properly to eliminate the invading pathogen. But, when these defence mechanisms are impaired or, if they are overcome due to a high inoculum of virulent organisms, pneumonitis results.

The pathogenesis of ventilator-associated pneumonia is due to[7]:

Bacterial colonization of the aero digestive tract and

Aspiration of contaminated secretions into the lower airways.

The presence of invasive devices is an important contributor to the pathogenesis and development of VAP. Presence of naso-gastric tube impairs the function of gastro-esophageal sphincter and increases gastric reflux. Naso-gastric tubes also predispose to VAP by elevating gastric pH, leading to gastric colonization and increased incidence of reflux causing aspiration of gastric contents[8]. Endotracheal tubes facilitate bacterial colonization of the tracheo-bronchial tree and lower airways and aspiration of secretions

through mucosal injury. Cuff on the tracheal tube helps in the passage of infected material into the airway. Pooling of the contaminated secretions above the cuff aids in the passage of the secretions along their folds thereby colonizing the trachea and eventually formation of biofilms[8]. Sometimes the ventilatory circuits, nebulizers or humidifiers contribute to the pathogenesis of VAP if they are contaminated with bacteria[9].

All these factors eventually cause lung injury and impair the ability of the pulmonary defence mechanism to deal with the pathogens. Pulmonary odema and alveolar hemorrhage provide a favorable environment for the proliferation of bacteria and development of pneumonia[12]

RISK FACTORS

The various risk factors which increase the likelihood of development of VAP are as follows.

1) **AGE**: Risk of development of VAP is more at the extreme of ages. Younger age group due to lack of well developed defence mechanisms and elderly due to waning immune mechanism and decreased gag and cough reflex[10].
2) **SMOKING**: Smokers have a higher preponderance of development of pulmonary infection as it impairs the muco-ciliary clearance and inhibits phagocytosis.
3) **ALCOHOL**: Alcohol is associated with increased risk of nosocomial pneumonia as it impairs pulmonary defence mechanism by depressing mucociliary clearance, decreased surfactant production and decreased alveolar macrophage function[11]. It also increases the chances of aspiration.
4) **SURGERY**: Postsurgical patients have a higher risk of development of VAP. History of smoking, longer preoperative stay, longer surgical procedures and thoracic or upper abdominal surgery are significant risk factors for postsurgical pneumonia[9]. The risk of development of VAP increases by about 14 times for thoracic and 3- 4 times for abdominal surgeries[9].
5) **UNDERLYING DISAEASE**: The incidence of VAP is more in patients with an underlying lung disease, cardiac disease.
6) **POSITION OF THE PATIENT**: Supine position promotes the development of VAP by increasing the chances of aspiration of gastric contents. The chances of

aspiration can be reduced by maintaining the patient in semi-recumbent position[13] or by using rotational beds

7) **AIRWAY INTUBATION**: Endotracheal tube causes a breach in the in the continuity of the mucous membrane. The local trauma and inflammation caused due to the endotracheal tube increases the chances of colonization of the organisms in the trachea with reduced clearance of organisms and secretions from the lower respiratory tract [9]. Presence of Endotracheal tube also impedes cough reflex and allows leakage around the cuff which leads to pooling of the secretions into the trachea-bronchial tree[9]. Formation of bio-film over time due to prolonged tube insitu[14] may increase the risk of bacterial embolization into the alveoli following rigorous suctioning or bronchoscopy.
8) **RE-INTUBATION**: In addition to the presence of endo-tracheal tube, re-intubation per se is an important risk factor for the development of VAP. Re-intubation causes aspiration of colonized oro-pharyngeal secretions into the lower airways and direct aspiration of gastric contents into the lower airways, when the naso-gastric tube also is insitu[9,8].
9) **DURATION OF MECHANICAL VENTILATION**: The risk of acquiring VAP in patients receiving mechanical ventilation increases with time. Fagon et al. suggested that the incidence of VAP increases by 1% per day of IMV[15].
10) **EQUIPMENT RELATED**: Ventilatory circuits, Bronchoscopes, nebulizers or humidifiers may contribute to the pathogenesis of VAP if they are contaminated with bacteria[10]. The major risk of infection was associated with contaminated reservoir nebulizers, designed to deliver drugs[10]. The ventilator tubing circuit gets frequently colonized by bacteria. Condensate collecting in the circuit can become contaminated from patient's secretions or by opening the circuit[11]. Simple procedures like turning the patient or raising the bed rail may accidentally spill contaminated condensate directly into the patient's trachea-bronchial tree. Bronchoscopy used for diagnostic purposes or for removal of secretions may cause embolization of bacteria from the bio-film to the trachea-bronchial tree.
11) **NASOGASTRIC TUBE / ENTERAL FEEDING**: Naso-gastric tube disrupts the oesophageal gastric barrier causing gastro-esophageal reflux and aspiration.

Enteral feeding by naso-gastric tube may predispose to VAP by elevating gastric pH, leading to gastric colonization and increasing the risk of reflux and aspiration by causing gastric distension[9].

12) **STRESS ULCER PROPHYLAXIS**: Treatment with antacids and Histamine receptor antagonists is associated with increased risk of infection. These agents inhibit the peptic activity and decreases acid secretion allowing overgrowth of gram negative bacteria and retrograde colonization of the oro-pharynx. A direct relationship between alkaline gastric pH and gastric bacterial colonization has been demonstrated in several studies[5]. However in some studies the rate of development of VAP was lower in patients receiving Sucralfate, but it is controversial[5].

13) **PRIOR ANTIBIOTIC THERAPY**: The effect of prior antibiotic therapy is controversial. Prior antibiotic therapy reduces the risk of early-onset ventilator associated pneumonia. However, prior prolonged antibiotic administration favours selection and subsequent colonization with resistant pathogens responsible for super infections[5,9].

ETIOLOGIC AGENTS

VAP is poly-microbial caused by a wide spectrum of bacterial pathogens and rarely due to viral or fungal pathogens. Common pathogens include gram-negative bacilli such as Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Acinetobacter species[2]. Infections due to gram-positive cocci, such as Staphylococcus aureus, particularly methicillin-resistant S. aureus (MRSA) have been rapidly emerging in recent years[2]. The frequency of specific Multi Drug Resistant pathogens causing VAP may vary by hospital, patient population, exposure to antibiotics, type of ICU patient and changes over time, emphasizing the need for timely surveillance[4,2].

Pseudomonas aeruginosa: Pseudomonas aeruginosa is an aerobic non-fermenting Gram-negative bacillus with intrinsic resistant to many classes of antibiotics. It is the most common antibiotic-resistant pathogen causing VAP and the most common cause of

fatal episodes of VAP. Pseudomonas has numerous virulence factors, which facilitate lung infection .The most important are a family of secreted exotoxins [ExoS, ExoT, ExoU (PepA), and ExoY] that are injected directly into the cytoplasm of host cells[17,18]. In a mouse model of pneumonia, the strains expressing ExoU appeared to have the greatest virulence. These exo-toxins cause lysis of alveolar epithelial and macrophage-like cell lines. It is resistant to many classes of antibiotics which is mediated by efflux pumps or by mutation[17,19].

Enterobacteriaceae: The Enterobacteriaceae or enteric Gram-negative bacilli, are a group of aerobic Gram-negative bacilli that normally reside in the lower gastrointestinal tract. Antibiotic therapy and critical illness can suppress the normal bacterial flora and lead to an overgrowth of Enterobacteriaceae in the gut and colonization of the skin and the upper gastrointestinal and respiratory tracts. The most common agents causing VAP are Klebsiella species, Enterobacter species and E.coli[1,17,19]. Individual members of this genus have unique intrinsic antimicrobial susceptibility patterns, but the most concerning issue is acquisition of extended-spectrum β-lactamases that render the bacteria resistant to penicillin and cephalosporin antibiotics [17,19].

Acinetobacter species: Acinetobacter species (predominantly baumannii and calcoaceticus) are aerobic nonfermenting Gram-negative bacilli present in soil and fresh-water sources. Recently there has been increasing recognition of Acinetobacter species as important causes of nosocomial infection, particularly in critically ill intensive care unit patients. Although generally less virulent than P. aeruginosa, Acinetobacter species have nonetheless become problem pathogens because of increasing resistance to commonly used antimicrobial agents. More than 85% of isolates are susceptible to carbapenems, but resistance is increasing due to IMP-type metalloenzymes or carbapenemases of the OXA type[18].

Staphylococcus Aureus: Staphylococcus aureus is a Gram-positive coccus that frequently colonizes the anterior nares and is one of the most important causes of nosocomial infection and of VAP. Risk factors for VAP caused by methicillin-sensitive

S.aureus include younger age, traumatic coma, and neurosurgical problems. Risk factors for VAP caused by MRSA include COPD, longer duration of mechanical ventilation, prior antibiotic therapy, prior steroid treatment, and prior bronchoscopy[17,19]. S. aureus possesses a number of important virulence factors, the most important being Panton-Valentine leukocidin gene. Panton-Valentine leukocidin gene is a extracellular staphylococcal toxin that has been associated with skin and soft-tissue infections and severe necrotizing pneumonia[19]. Traditionally, most strains have been susceptible to pencillinase-resistant β lactam antibiotics (methicillin-sensitive S. aureus), but the prevalence of methicillin-resistant S. aureus (MRSA) strains is increasing, even in community isolates [17,19].

Streptococcus pneumoniae: Streptococcus pneumoniae is a Gram-positive capsulated diplococcus. It colonizes the upper respiratory tract and invades the lung after microaspiration of oropharyngeal secretions. Capsule prevents opsonization and phagocytosis. It is the most common cause of community-acquired pneumonia and is usually associated with early onset VAP. The main risk factors for VAP are smoking, chronic obstructive pulmonary disease (COPD), and the absence of prior antibiotic therapy[17,19].

Fungal pathogens: Nosocomial pneumonia due to fungi, such as Candida speciesand Aspergillus fumigatus are most common in organ transplant or immunocompromised and neutropenic patients but uncommon in immunocompetent patients [20].Nosocomial infection by Aspergillus species suggest possible airborne transmission by spores, and may be associated with an environmental sources such as contaminated air ducts or hospital construction[25].

Isolation of Candida albicans and other Candida species from endotracheal aspirates is common, but usually represents colonization of the airways, rather than pneumonia in immunocompetent patients, and rarely requires treatment with antifungal therapy[19,20].

Viral pathogens: The incidence of HAP and VAP due to viruses is very low in immune-competent hosts. Influenza virus, Parainfluenza virus, Adenovirus and Respiratory syncytial virus account for 70% of the nosocomial viral causes of HAP and VAP[17,19].

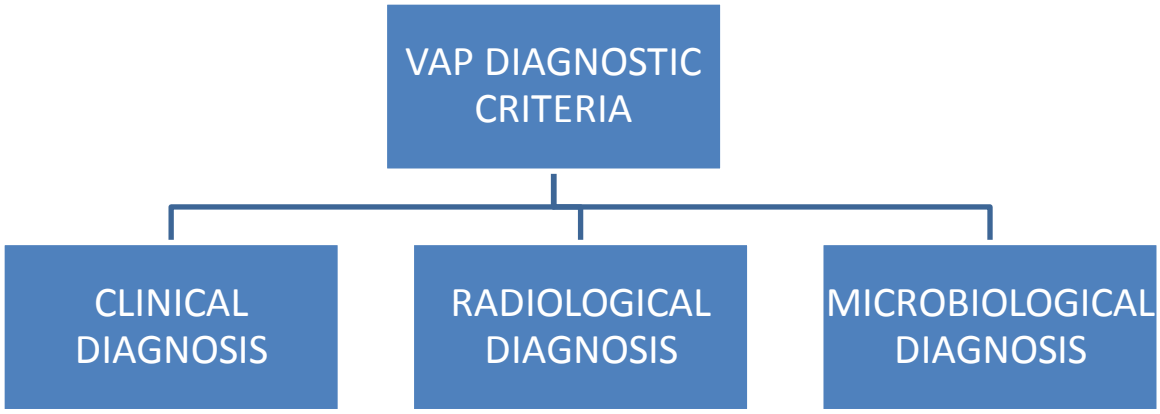
Respiratory syncytial virus outbreaks of bronchiolitis and pneumonia are more common in Pediatric wards and rare in immune-competent adults. Diagnosis of these viral infections is often made by rapid antigen testing and viral culture or serologic assay. Influenza A is probably the most common viral cause of HAP in adult patients. Pneumonia in patients with influenza A or B may be due to the virus or secondary bacterial infection or both. The use of influenza vaccine along with prophylactic and early antiviral therapy among healthcare workers and high-risk patients with amantadine, rimantadine has reduced the spread of influenza within hospital and healthcare facilities[21].

DIAGNOSIS

There is no gold standard test for the diagnosis of VAP[5,1,22]. The diagnosis and management of VAP remains one of the most controversial and challenging topics in management of critically ill patients. Aggressive surveillance is vital in understanding local factors leading to VAP and the microbiologic milieu of a given unit.

The diagnosis of VAP is usually based on clinical, radiological signs and bacteriologic evidence of lower respiratory tract infection[1]. New fever, purulent endo-tracheal secretion, and leukocytosis are the usual warning clinical signs[17,22]. Chest radiograph remains an important component for suspicion of VAP. Detection of a new infiltrate is often a real challenge in cases of acute respiratory distress syndrome or previous severe pulmonary injury. Moreover, an infiltrate may be due to a variety of causes[23]. Because these signs of suspicion have good sensitivity but poor specificity for diagnosing VAP, collection of a respiratory tract sample namely endotracheal aspirates, BAL or protected-specimen brushes are performed to isolate microorganism, to confirm infection and specify etiology[1,17,24]. Blood or pleural fluid cultures rarely contribute to etiologic diagnosis[1,24].

VAP DIAGNOSTIC CRITERIA ACCORDING TO CDC[22,25]



CLINICAL SIGNS

Ventilator-associated pneumonia is usually suspected when the patient develops fever, tachycardia, leukocytosis or leucopenia, and purulent tracheobronchial secretions. These systemic signs such as fever, tachycardia, and leukocytosis, are nonspecific and can be caused by any inflammatory condition that releases cytokines[17,23]. These clinical criteria when considered alone had a poor efficacy and have a limited diagnostic value in definitive diagnosis of VAP. Hence clinical diagnosis of VAP is associated with 30–35% false negative and 20–25% false-positive results.[23,26]

RADIOLOGICAL SIGNS

Chest roentgenogram is an important component in the evaluation of hospitalized patients with suspected pneumonia. Presence of new or progressive and persistent infiltrate, Consolidation or Cavitation arouses a suspicion of VAP[5,23,26].

MICROBIOLOGICAL DIAGNOSIS

Microbiological diagnosis of VAP is based on detection of causative organism by collecting respiratory secretions. The commonly used sampling techniques are either by invasive [Blind Bronchial Sampling (BBS), protected sample brush (PSB) or broncho alveolar lavage (BAL)] or noninvasive [endotracheal aspirate (ETA)] methods and culturing quantitatively and semi-quantitatively [23,27]. The best of these diagnostic procedures is still controversial. The rationale for quantitative culture is to differentiate

between infection and colonization because colonization is most common in mechanically ventilated patients [5,23].

NON INVASIVE DIAGNOSTIC TECHNIQUES

Pleural fluid or blood culture: VAP spreads to the blood or pleural space in less than 10% of cases[1]. Most experts recommend two sets of blood cultures and a thoracocentesis of non-loculated pleural effusions for the evaluation of suspected VAP [17,25] . However, the sensitivity of blood cultures for the diagnosis of VAP is less than 25% and also when positive, the organisms may originate from an extra-pulmonary site[17].

Endo-Tracheal Aspirate: Endo tracheal aspirate is the most common sampling technique employed in intubated patients with suspicion of VAP. Gram staining and non-quantitative cultures of tracheal secretions have the advantages of reproducibility and it require little technical expertise [5,23]. However non-quantitative culture is less sensitive as the upper respiratory tract gets rapidly colonized by potential pulmonary pathogens even when pneumonia is not present. Thus, if an organism is cultured or noted on Gram stain it is difficult to identify whether the isolate is a pathogen or colonizer.

Because of the poor specificity of the clinical diagnosis of VAP and of qualitative evaluation of ETAs, Pugin et al. developed a composite clinical score, called the clinical pulmonary infection score (CPIS), based on six variables: temperature, blood leukocyte count, volume and purulence of tracheal secretions, oxygenation, pulmonary radiography, and semi- quantitative culture of tracheal aspirate[24].

Quantitative culture of ETA: To improve the specificity of the diagnosis of VAP and avoid unnecessary antibiotic use, numerous studies have investigated the role of quantitative cultures of respiratory secretions [1,5,23]. Quantitative cultures have a good diagnostic utility especially in patients with a low or equivocal clinical suspicion of VAP. Any growth $>10^5$ colony forming units (cfu)/ml are considered as pathogens and any

growth below this threshold are considered as colonizers or contaminants [1,17,22,25]. Using this threshold value the Sensitivity ranged from 38% to 82% with specificity ranged from 72% to 85%[17,25].

ADVANTAGES: Inherent advantages of the non invasive techniques are less invasiveness, lower cost, the lack of potential contamination by the bronchoscope, less compromise of patient gas exchange during the procedure.[9,17]

DISADVANTAGES: sampling errors as it is a blind technique and lack of airway visualization.[9,17]

INVASIVE TECHNIQUES

Bronchoscopic techniques: Fiber-optic bronchoscopy is a safe and accurate technique for the diagnosis of various pulmonary lesions. During the last two decades it has been extensively used for diagnosing HAP and particularly VAP. Advantages of using a bronchoscope include collection of lower airways secretions from the site of presumed infection thereby by-passing the upper airways which is usually colonized by non-pathogenic micro-organisms and leading to a potential misinterpretation of the cultures.

PSB and broncho alveolar lavage (BAL) sampling technique have improved the sensitivity and specificity of diagnosis of VAP. Furthermore, the quantitative culture of the samples obtained by these methods allows a better differentiation between colonization and infection [27].

In mechanically ventilated patients, fiber-optic bronchoscopy is performed through the endotracheal tube, so the inner bore of the endotracheal tube is wide enough to permit the progression of the bronchoscope [27,28]. Patients should be sedated and paralyzed to allow effective ventilation and to prevent damage to the bronchial mucosa during the procedure.

1. PROTECTED SPECIMEN BRUSH: The PSB consists of a double telescoping catheter containing a metallic brush within the inner cannula. The technique involves positioning the bronchoscope next to the orifice of the sampling area and advancing the PSB catheter 3 cm beyond the fiberoptic bronchoscope to avoid collection of pooled secretion on the catheter tip [27,28]. A small quantity of brushed secretions is used for direct examination after staining by Gram methods which facilitates the evaluation of the quality of the sample and later subjected for culture[17,27,28]. About ~0.001 ml (range 0.01–0.001) of lower respiratory secretions is aspirated. Quantitative bacterial cultures of PSB allow the distinction between colonization and infection[27]. Quantitative cultures represent serial dilutions of the respiratory samples. The colony counts are calculated by the number of colonies visible on the agar plate in relation to the dilution. In PSB, 0.001 mL of secretions are collected and the presence of $>10^3$ cfu/mL bacteria has 80–90% sensitivity and 95% specificity for the diagnosis of VAP [27].

2. BRONCHOALVEOLAR LAVAGE: BAL is an important diagnostic tool for the diagnosis of VAP. Quantitative cultures of the samples retrieved by protected and non-protected Bronchoscopic BAL provide a good diagnostic yield. Quantitative bacterial cultures of BAL samples facilitate the differentiation between colonization and infection. In BAL, larger proportion of lung can be sampled and the diagnostic threshold is $>10^4$ cfu/ml. The sensitivity and specificity of BAL are 86– 100% and 95–100% resp[17,25].

DIAGNOSTIC ACCURACY:

Various studies having had evaluated the accuracies of ETA, PSB and BAL to diagnose VAP have shown a significant degree of variability in sensitivity, specificity and positive and negative predictive values for each of the techniques. This variability has resulted from the use of different "gold standards" for the diagnosis of VAP, the use of different cut off thresholds for quantitative cultures, differences in equipment and protocols, and differences between the

populations studied and prior use of antibiotics[1,5,17].

Numerous studies have demonstrated that prior and concurrent antibiotic therapy decrease the accuracy, sensitivity and negative predictive value of Gram staining and quantitative, semi-quantitative and non-quantitative cultures [5,17,22]. Even 24 h of administration of an antibiotic can affect culture results. This effect of prior antibiotics on the false-negative rate of microbiologic studies is of great concern, particularly since VAP is a potentially lethal disorder. However, if antibiotics have not been changed in the last 72 h, the diagnostic yield of any culture technique is unaffected[29].

TREATMENT

Treatment and prevention of VAP is the major priority because VAP leads to longer duration of mechanical ventilation, longer stay in the ICU, increased use of antibiotics, higher costs for healthcare, and higher mortality[1,17,25]. Despite broad clinical experience, no consensus has been reached concerning issues regarding optimal antimicrobial regimen or its duration[11]. The criteria for a definitive diagnosis of VAP in critically ill patients remain to be established.

Although it is difficult to clinically distinguish between bacterial colonization of the trachea-bronchial tree and true nosocomial pneumonia all previous therapeutic investigations rely on clinical diagnostic criteria. In most of the studies, tracheal secretions are collected for culture, though the upper respiratory tract of ventilated patients is usually colonized with multiple potential pathogens. The lack of a standard technique to directly sample the infection site in the lung has hampered the study of the ability or inability of antibiotics to eradicate the causative pathogens from the lower

respiratory tract and, therefore, to predict their bacteriologic efficacy[1,17].

INITIAL EMPHERICAL ANTIBIOTIC THERAPY: Once the clinical diagnosed of VAP is done, therapy should be initiated. The following factors have to be kept in mind before initiating antibiotics. The American Thoracic Society[17] has laid down a few recommendations depending on the organism and the time of onset of VAP

1) Selection of an initial empiric therapy should be based on the presence or absence of risk factors for MDR pathogens.
2) Choice of specific agents should be dictated by local microbiology, cost, availability, and formulary restrictions.
3) Patients with healthcare-related pneumonia should be treated for potentially drug-resistant organisms, regardless of duration of stay.
4) Inappropriate therapy is a major risk factor for increased mortality and prolonged length of stay for patients with Hospital Acquired Pneumonia (HAP), and antibiotic-resistant organisms are most commonly associated with inappropriate therapy.
5) In selecting empiric therapy for patients who have recently received an antibiotic, an effort should be made to use an agent from a different antibiotic class, because recent therapy increases the probability of inappropriate therapy and resistant to same class of antibiotic.
6. Initial antibiotic therapy should be given promptly because delay in administration may add to excess mortality.

OPTIMIZATION OF ANTIBIOTIC THERAPY: Initial antibiotic treatment regimen must be re-evaluated to either stop the treatment if the infection is not confirmed or narrow the antibiotic spectrum (ie, de-escalation strategy).Optimal outcome in patients with HAP can best be achieved with the combination of appropriate initial therapy and an adequate therapy regimen. To achieve adequate therapy, it is necessary not only to use the correct antibiotic, but also the optimal dose and the correct route of administration (oral, intravenous, or aerosol) to ensure that the antibiotic penetrates to the site of infection, and to use combination therapy if necessary[1,17]. Pharmacodynamic properties of specific antibiotics should also be considered in selecting an adequate dosing regimen[30]. Some antibiotics penetrate well and achieve high local concentrations in the

lung whereas others do not. For example, most β-lactam antibiotics achieve less than 50% of their serum concentration in the lung, whereas fluoroquinolones and linezolid attain same concentrations in the serum and lung [30].

MONOTHERAPY OR COMBINATION THERAPY: Combination therapy is common practice in the therapy of suspected and proven gram-negative VAP. Combination of antibiotics allows broadening of the spectrum of initial empirical therapy and achieves synergy in the treatment of some bacteria such as P.aeruginosa and prevents the emergence of resistance during therapy[1]. Combination therapy should include agents from different antibiotic classes to avoid antagonism of therapeutic mechanisms[29,30]. For GNB, regimens usually involve combining 2 drugs from the β-lactam, quinolone, or aminoglycoside classes.

Monotherapy should be used when required because combination therapy is often expensive and exposes patients to unnecessary antibiotics, thereby increasing the risk of MDR pathogens and adverse outcomes. Patients who develop nosocomial pneumonia with no risk factors for drug-resistant organisms are likely to respond to monotherapy. For monotherapy,the dosage of antibiotics must be optimum. Patients with severe VAP should initially receive combination therapy , but later focused to a single agent if lower respiratory tract cultures does not demonstrate a resistant pathogen .

DURATION OF THERAPY: No consensus exists regarding treatment duration, but epidemiologic data have shown that some microorganisms can be rapidly eradicated, such as H influenzae, S pneumoniae, or Moraxella catarrhalis, whereas Enterobacteriaceae, S aureus, and P aeruginosa may persist despite in vitro susceptibility to the antibiotics administered. In recent years randomized trials done by Pugh and Singh et al showed that shorter durations of treatment (≤ 1 week) are as efficacious as longer durations of treatment (≥ 2 weeks) for patients with VAP[33,32]Most patients with VAP who receive appropriate antimicrobial therapy have a good clinical response within the first 6 days[17,31,32]. A multicenter, randomized, controlled trial demonstrated that patients who received appropriate, initial empiric therapy of VAP for 8 days had outcomes similar to those of patients who received therapy for 14 days. Prolonged therapy leads to colonization with antibiotic resistant bacteria, which may precede a recurrent episode of

VAP.

Another approach consists of guiding treatment duration using biochemical markers. A recent multicenter trial conducted by PRORATA trial group including critically ill patients with many types of infection have shown that treatment guided by repeated procalcitonin tests can help physicians stop antibiotic therapy earlier without risk to patients and with less antibiotic use[34].

Recent approach is use of aerozolised medications . though it directly delivers the drug into the site of infection and decreases side effects. The disadvantages include use of costly device for drug delivery, increased use of sedatives which inhibits reflux and increases the chance of aspiration.

ANTIBIOTIC CYCLING: Antibiotic cycling or rotation has been advocated as a potential strategy for reducing the emergence of antimicrobial resistance[1,17]. A class of antibiotics or a specific antibiotic is withdrawn from use for a defined time period and reintroduced at a later point in time in an attempt to limit bacterial resistance[35].

Gruson and colleagues observed a reduction in the incidence of VAP after introducing an antimicrobial program that consisted of supervised rotation and restricted use of ceftazidime
and ciprofloxacin . They observed a decrease in the incidence of VAP, primarily because of a reduction in the number of episodes attributed to antibiotic resistant gram-negative bacteria[36].

PREVENTION

As VAP is associated with increased mortality and morbidity despite adequate antibiotic therapy, prevention is mandatory. The preventive strategies are listed below:

1) GENERAL MEASURES
    i) Staff education: All hospitals should have a programme of ongoing education in infection prevention and control for all clinical staff caring for patients undergoing mechanical ventilation[23,37]. Mandatory induction

training for all clinical staff should incorporate training in infection prevention and control, including hand hygiene and the appropriate use of personal protective equipment[37]

ii) Staffing levels: Most important and underappreciated prevention strategy is adequate staffing. Reduced levels of nurse staffing are associated with higher rates of Hospital Care Associated Infection (HCAI). In critical care settings, maintenance of a higher nurse to patient ratio was associated with a reduction in incidence of HCAI and with a decreased risk of late-onset VAP.[38]

iii) Infection prevention and control strategies: Effective targeted surveillance for high risk patients coupled with staff education, use of proper isolation techniques and infection control practices are cornerstones for prevention of VAP[3,37]. Infection control programs are effective in reducing infection and colonization due to MDR organisms along with staff education and infection control measures. Surveillance of ICU infections to identify the prevalent organisms and new MDR organisms is required to take necessary action to prevent the spread of MDR strains[1,17,37]. Regular communication with clinicians, laboratory, infection control staff and pharmacy is essential. Cross infection and cross colonization are the important mechanisms for the pathogenesis of VAP.

Transmission-based precautions should be used in addition to standard precautions when caring for patients who are known or suspected to be colonised or infected with organisms which can be transmitted via direct or indirect contact, or by droplet and airborne routes. The Intensive care unit should be cleaned regularly to reduce the possibility of transmission of organisms from the environment to the patient[17,37].

iv) Antibiotic stewardship programmes: play a major role in controlling health care associated infections, reduce the emergence of drug resistant strains and reduce health care cost.

2) PREVENTION OF ASPIRATION:

i) Aspiration of oropharyngeal secretions into the bronchial tree is a major factor in the development of VAP [8,10] therefore, strategies to prevent aspiration are important in the prevention of VAP

ii) Semi-recumbent positioning of the patient has been shown to be associated with less aspiration into the lower airway and a lower incidence of VAP than supine positioning .[8,10]

iii) The pressure of the endotracheal tube cuff should be sufficiently high to minimise the leakage of bacterial pathogens around the cuff into the lower respiratory tract [8,10]

iv) Oropharyngeal secretions can accumulate above the endotracheal cuff and contribute to the risk of aspiration. A meta-analysis found that establishing sub-glottic drainage of oropharyngeal secretions using a specialised endotracheal tube was effective in reducing early-onset VAP in patients expected to be ventilated for more than 72 hours[10].

v) Use of an appropriately inflated cuffed endo-tracheal or tracheostomy tube should be used in patients who require mechanical ventilation and are at high risk of aspiration.

vi) The use of rotating beds may be considered in mechanically ventilated patients who cannot tolerate the semi-recumbent position.

vii) Gastric distension should be avoided in mechanically ventilated patients who are being fed enterally[8].

3) PREVENTION OF CONTAMINATION OF EQUIPMENT

i) All equipment involved in patient care should be cleaned, decontaminated and stored in accordance with the manufacturer's instructions.

ii) Sterile water should be used to rinse reusable non invasive respiratory equipment. Healthcare workers should use facial protection when disconnecting closed breathing circuits

iii) Nebulisers and resuscitation equipment should be single patient use only. Items designated for 'single-use' must never be reused[9]

iv) Outbreaks of nosocomial pneumonia due to contaminated respiratory devices, including nebulisers, have been reported[9,10.] All reusable patient care equipment should be appropriately decontaminated between each patient use to prevent cross-infection

v) The ventilator circuit should be changed only if soiled or damaged

vi) Condensate accumulating within the ventilator circuit may be contaminated and should be drained and disposed carefully.

4) PREVENTION OF COLONISATION OF THE AERODIGESTIVE TRACT

i) Histamine 2 receptor antagonists or proton pump inhibitors should be used in mechanically ventilated patients at high risk of developing upper gastrointestinal bleeding. Sucralfate may be considered in patients at low to moderate risk of bleeding [17].

ii) Regular oral hygiene should be carried out in all mechanically ventilated patients. A soft toothbrush should be used to clean the oral mucosa at least 12-hourly, except where contraindicated (e.g., increased risk of bleeding, thrombocytopenia).

iii) Modulation of oropharyngeal colonisation by selective digestive decontamination (SDD) using a combination of topical application of oral antibiotics along with systemic administration of antibiotics has been studied[37,38]. However SDD should be used in targeted clinical scenarios but not employed routinely for prevention of VAP due to rapid increase in antimicrobial resistance[37,38]

5) IMPLEMENTATION OF VAP CARE BUNDLE

A VAP care bundle should be implemented in all critical care areas caring for mechanically ventilated patients[38].

6) SURVEILLANCE OF VENTILATOR-ASSOCIATED PNEUMONIA

i) Surveillance of VAP should be carried out in all critical care units caring for mechanically ventilated patients.

ii) A multidisciplinary committee should be established with relevant representatives including intensivists, ICU nursing, ICU audit, microbiology, infectious diseases, infection prevention and control, surveillance staff and healthcare facility management. This committee should lead the surveillance project, encourage compliance and monitor the effectiveness of preventative programmes.[37,38]

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iii) VAP rates for each unit should be expressed as the number of VAP per 1000 ventilator days and informed to the surveillance unit regularly.

iv) VAP protocols and case definitions should be standardised and adhere to other international frameworks for comparative analysis of VAP incidence rates.

MATERIALS AND METHODS

A total of hundred clinically suspected patients who were mechanically ventilated for more than 48 hours were taken up for the study.

STUDY DESIGN: Prospective study

SOURCE OF CLINICAL MATERIAL: Patients admitted to Intensive Care unit at R L Jalappa Hospital and Research centre, Tamaka, Kolar.

DURATION OF STUDY: The study was conducted for a period of 1 year 7 months, from January 2010 to August 2012.

INCLUSION CRITERIA

- Age more than 18 years
- Clinically suspected patients receiving mechanical ventilation for >48 hours admitted to ICU according to the CDC criteria.

CRITERIA FOR SELECTION OF PATIENTS: (according to CDC) [22,25]

Radiology signs (2 or more serial chest x-rays with at least one of the following)

- New or progressive and persistent infiltrate
- Consolidation
- Cavitation

Clinical signs

At least one of the following,

- Fever (temperature > 38 deg C) with no other recognised cause
- Leucocytosis (> 12000cells/cmm) or leucopenia (<4000 cells/cmm)
- For adults above 70 years , altered mental status with no other recognisable cause

AND at least 2 of the following

- New onset of purulent sputum or change in character of sputum or increased respiratory secretions or increased suctioning requirements.
- New-onset or worsening cough or dyspnoea or tachypnoea .
- Rales or bronchial breath sounds .
- Worsening gas exchange (eg. O2 desaturations [PaO2/FiO2 ≤ 240], increased O2 requirements or increased ventilation demand.

Clinically suspected patients according to CDC criteria were scored by the Clinical Pulmonary Infection Scoring (CPIS)[4] system according to the clinical , microbiological and radiological signs . Patients with the CPIS > 6 are considered as confirmed VAP[4] cases and microbiological processing was done. CPIS score is as follows

CPIS points	**0**	**1**	**2**
Temperature (oC)	≥ 36.5and ≤ 38.4	≥ 38.5 and ≤ 38.9	≥ 39 or ≤ 36
Leucocyte count (per mm3)	4,000 - 11,000	< 4,000 or > 11,000	< 4,000 or > 11,000 + band forms ≥ 500
Tracheal secretions	Rare	Abundant	Abundant+ Purulent
PaO2/ FiO2 mm Hg	> 240 or ARDS	-	≤ 240 and no ARDS
Chest radiograph	No infiltrate	Diffuse infiltrate	Localized infiltrate
Culture of tracheal aspirate	Negative	-	Positive

EXCLUSION CRITERIA

Pediatric patients and adolescents' less than 18 years of age

Patients who were intubated before admission.

Patients who are on mechanical ventilation for < 48 hours

METHOD OF COLLECTION OF DATA

Patients with clinical suspicion of VAP were selected according to the above VAP criteria as per the CDC guidelines. All relevant data including age, gender, and clinical diagnosis were considered.

SPECIMEN COLLECTION [39]

Endotracheal aspirate was collected from clinically diagnosed cases. ETA was collected using two catheters where-in a Ramson's 8F suction catheter was guided through a Ramson's 16f suction catheter and gently introduced through the endo-tracheal tube for approximately 24 cm. The sample was gently aspirated without installing saline and the suction catheters were withdrawn.

The sample was transferred into a clean labeled container. The sample was immediately transported to the laboratory for microbiological processing.

MICROBIOLOGICAL PROCESSING OF THE SAMPLE:

Samples were mechanically liquefied and homogenized by vortexing or shaking vigorously for 1 minute and then subjected to grams stain.

GRAM STAINING TECHNIQUE[40]:

Preparation of smear:

A loopful of the undiluted sample was smeared on a clean labeled slide.

Sample was air dried and heat fixed and kept on the staining rack,

Step1: Crystal violet dye was added and allowed to stand for 2 minutes and washed with water,

Step 2: grams iodine was added for 1 minute and washed with water,

Step 3: De-colourization using acetone (Flush and Wash Technique).

The slide was held in slanting position near the water tap. Acetone was added and immediately washed with water.

Step 4: Safranin was added and allowed to stand for 30 sec and washed with water.

The slide was blotted dry and observed under oil immersion objective.

Interpretation of grams stain[25]:

The Gram's stain of the respiratory secretions showing > 10 polymorpho-nuclear cells (PMN) / high power field (HPF) and more than one bacterium /oil immersion field was considered as significant and processed.

CULTURE:

Samples were serially diluted in 0.9% sterile saline solution with final dilutions of 10^{-2}, 10^{-3}, 10^{-4}.[39] The method of quantitative dilutions is as follows:

QUANTITATIVE DILUTIONS OF THE ENDOTRACHEAL ASPIRATE

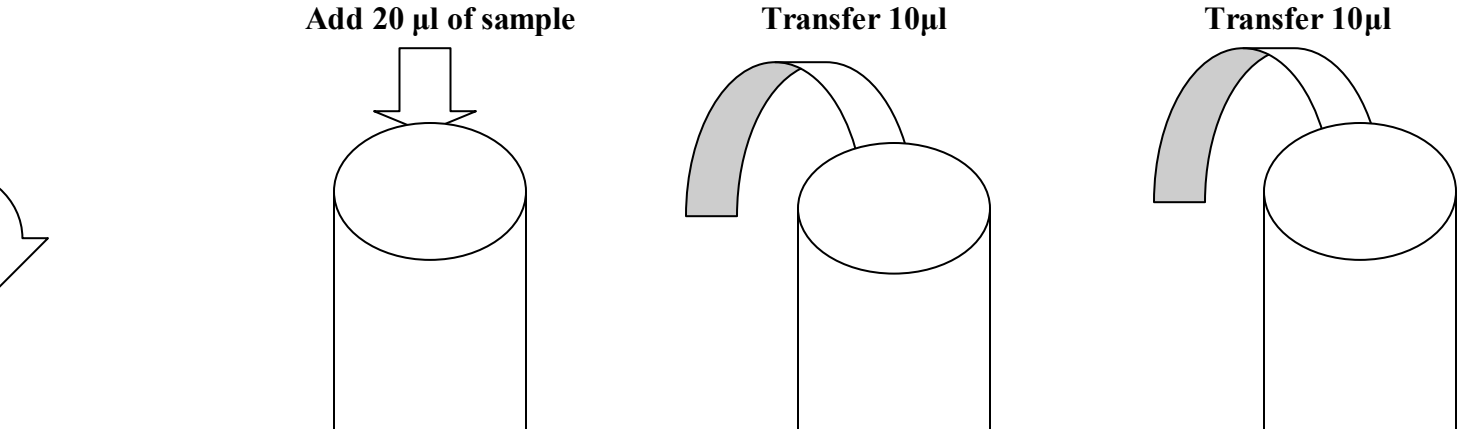
Three test tubes were taken and labeled as 1, 2 and 3 suggesting 1:100, 1:1000 and 1:10000 dilutions respectively.

180µl, 90µl and 9µl of 0.9% saline was added respectively in tubes 1, 2 and 3.

About 20µl of well homogenized endotracheal aspirate was added in the first tube containing 180µl of saline and mixed well

About 10 µl of aspirate was transferred from tube 1 to tube2 and mixed well

And 10 µl of aspirate was transferred from tube 2 to tube 3 and mixed well



TUBE 1	TUBE 2	TUBE 3
180µl of saline	**90µl of saline**	**9µl of saline**

A loopful containing 0.01 ml of the endotracheal aspirate from each of the serial dilutions were plated on blood agar, chocolate agar and Mac-conkey agar and incubated overnight at 37^0C.

If fungal elements were seen on gram's stain, sample was plated on Saboraud's dextrose agar.

Any growth in the final dilution (10^{-4}) was considered as pathogenic and processed for identification.

The total number of colonies in the final dilution was expressed as colony forming unit/ml.

The isolates were identified by standard biochemical reactions and subjected to antibiotic sensitivity testing by Kirby Bauer disc diffusion method.

The Biochemical tests[41] used for identification are:

Staphylococcus aureus:

i) Catalase test
ii) Coagulase test
iii) Mannitol fermentation
iv) Urease test

Gram negative bacilli:

i) Catalase test
ii) Oxidase test
iii) Indole test

iv) Mannitol motility test

v) Triple sugar iron agar test

vi) Christensens Urease test

vii) Simmons Citrate test

viii) Lysine iron agar

ANTIBIOTIC SENSITIVITY TESTING

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:

Inoculum preparation: Using a straight wire the 3-4 similar looking colonies of the test strain were picked and inoculated into a test tube containing peptone water and incubated at 37^0C for 2 hours

The turbidity of the test strain was matched with 0.5 Mc Farland turbidity standard.

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.

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.

The antibiotic discs were placed on the inoculated plates using sterile forceps with a distance of 25mm from the centre of the disc.

The plates were incubated overnight at 37^0C.

The diameter of each zone of inhibition was measured in millimeter (mm). Interpretation of the zone of inhibition was according to CLSI guidelines.

The antibiotics listed below for testing was according to CLSI guidelines[43.]

The list of antibiotics tested is as follows[43]:

ENTEROBACTERIACAE	Disc Content (µg)	STAPHYLOCOCCUS AUREUS	Disc Content (µg)
Ampicillin(A)	10	Penicillin(P)	10U
Piperacillin(Pi)	100	Amoxycillin-clavulanic acid(Amc)	20/10
Amoxycillin-clavulanic acid(Amc)	20/10	Cefoxitin(Cx),	30
		Erythromycin(E)	15
Piperacillin-tazobactum(Pt)	100/10	Clindamycin(Cd)	2
Cefoxitin(Cx)	30	Trimethoprim- Sulfamethoxazole (Cot)	1.25/23.75
Cefotaxime(Ctx)	30	Tetracycline(T)	30
Ceftriaxone(Ctr)	30	Doxycycline(Do)	30
Ceftazidime(Caz)	30	Ciprofloxacin(Cip)	5
Ceftazidime-clavulanic acid(Cac)	30/10	Chloramphenicol(C)	30
		Gentamicin(G)	10
Gentamicin(G)	10	Linezolid(Lz)	30
Tobramycin(T)	10	Vancomycin (Va)	30
Amikacin(Ak)	10		
Tetracycline(T)	30		
Trimethoprim-Sulfamethoxazole (Cot)	1.25/23.7		
Chloramphenicol (C)	5		
Ciprofloxacin(Cip)	30		
Levofloxacin(Le)	5		
Imipenen(Ipm)	5		
Meropenem(Mrp)	10		

PSEUDOMONAS AERUGINOSA	Disc Content (µg)	ACINETOBACTER SPECIES	Disc Content (µg)
Piperacillin(Pi)	100	Piperacillin(Pi)	100
Piperacillin tazobactum(Pt)	100/10	Piperacillin tazobactum(Pt)	100/10
Ceftazidime(Caz)	30	Cefotaxime(Ctx)	30
Gentamicin(G)	10	Ceftriaxone(Ctr)	30
Tobramycin(T)	10	Ceftazidime(Caz)	30
Amikacin(Ak)	10	Gentamicin(G)	10
Ciprofloxacin(Cip)	5	Tobramycin(T)	10
Levofloxacin(Le)	5	Amikacin(Ak)	10
Imipenen(Ipm)	10	Tetracycline(T)	30
Meropenem(Mrp)	10	Doxycycline(Do)	30
Polymyxin B (P b)	300U	Trimethoprim-Sulfamethoxazole	1.25/23.75
Colistin (Cl)	10	(Cot)	
		Ciprofloxacin(Cip)	5
		Levofloxacin(Le)	5
		Imipenen(Ipm)	10
		Meropenem(Mrp)	10
		Polymyxin B (P b)	300U
		Colistin (Cl)	10

DETECTION OF MRSA AND MSSA[44]:

Susceptibility of S. aureus to Methicillin was determined using Cefoxitin (30µg) disk, 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[45]:

Double disc synergy test using ceftazidime disk alone and in combination with clavulanic acid, was performed for detection of extended spectrum -lactamase (ESBL) among the members of Enterobacteriaceae . Five mm or more 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 [46]: Detection of Amp C β lactamase was done by placing Cefotaxime , Cefoxitin and Cefritaxone disc 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[47]:

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

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.

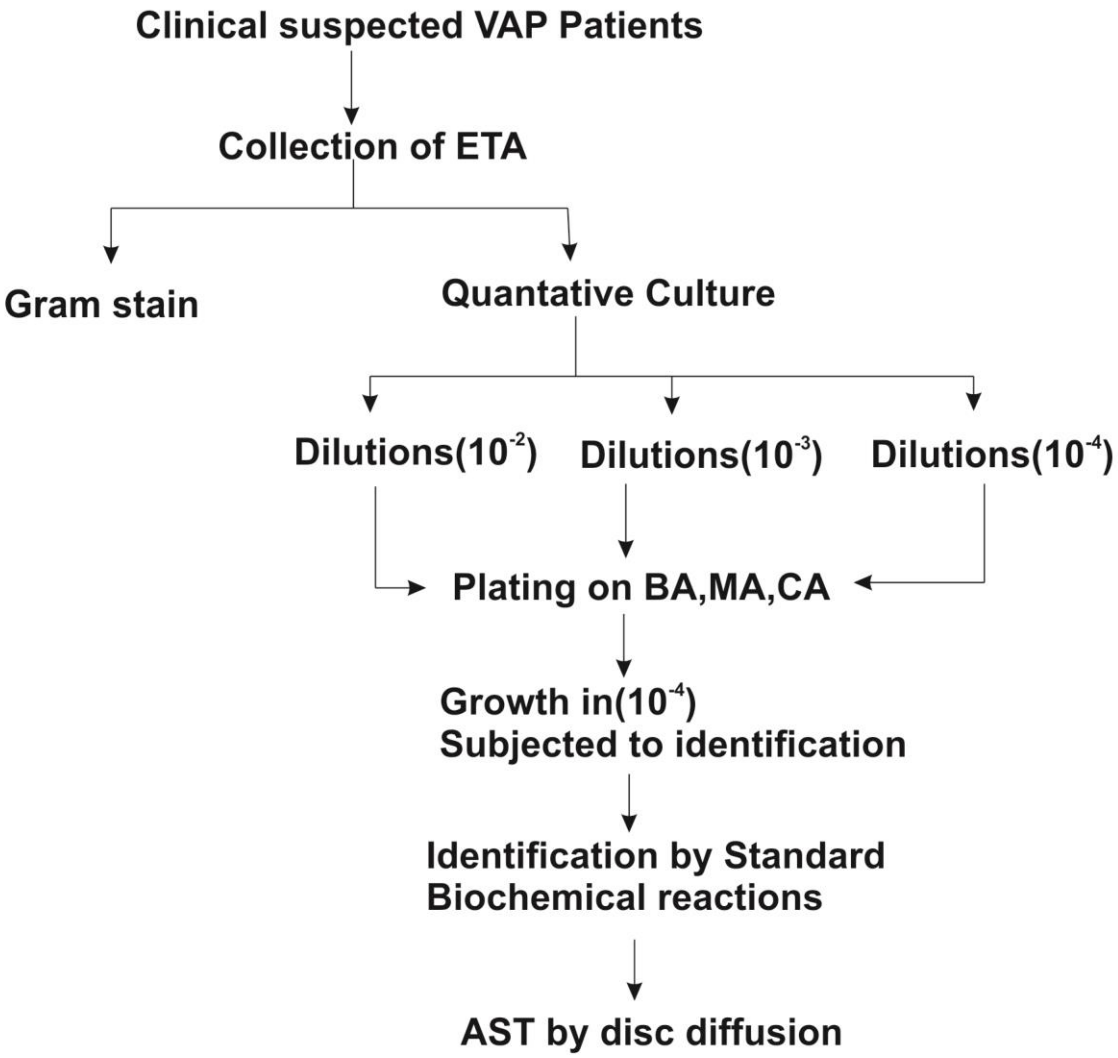
The plates were incubated overnight at 37^0C.

The presence of a cloverleaf-shaped zone of inhibition due to carbapenemase production by the test strain was considered positive.

CALCULATION OF VAP RATE: The VAP rate[48] was collected every month by using the formula to calculate the incidence.

$$\text{VAP RATE} = \frac{\text{Total no of VAP cases per month}}{\text{Total no of ventilator days per month.}} \times 1000$$

SAMPLE PROCESSING

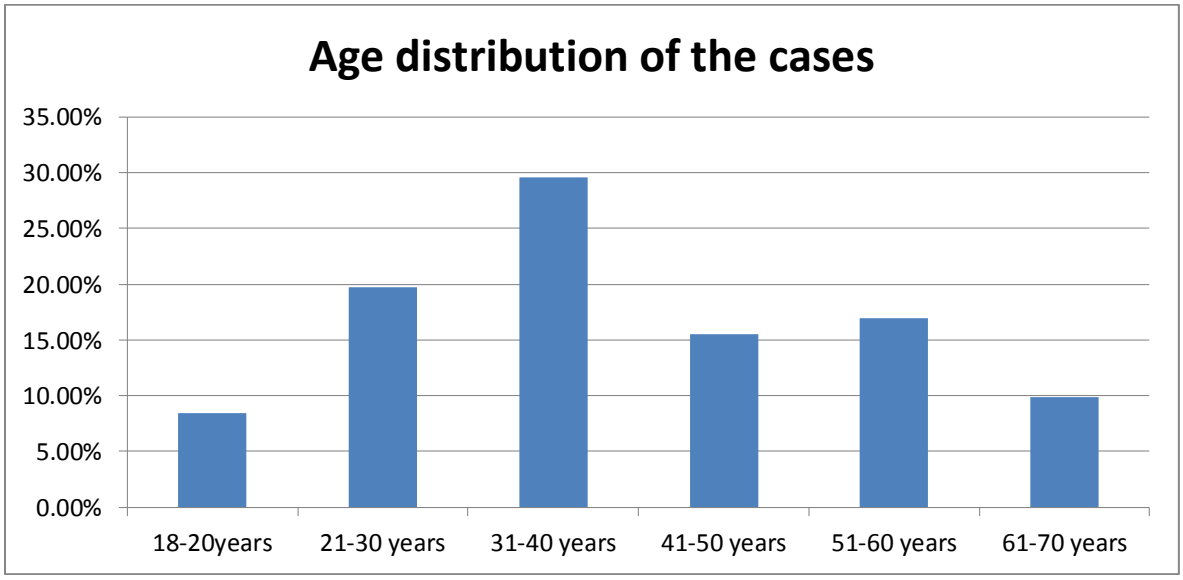


RESULTS

Of the 100 clinically suspected VAP patients, 71 were diagnosed as VAP as per the CPIS.

Table 1: Age distribution of patients

Age in years	Number of patients	Percentage
18-20	6	8.45%
21-30	14	19.71%
31-40	21	29.57%
41-50	11	15.49%
51-60	12	16.90%
61-70	7	9.85%
Total	**71**	**100%**

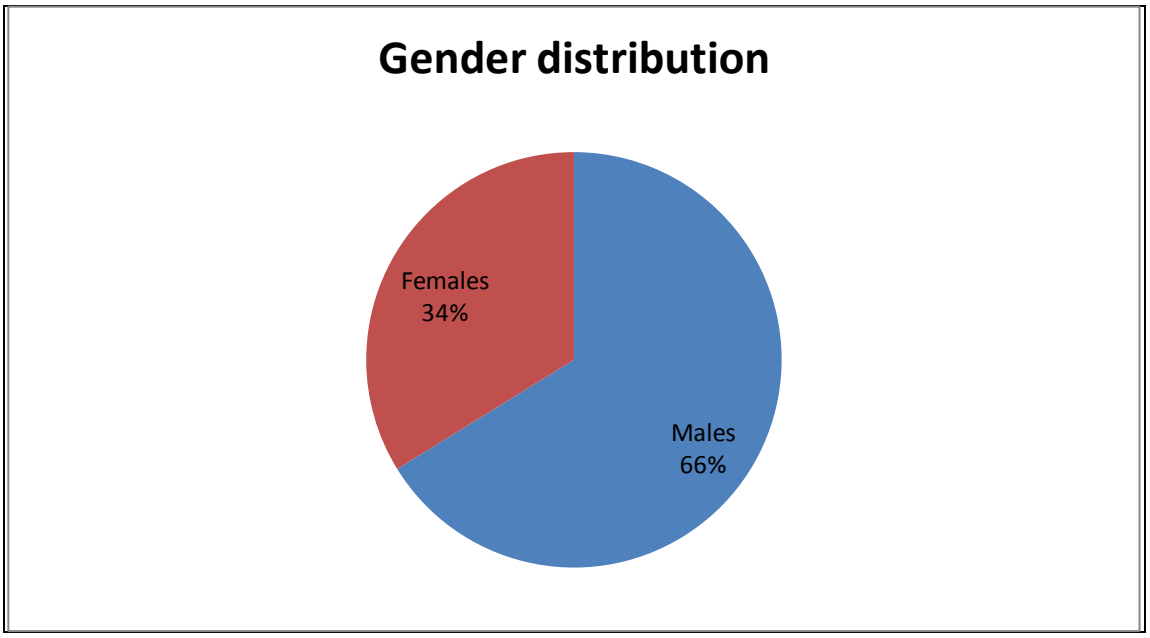


Maximum number of cases was seen in the age group 31-40 years. The mean age is 41.13±15.3 with a minimum age of 18 years and a maximum of 75 years.

Table 2: Gender distribution of patients studied

Gender	Number of patients	Percentage
Male	47	66.19%
Female	24	33.80%
Total	71	100.0

Figure 2: Pie chart showing gender distribution



66% of the VAP patients were males while 34% were females.

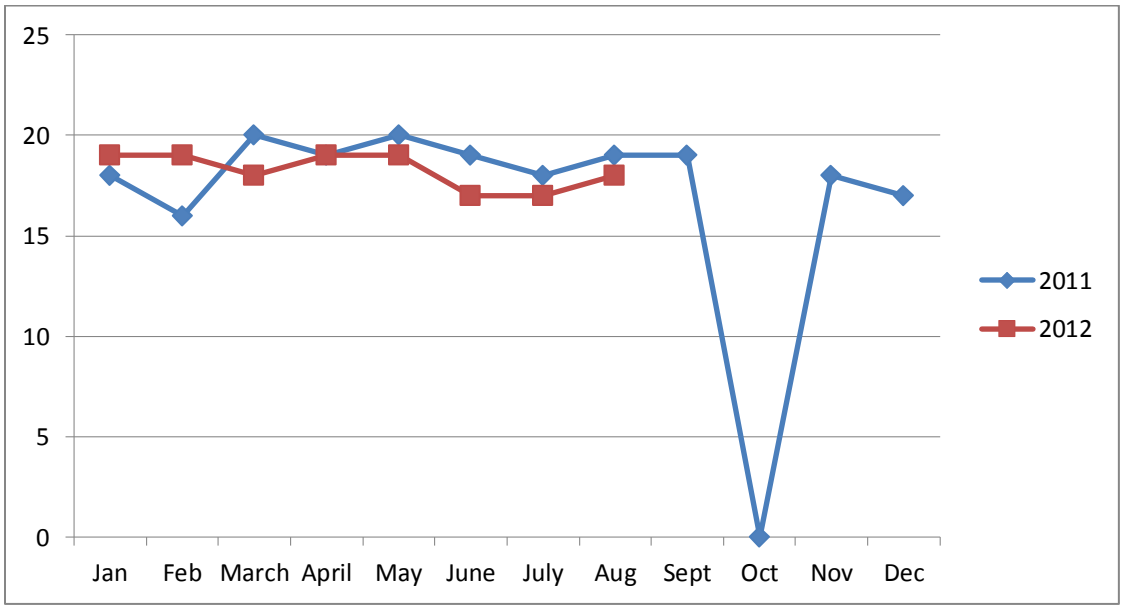
Table3: Showing the VAP rate during the study period

$$\text{VAP RATE} = \frac{\text{Total no of VAP cases per month}}{\text{Total no of ventilated days per month}} \times 1000$$

VAP RATE	JAN	FEB	MARCH	APRIL	MAY	JUNE	JULY	AUG	SEPT	OCT*	NOV	DEC
2011	21	17	22	19	21	17	18	19	19	ND	21	17
2012	18	19	21	22	21	19	17	18	-	-	-	-

Mean VAP rate = 25.6 /1000 patient ventilated days.

Figure 3: Distribution of the VAP rate over the months

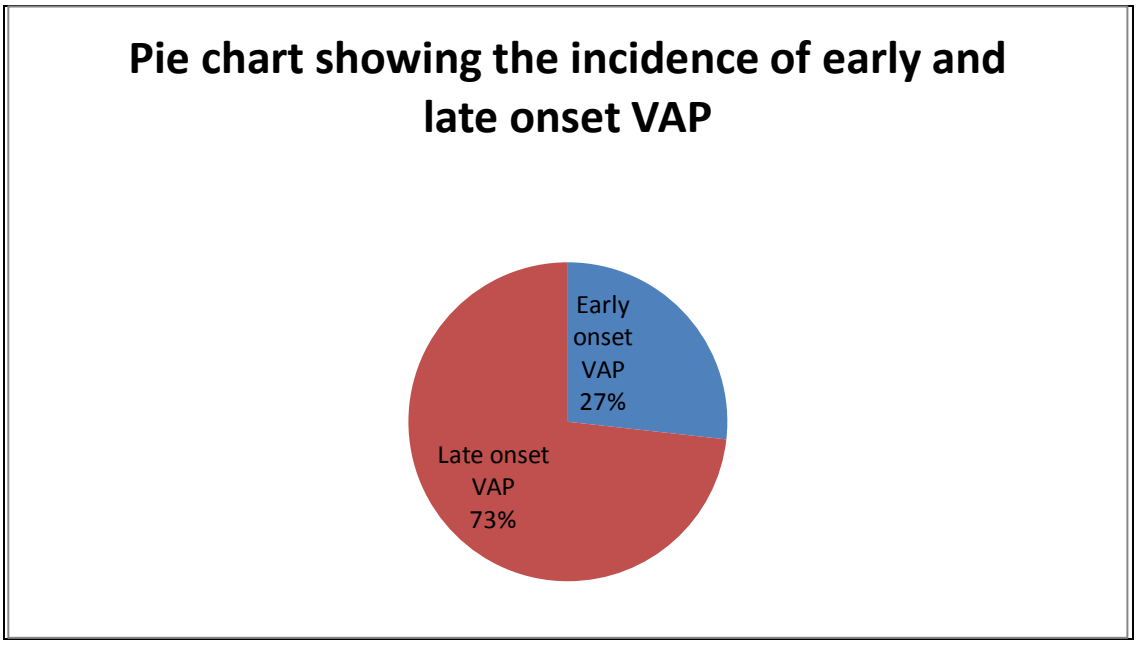


The VAP rate ranged between 17 to 22/1000 patient ventilated days during the various months of the study period.
*In the month of October no samples were collected due to external postings VAP rate was not calculated.

Table 3: Distribution of patients depending onset of VAP

Onset of VAP	Number of patients	Percentage
Early onset (<96 hours)	19	26.76%
Late onset (>96 hours)	52	73.23%
Total	**71**	**100%**

Figure 3: Pie chart showing the incidence of Early onset and Late onset VAP



The incidence of Early onset VAP was 27% while Late onset VAP was 73%. Late onset VAP is

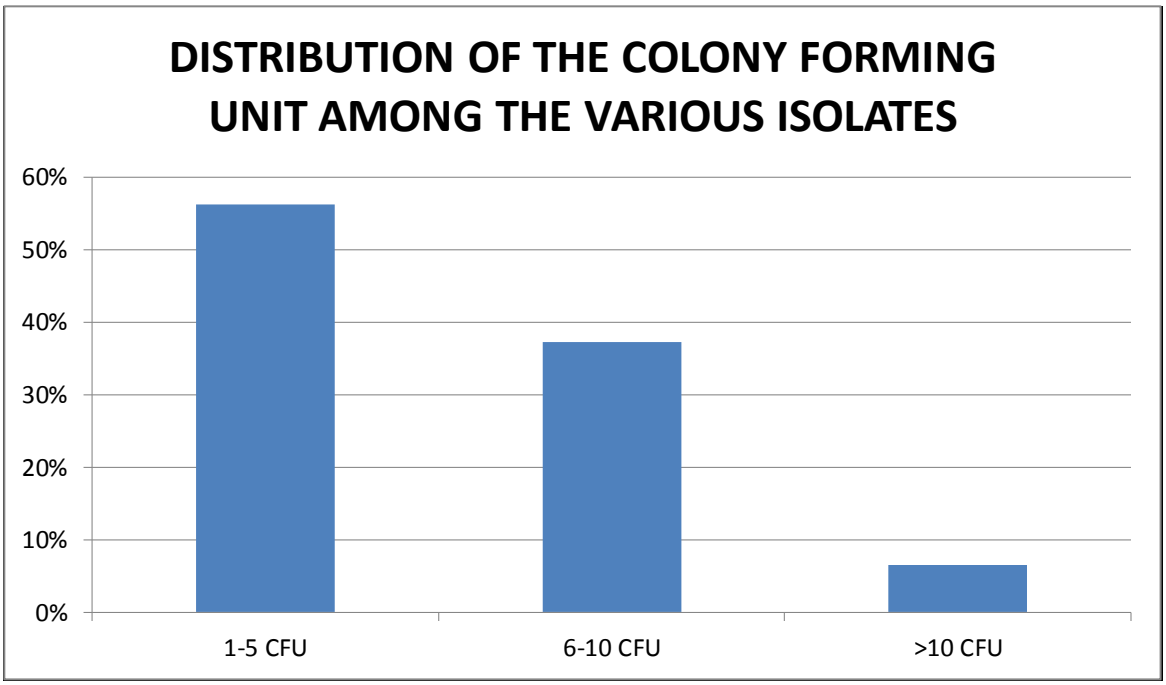
more common than Early onset VAP.

Table 4: Distribution of colony forming unit of patients studied

Colony forming unit	Number of isolates	Percentage
1-5	77	56.20%
6-10	51	37.22%
>10	9	6.56%

Mean colony forming unit = 5.98 ±3.20

Figure 4: Distribution of the colony forming units among the various isolates

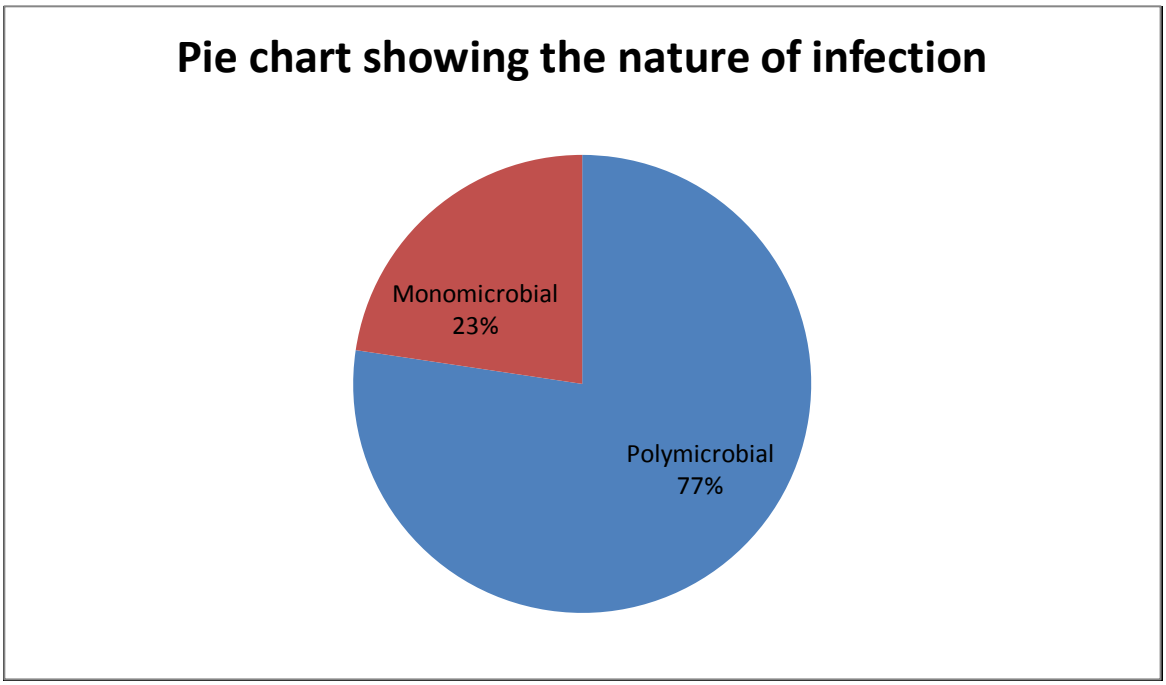


Quantitative culture of the ET aspirate showed CFU ranging between 1-14 cfu/ml. In 56% of the isolates cfu ranged between 1 to5, while 37.22% of the isolates yielded 6-10 cfu/ml and 6.56% yielded more than 10 cfu.

Table 5: showing the distribution of patients depending on the nature of infection

Nature of infection	Number of cases	percentage
Polymicrobial	55	77.46%
Monomicrobial	16	22.54%
Total	**71**	**100%**

Figure 5: Pie chart showing the nature of infection

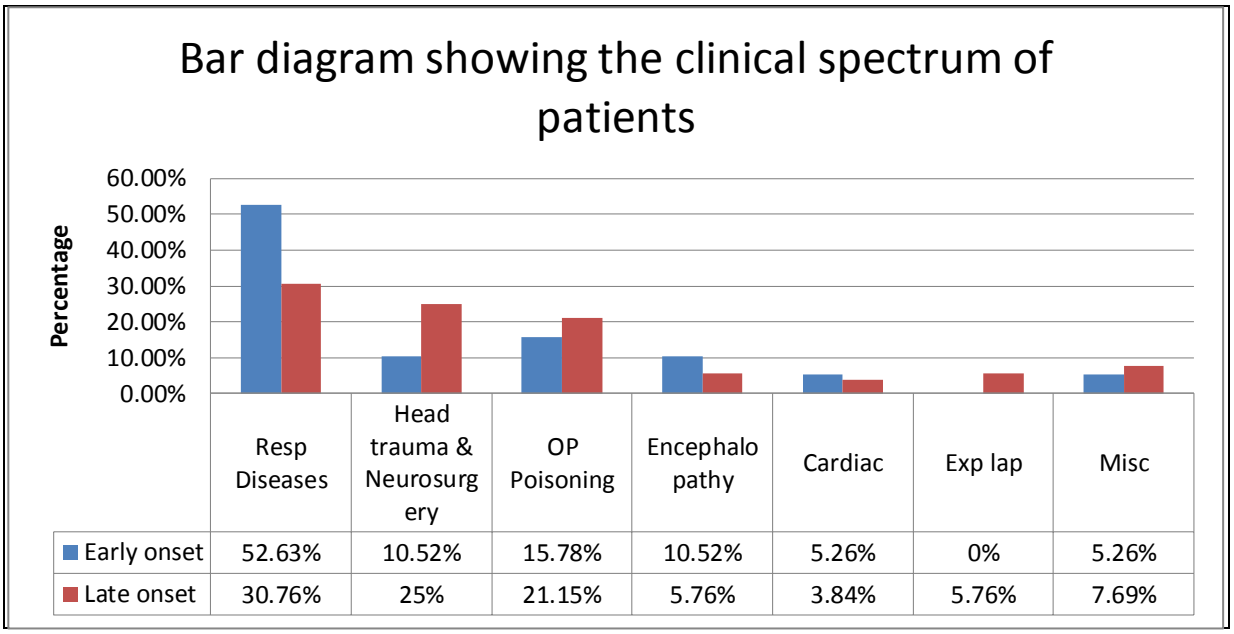


77% of the infections were polymicrobial while 23% was monomicrobial.

Table 6: Clinical spectrum of patients with VAP

Clinical spectrum	Early onset VAP	Late onset VAP
Respiratory diseases (ARDS,COPD,Pneumonia)	10(52.63%)	16(30.76%)
Head trauma and neurosurgery	2(10.52%)	13(25%)
OP Poisoning	3(15.78%)	11(21.15%)
Encephalopathy	2(10.52%)	3(5.76%)
Cardiac causes (pulmonary odema,CCF,IHD)	1(5.26%)	2(3.84%)
Exploratory Laparotomy	0	3(5.76%)
Miscellaneous(PUO,Snake bite, Septicemia)	1(5.26%)	4(7.69%)
Total	**19(100%)**	**52(100%)**

Figure 6: Bar diagram showing the clinical spectrum of the patients with VAP

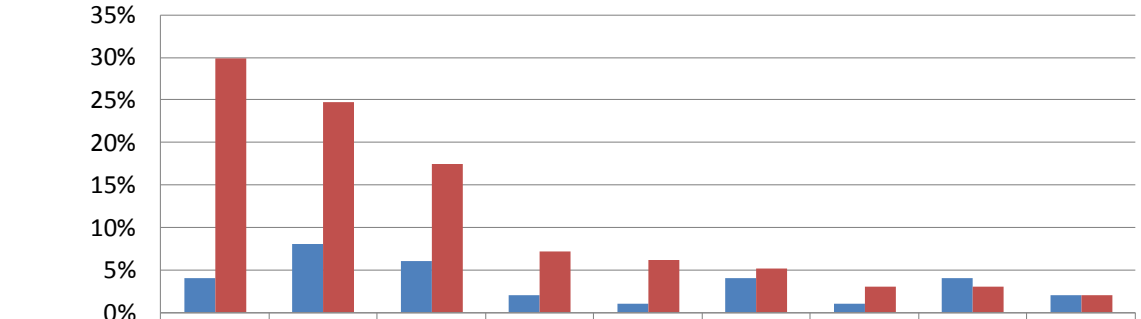


Incidence of VAP was most commonly seen in patients with underlying Respiratory diseases followed by Head trauma & neurosurgery and OP Poisoning. None of patients following Exploratory laporotomy developed early onset VAP.

Table 7: Distribution of organisms causing VAP

Organism	Early onset VAP	Late onset VAP
Acinetobacter species	10(4%)	29(29.89%)
Pseudomonas aeruginosa	8(8%)	24(24.74%)
Klebsiella pneumonia	6(6%)	17(17.52%)
MRSA	2(2%)	7(7.21%)
Non Albicans Candida	1(1%)	6(6.18%)
Enterobacter species	4(4%)	5(5.15%)
MSSA	4(4%)	3(3.09%)
E. coli	1(1%)	3(3.09%)
Citrobacter diversus	2(2%)	2(2.06%)
Candida albicans	2(2%)	1(1.03%)
Total	**40(100%)**	**97(100%)**

Figure 7: Bar diagram showing the distribution of organisms



	Acinetobacter	Ps aeruginosa	Kleb pneumoniae	MRSA	Non Albicans Candida	Enterobacter species	E.coli	MSSA	Citrobacter diversus
Early onset	4%	8%	6%	2%	1%	4%	1%	4%	2%
Late onset	29.89%	24.74%	17.52%	7.21%	6.18%	5.15%	3.09%	3.09%	2.06%

Acinetobacter is the common species causing Early and Late onset VAP followed by Pseudomonas aeruginosa and Klebsiella pneumonia. Incidence of MRSA and Non albicans Candida was more common in Late onset VAP.

Table 8: Distribution of organism according to the clinical diagnosis

	Acinetobacter Species	Pseudomonas Aeruginosa	K.pneumoniae	E.coli	Enterobacter species	Citrobacter diversus	MRSA	MSSA	Candid species
Respiratory diseases	11	10	11	0	4	2	4	2	7
Head Trauma & Neurosurgery	11	9	6	1	1	1	2	3	1
OP Poisoning	8	7	2	0	4	0	2	0	0
Encephalopathy	3	2	1	1	0	0	1	0	0
Cardiac causes	3	2	2	0	0	0	0	0	0
Exploratory Lap	2	0	0	2	0	1	0	0	0
Miscellaneous	1	2	1	0	0	0	0	2	2
Total	39	32	23	4	9	4	9	7	10

Acinetobacter species and Pseudomonas aeruginosa was the most common organism associated with Respiratory diseases and Head trauma & neurosurgery cases. Enterobacter species was associated with Respiratory diseases and OP poisoning. Candida species was most commonly associated with underlying Respiratory diseases.

Table 9: Showing the association of organisms with clinical disease in Early onset VAP

	Acinetobacter species	Pseudomonas aeruginosa	K.pneumoniae	MRSA	MSSA	E.coli	Enterobacter species	Candida species	Citrobacter diversus
Respiratory diseases	4	3	5	1	2	0	3	3	1
Head trauma & Neuro surgery	1	0	0	0	1	0	0	0	0
OP Poisoning	4	2	0	1	0	0	1	0	0
Encephalopathy		1	0	0	0	0	0	0	0
Cardiac causes	1	1	1	0	0	0	0	0	0
Exploratory Lap	0	0	0	0	0	1	0	0	1
Others	0	1	0	0	1	0	0	0	0
Total	10	8	6	2	4	1	4	3	2

In the Early onset group Klebsiella pneumoniae was most commonly associated with Respiratory causes. Acinetobacter was associated with Respiratory causes and OP Poisoning. All the Candida species isolated was associated with respiratory causes.

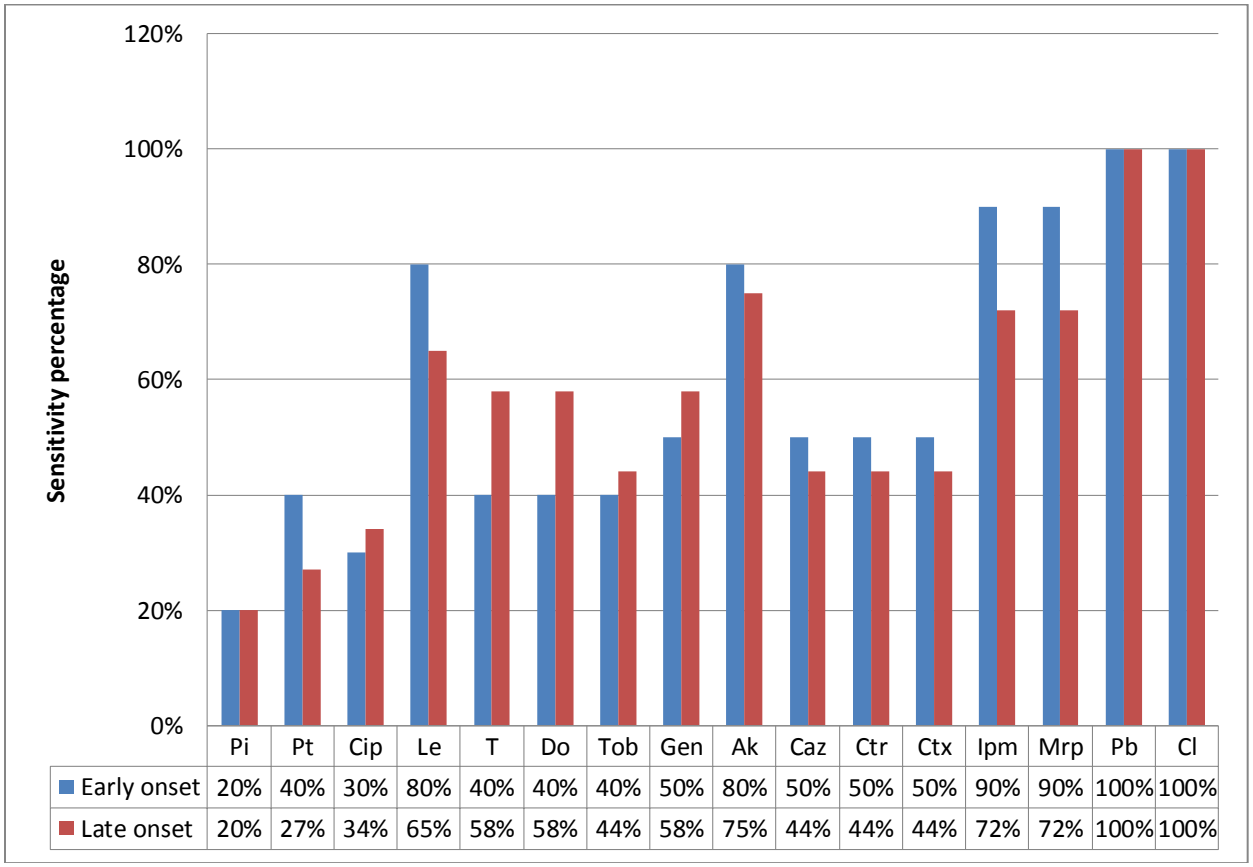
Table 10: Showing the association of organisms with clinical disease in Late onset VAP

	Acinetobacter Species	Pseudomonas aeruginosa	K.Pneumoniae	MRSA	MSSA	E.coli	Enterobacter species	Candida species	Citrobacter diversus
Respiratory causes	7	7	6	3	0	0	1	4	1
Head trauma & Neurosurgery	10	9	6	2	2	1	1	1	1
OP Poisoning	4	5	2	1	0	0	3	0	0
Encephalopathy	3	1	1	1	0	1	0	0	0
Cardiac causes	2	1	1	0	0	0	0	0	0
Exploratory Lap	2	0	0	0	0	1	0	0	0
Others	1	1	1	0	1	0	0	2	0
Total	**29**	**24**	**17**	**7**	**3**	**3**	**5**	**7**	**2**

In the late onset group Head trauma and Neurosurgery was commonly associated with Acinetobacter species followed by Ps aeruginosa and Kleb pneumoniae.MRSA was the predominant gram positive cocci associated with respiratory causes.

Figure 8: Bar diagram showing the sensitivity pattern of Acinetobacter species in the

Early onset and Late onset group

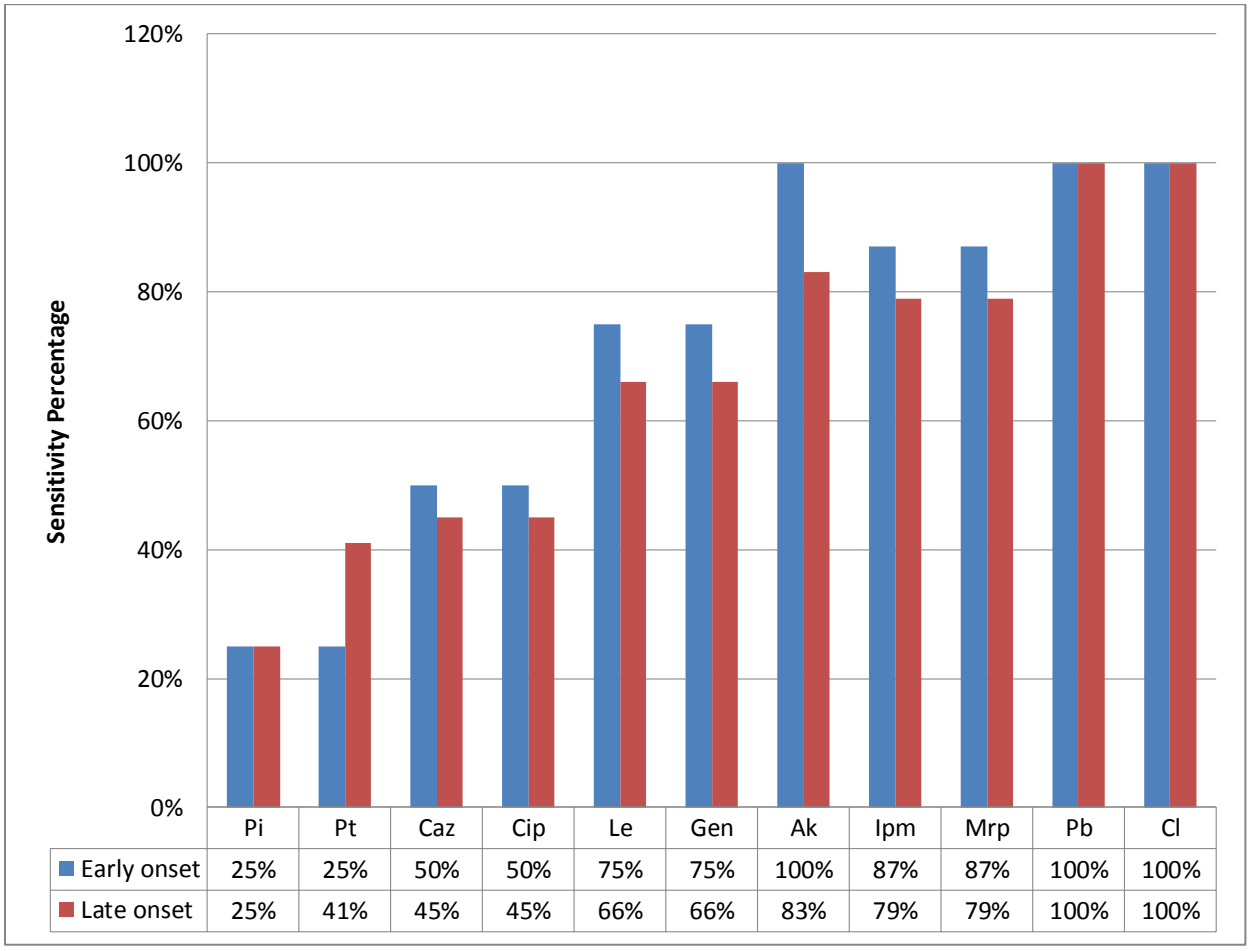


	Pi	Pt	Cip	Le	T	Do	Tob	Gen	Ak	Caz	Ctr	Ctx	Ipm	Mrp	Pb	Cl
Early onset	20%	40%	30%	80%	40%	40%	40%	50%	80%	50%	50%	50%	90%	90%	100%	100%
Late onset	20%	27%	34%	65%	58%	58%	44%	58%	75%	44%	44%	44%	72%	72%	100%	100%

Pi-Piperacillin, Pt-Piperacillin tazobactum, Cip-Ciprofloxacin, Le-Levofloxacin ,Te-Tetracycline, Do-doxycycline, Gen-Gentamycin ,Tob-Tobramycin, Ak-Amikacin, Caz-Ceftazadime , Ctx-Cefotaxime, Ctr-Ceftriaxone , Imp-Imipenem, Mrp-Meropenem., Pb-Polymyxin, Cl-Colistin

Early onset VAP was associated with more sensitive strains when compared to Late onset VAP. Sensitivity to Tetracycline, Doxycycline, Gentamycin and Tobramycin was more in Late onset group. None of the strains were resistant to Polymyxin or Colistin.

Figure 9: Bar diagram showing the sensitivity pattern of Pseudomonas aeruginosa in the Early onset and Late onset group.

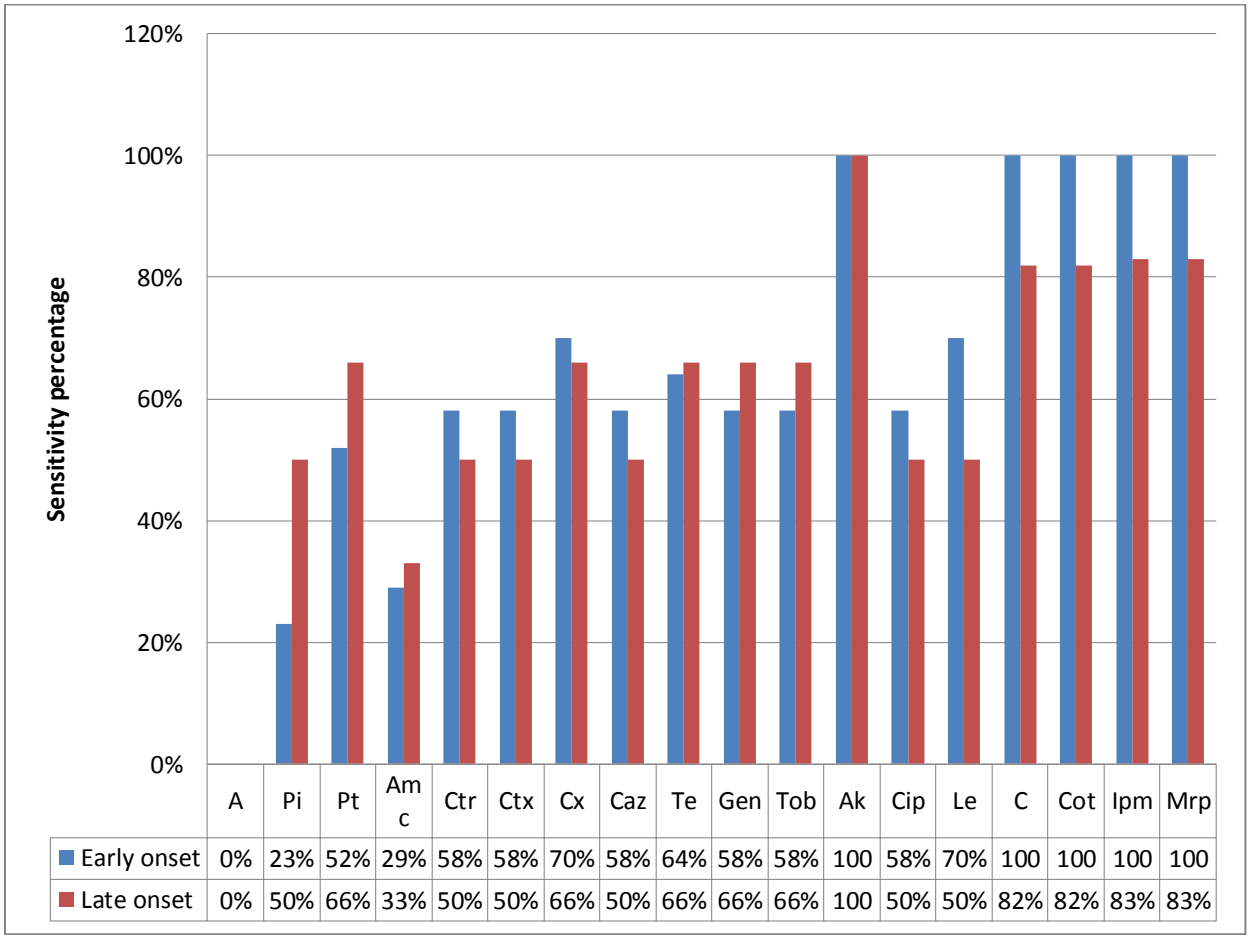


	Pi	Pt	Caz	Cip	Le	Gen	Ak	Ipm	Mrp	Pb	Cl
Early onset	25%	25%	50%	50%	75%	75%	100%	87%	87%	100%	100%
Late onset	25%	41%	45%	45%	66%	66%	83%	79%	79%	100%	100%

Pi -Piperacillin, Pt-Piperacillin tazobactum, Caz- Ceftazidime, Cip-Ciprofloxacin, Le-Levofloxacin ,
Gen-Gentamycin, Ak-Amikacin, Imp-Imipenem, Mrp-Meropene, Pb-Polymyxin, Cl-Colistin.

Pseudomonas aeruginosa causing Early onset VAP was more sensitive when compared to the strains causing Late onset VAP. None of the strains were resistant to Polymyxin B and Colistin. 100% sensitivity was noted to Amikacin, in the Early onset group.

Figure 10: Sensitivity of Klebsiella pneumonia in the Early and Late onset VAP.

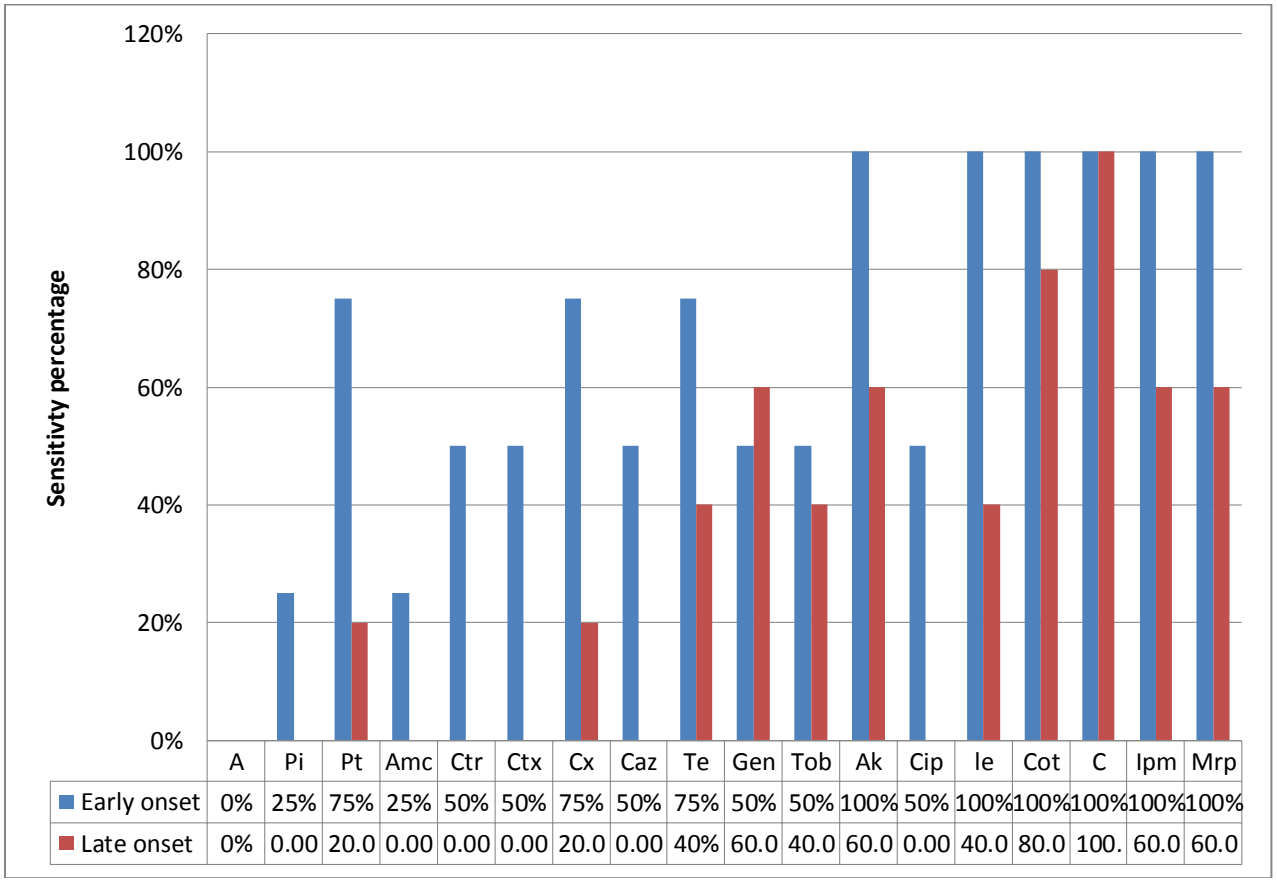


	A	Pi	Pt	Amc	Ctr	Ctx	Cx	Caz	Te	Gen	Tob	Ak	Cip	Le	C	Cot	Ipm	Mrp
Early onset	0%	23%	52%	29%	58%	58%	70%	58%	64%	58%	58%	100	58%	70%	100	100	100	100
Late onset	0%	50%	66%	33%	50%	50%	66%	50%	66%	66%	66%	100	50%	50%	82%	82%	83%	83%

A-Ampicillin, Pi-Piperacillin,Pt-Piperacillin tazobactum, Amc-Amoxyclav , Ctr-Ceftriaxone,Caz-Ceftazadime ,Cx-Cefoxitin, Ctx-Cefotaxime, Te-Tetracycline, Gen-Gentamycin ,Tob-Tobramycin, Ak-Amikacin, Cip-Ciprofloxacin, Le-Levofloxacin, Cot-Cotrimoxazole, C-Chloramphenicol, Imp-Imipenem, Mrp-Meropenem.

Late onset VAP was associated with resistant strains when compared to Early onset. 100% sensitivity to Amikacin was noted in both the groups. None of the strains were sensitive to Ampicillin.

Figure 11: Bar diagram showing the sensitivity pattern of Enterobacter species

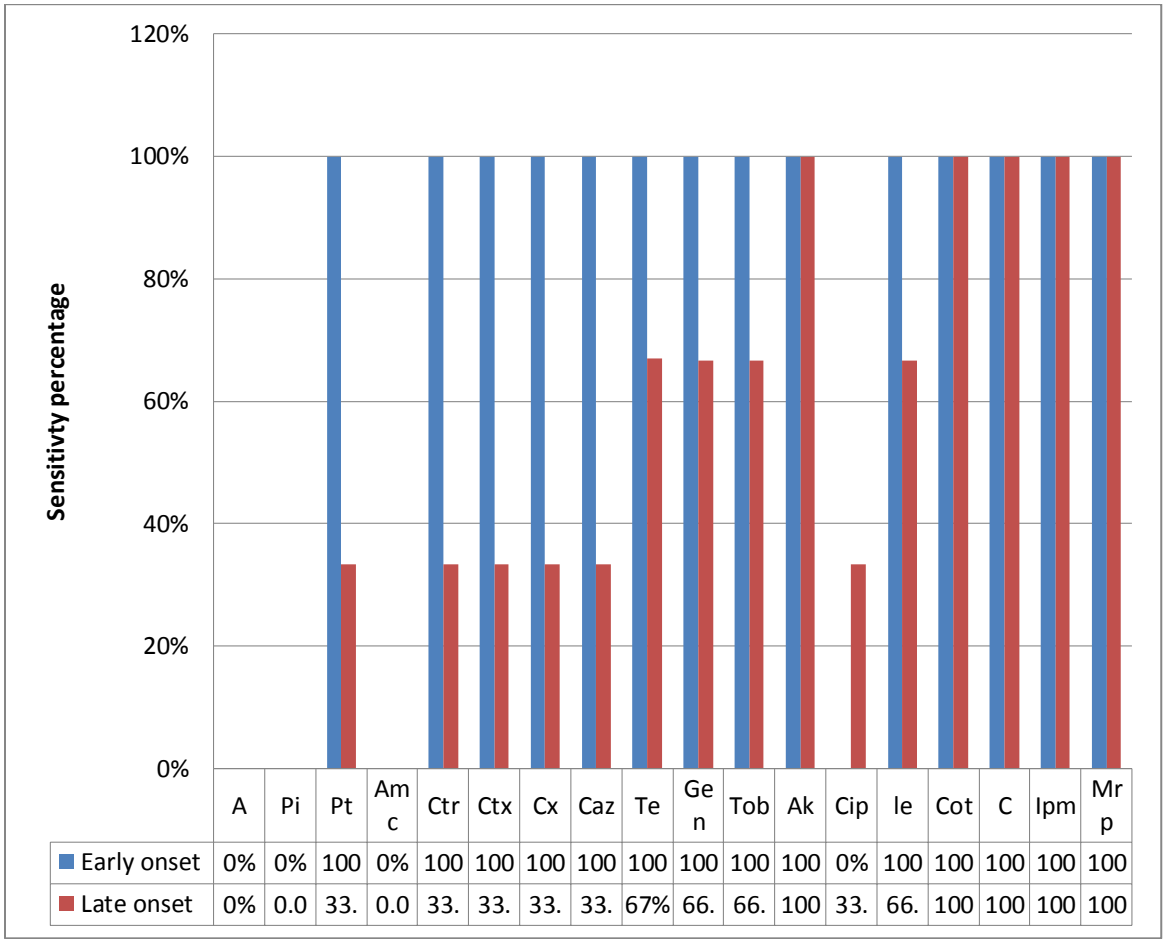


	A	Pi	Pt	Amc	Ctr	Ctx	Cx	Caz	Te	Gen	Tob	Ak	Cip	Ie	Cot	C	Ipm	Mrp
Early onset	0%	25%	75%	25%	50%	50%	75%	50%	75%	50%	50%	100%	50%	100%	100%	100%	100%	100%
Late onset	0%	0.00	20.0	0.00	0.00	0.00	20.0	0.00	40%	60.0	40.0	60.0	0.00	40.0	80.0	100.	60.0	60.0

A-Ampicillin, Pi-Piperacillin,Pt-Piperacillin tazobactum, Amc-Amoxyclav , Ctr-Ceftriaxone,Caz-Ceftazadime ,Cx-Cefoxitin, Ctx-Cefotaxime, Te-Tetracycline, Gen-Gentamycin ,Tob-Tobramycin, Ak-Amikacin, Cip-Ciprofloxacin, Le-Levofloxacin, Cot-Cotrimoxazole, C-Chloramphenicol, Imp-Imipenem, Mrp-Meropenem.

Most of the isolates in the late onset group were resistant. 60% sensitivity was seen to Carbapenems in the late onset group.

Figure 12: Bar diagram showing the sensitivity pattern of E. coli

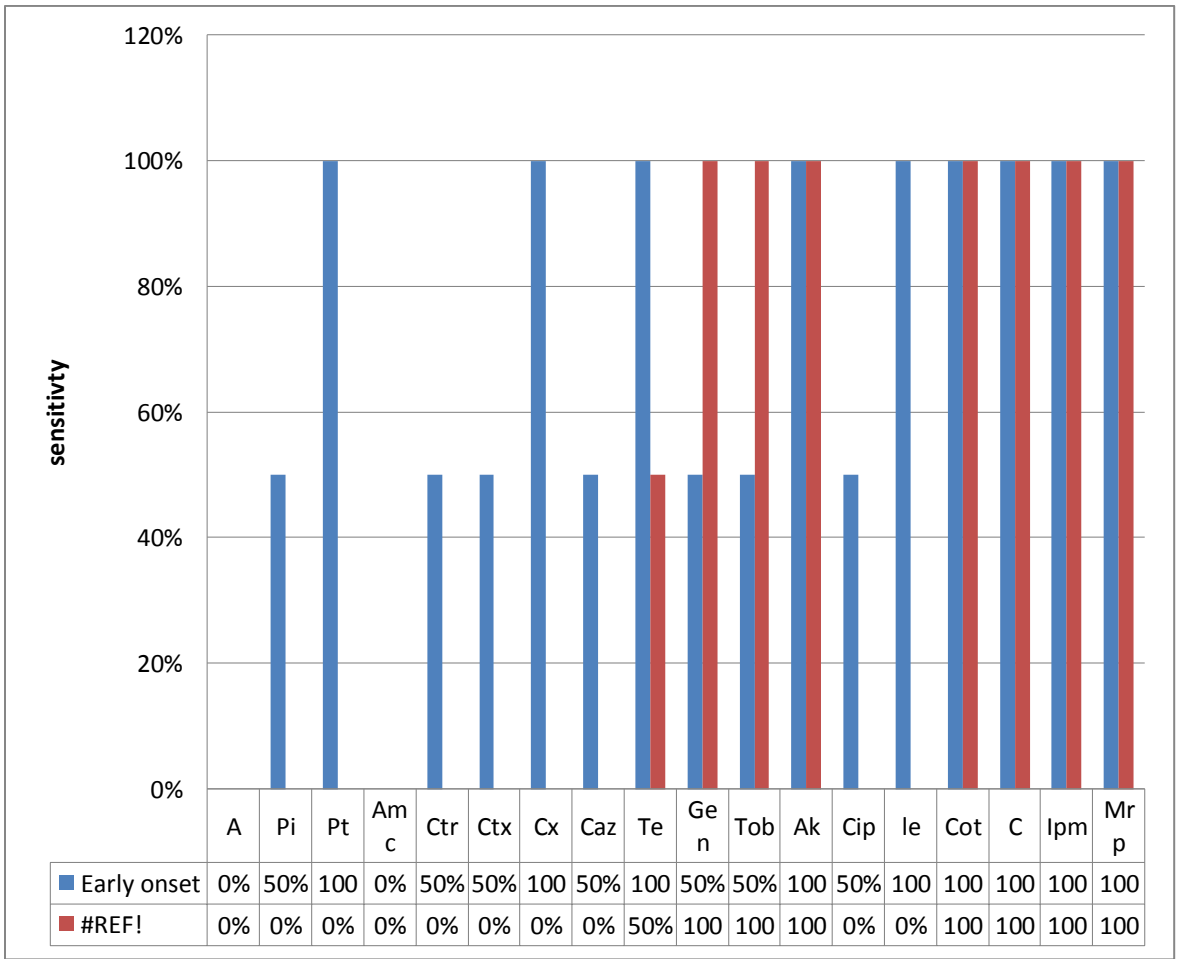


	A	Pi	Pt	Amc	Ctr	Ctx	Cx	Caz	Te	Gen	Tob	Ak	Cip	Ie	Cot	C	Ipm	Mrp
Early onset	0%	0%	100	0%	100	100	100	100	100	100	100	100	0%	100	100	100	100	100
Late onset	0%	0.0	33.	0.0	33.	33.	33.	33.	67%	66.	66.	100	33.	66.	100	100	100	100

A-Ampicillin, Pi-Piperacillin,Pt-Piperacillin tazobactum, Amc-Amoxyclav , Ctr-Ceftriaxone,Caz-Ceftazadime ,Cx-Cefoxitin, Ctx-Cefotaxime, Te-Tetracycline, Gen-Gentamycin ,Tob-Tobramycin, Ak-Amikacin, Cip-Ciprofloxacin, Le-Levofloxacin, Cot-Cotrimoxazole, C-Chloramphenicol, Imp-Imipenem, Mrp-Meropenem.

Late onset VAP was associated with more resistant strains. None of the isolates were resistant to Carbapenems.100% sensitivity to Amikacin was seen in both the groups.

Figure 13: Bar diagram showing the sensitivity of Citrobacter diversus

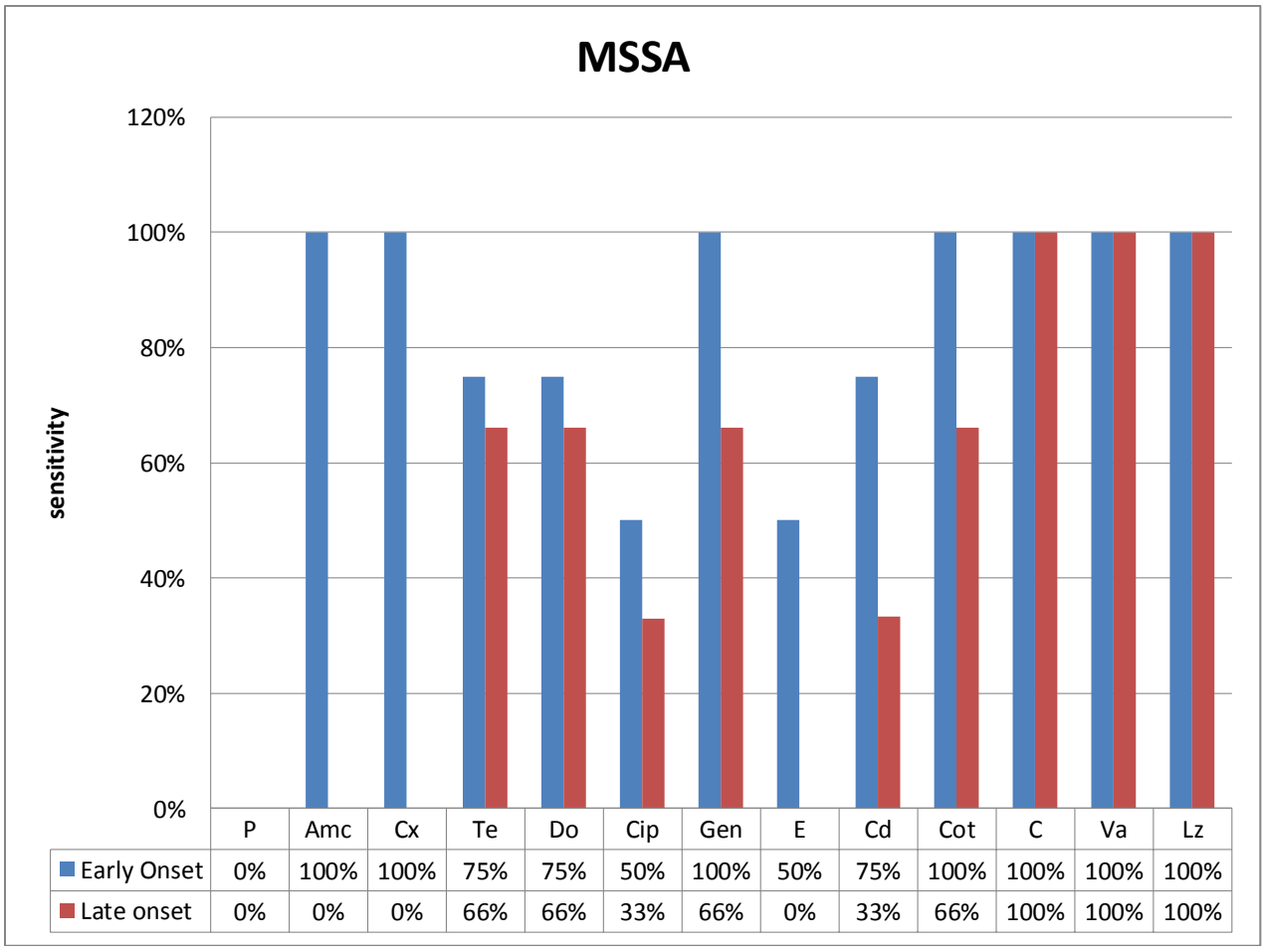


	A	Pi	Pt	Amc	Ctr	Ctx	Cx	Caz	Te	Gen	Tob	Ak	Cip	le	Cot	C	Ipm	Mrp
Early onset	0%	50%	100	0%	50%	50%	100	50%	100	50%	50%	100	50%	100	100	100	100	100
#REF!	0%	0%	0%	0%	0%	0%	0%	0%	50%	100	100	100	0%	0%	100	100	100	100

A-Ampicillin, Pi-Piperacillin,Pt-Piperacillin tazobactum, Amc-Amoxyclav , Ctr-Ceftriaxone,Caz-Ceftazadime ,Cx-Cefoxitin, Ctx-Cefotaxime, Te-Tetracycline, Gen-Gentamycin ,Tob-Tobramycin, Ak-Amikacin, Cip-Ciprofloxacin, Le-Levofloxacin, Cot-Cotrimoxazole, C-Chloramphenicol, Imp-Imipenem, Mrp-Meropenem.

Most of the isolates in the late onset group are resistant. None of the strains were resistant to Chloramphenicol, Co- trimoxazole, Amikacin and Carbapenems.

Figure 14: Bar diagram showing the sensitivity of MSSA in the Early onset and Late onset VAP.

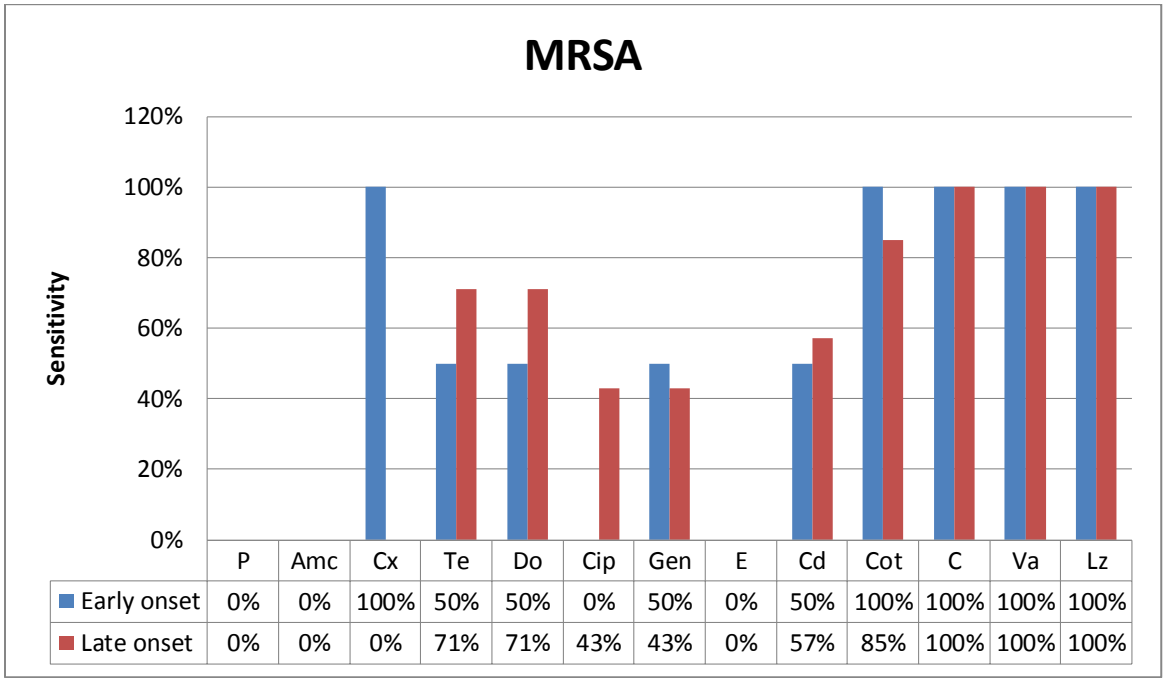


	P	Amc	Cx	Te	Do	Cip	Gen	E	Cd	Cot	C	Va	Lz
Early Onset	0%	100%	100%	75%	75%	50%	100%	50%	75%	100%	100%	100%	100%
Late onset	0%	0%	0%	66%	66%	33%	66%	0%	33%	66%	100%	100%	100%

P-Penicillin, Amc- Amoxyclav,Cx-Cefoxitin, Te-Tetracycline ,Do-Doxycycline, Cip-Ciprofloxacin, Gen-Gentamycin E-Erythromycin, Cd-Clindamycin, Cot-Cotrimoxazole,C-Chloramphenicol, Va-Vancomycin, Lz-Linezolid.

None of the MSSA isolates were resistant to Vancomycin and Linezolid. 100% sensitivity was noted to Chloramphenicol. None of the isolates in the Late onset group was sensitive to Erythromycin.

Figure 15: Bar diagram showing the sensitivity of MRSA in the Early onset and Late onset VAP.



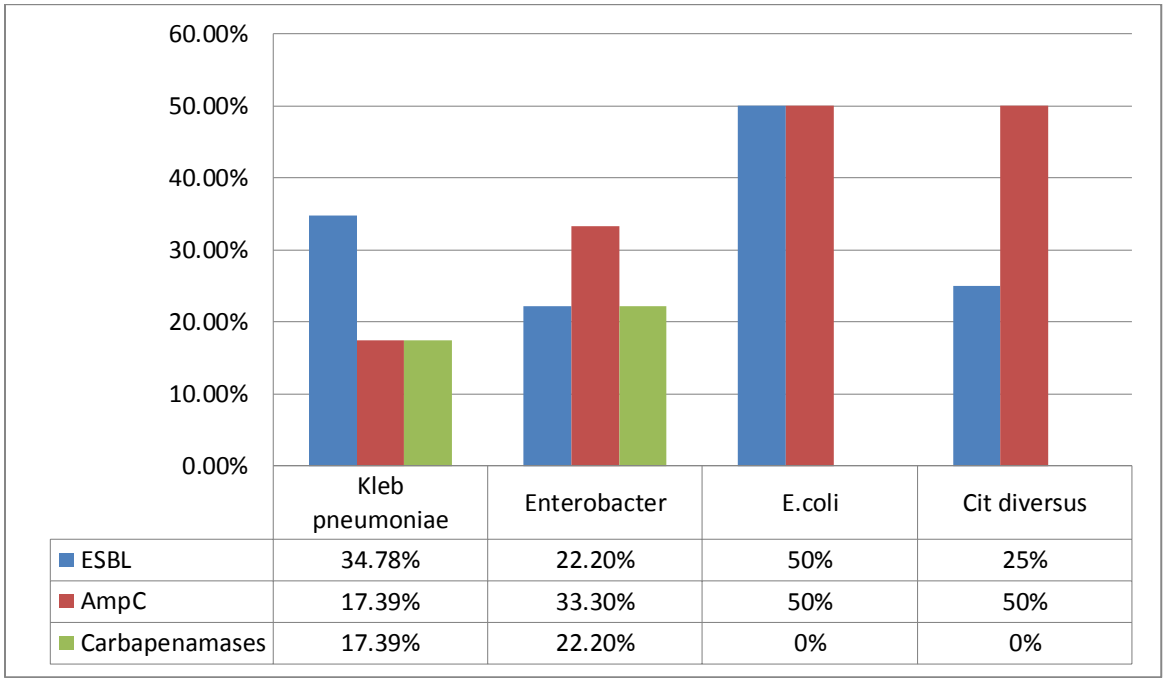
P-Penicillin, Amc- Amoxyclav, Cx-Cefoxitin, Te-Tetracycline, Do-Doxycycline, Cip-Ciprofloxacin, Gen-Gentamycin, E-Erythromycin, Cd-Clindamycin, Cot-Cotrimoxazole, C-Chloramphenicol, Va-Vancomycin, Lz-Linezolid.

None of the MRSA strains were resistant to Chloramphenicol, Vancomycin and Linezolid. 100% resistance to erythromycin was seen in both the groups.

Table 11: Showing the distribution of MDR strains among Enterobacteriacae.

Organism	Total no	ESBL	Amp C	Carbapenamase
Klebsiella pneumoniae	23	8(34.78%)	4(17.39%)	4(17.39%)
E.coli	4	2(50%)	2(50%)	0
Enterobacter	9	2(22.2%)	3(33.3%)	2(22.2%)
Citrobacter diversus	4	1(25%)	2(50%)	0
Total	**40**	**13(32.5%)**	**10(25%)**	**6(15%)**

Figure 16: Bar diagram showing the distribution of MDR strains

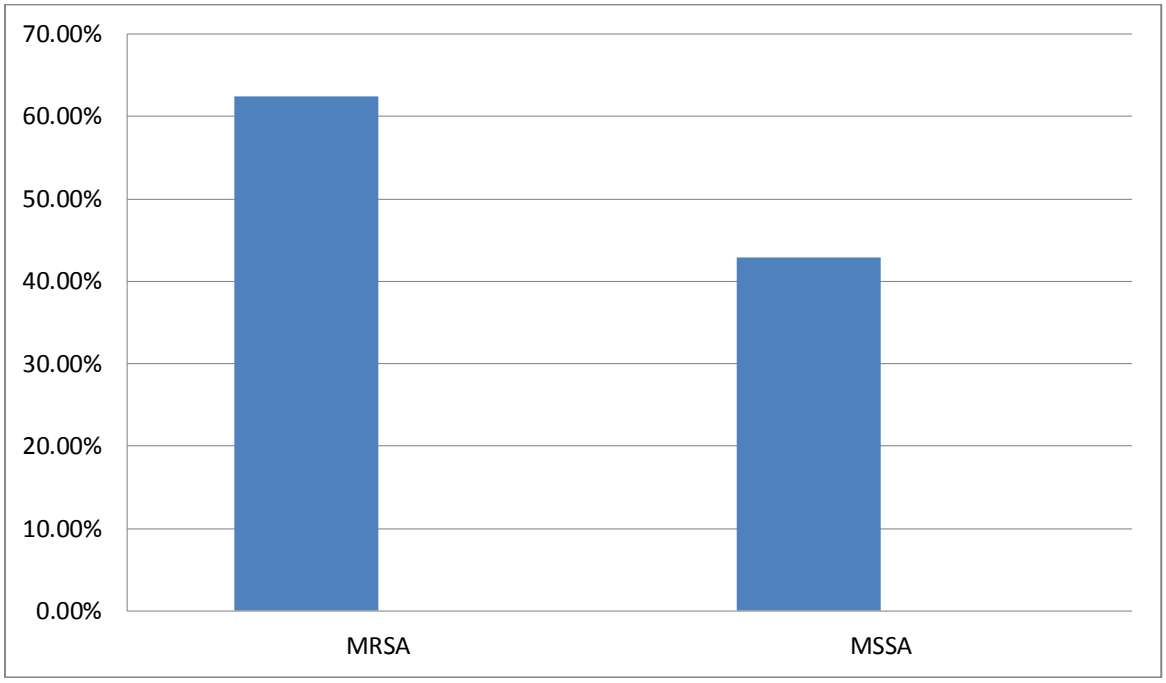


Majority of the ESBL and Carbapenamse producing strains was Klebsiella pneumonia followed by E.coli. Enterobacter was the most common species producing Amp C β lactamase.

Table 12: showing the distribution of Inducible Clindamycin resistance among Sataphylococcal isolates.

Organism	Total no	Inducible Clindamycin resistance
MRSA	8	5(62.5%)
MSSA	7	3(42.85%)

Figure 17: Bar diagram showing the distribution of Inducible Clindamycin resistance among Staphylococcal isolates.

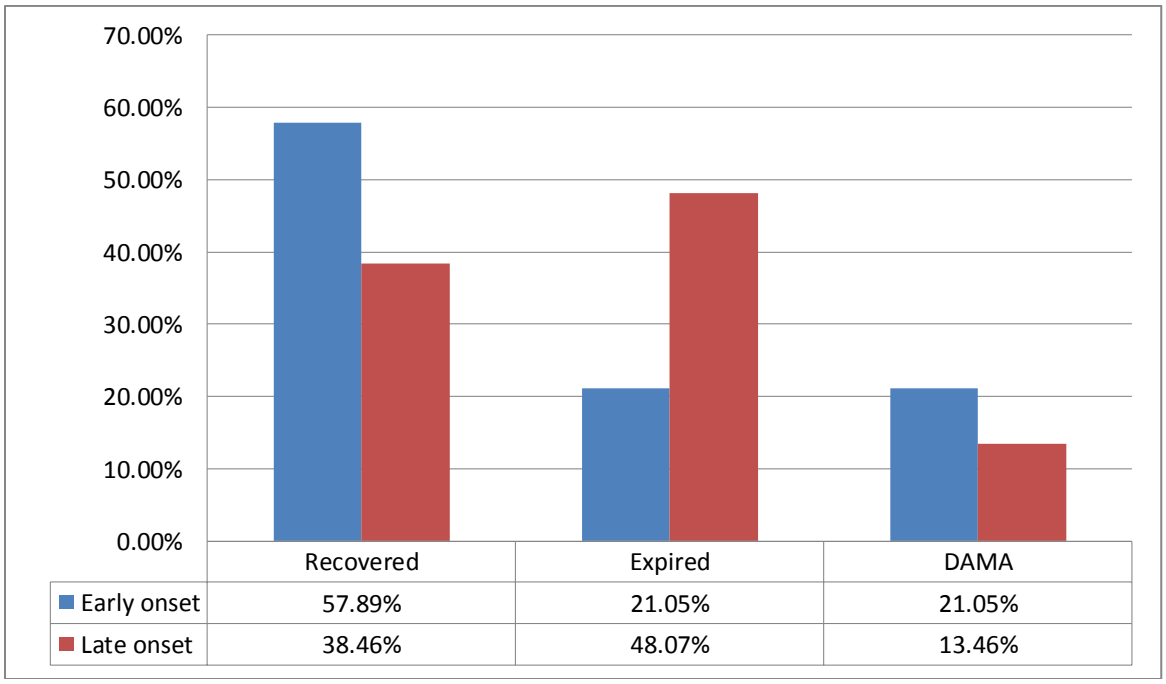


About 62.5% of the MRSA strains were Inducible Clindamycin producers while 42.85% of the MSSA strains were Inducible Clindamycin producers.

TABLE 13: OUTCOME OF THE PATIENTS:

	RECOVERED	EXPIRED	DAMA	Total
Early onset	11(57.89%)	4(21.05%)	4(21.05%)	19(100%)
Late onset	20(38.46%)	25(48.07%)	7(13.46%)	52(100%)

Figure 18: Bar diagram showing the outcome of patients



	Recovered	Expired	DAMA
Early onset	57.89%	21.05%	21.05%
Late onset	38.46%	48.07%	13.46%

Late onset VAP was associated with increased mortality when compared to Early onset VAP. Recovery rate was more in Early onset when compared to Late onset VAP.

DISCUSSION

VAP is the second most common nosocomial infection after UTI and one of the most common nosocomial infection in the Intensive care unit [1,4] . In contrast to other nosocomial infections the mortality rate occurring due to VAP ranges from 24% to 76%[9,10]. Pathogens causing VAP vary among hospitals and also in same centre over time. Therefore the frequency of occurrence of VAP and its microbial flora needs to be studied in each setting so as to guide the clinician in more effective and appropriate treatment and rational use of anti-microbial agents.This also helps to develop an antibiotic policy for empiric antibiotic therapy in VAP patients .Hence this study was undertaken to find out the occurrence of VAP, the organisms associated with it and their antibiotic sensitivity pattern.

INCIDENCE OF VAP:

A total of 100 clinically suspected VAP patients admitted in the ICU were enrolled in the study as per the inclusion criteria. Of the 100 patients analysed VAP was diagnosed in 71% patients as per the CPIS score.

The incidence of VAP in our study was 25.6/1000 patient ventilated days. According to a study by Rakshit et al the incidence of VAP was 26/ 1000 ventilated days which is similar to our study[4]. In our study 77.46% of the infections were polymicrobial in nature. In a study by Joseph et al 27.8% of the infections were polymicrobial [49]while Touillet reported 54.8% though it can range from 13% to 87%.[50]

Various studies have reported incidence rate ranging between 15.7 and 67[4]. Incidence rate depends on the duration of study and sample size. According to Rakshit et al increased incidence is associated with shorter duration and small sample size[4].

PATIENT CHARACTERISTICS:

Of the 71 diagnosed VAP cases, 66% were males and 34% were females with a mean age of 41.13 ±1 5.38(range 18 to 70 years).

TIME OF ONSET OF VAP

The risk of pneumonia in patients receiving mechanical ventilation increases with the duration of ventilation. Fagon et al showed that incidence of VAP rises with number days of mechanical ventilation [17,23].

In our study 26.76% (19) were categorized as Early onset VAP while 73.23% (52) were categorized as Late onset VAP with a mean duration of ventilation of 5.32 ± 1.36 days. In our ICU set up Late onset VAP was most common when compared to Early onset VAP. A study by

Valles J et al showed 27.5% of the patients had early-onset VAP and 72.5% had late-onset VAP[51]. While in a study by Rello et al and Vanhems et al the incidence of Early onset VAP was 12.8% and 8.3% which is lower than our study[52].

Early-onset VAP is usually due to the underlying pathology. On the other hand, late-onset VAP could be due to prolonged ventilation, evolution of the underlying disease, quality of nursing care, duration of antibiotic exposure or environmental ecology of the hospital[23]. Studies have shown that previous antibiotic useage decreases early-onset VAP but markedly increases multidrug-resistant (MDR) pathogens.

CLINICAL SPECTRUM OF PATIENTS:

Colonization of organism was commonly seen in patients with underlying respiratory disease(36.6%) which included ARDS, Bronchopneumonia and COPD, followed by head trauma and neurosurgical cases (21.12%) and OP poisoning(19.71%) . Cook et al reported an incidence of VAP in 17.8% of trauma and neurosurgical patients[53]. In a study by Joseph et al incidence of VAP was more in OP poisoning followed by Neurological disorders and CNS infections (P value 0.0046 and 0.0249 respectively) .[54]

Various studies have shown that the incidence of VAP is greater in patients with diseases requiring prolonged MV or in patients with diseases that predispose to pulmonary infection

Incidence of VAP is more common in patients with underlying respiratory diseases due to the following reasons:

i) impaired mucociliary clearance
ii) loss of mucosal integrity
iii) history of previous exposure to steroids and antibiotics which suppress the lung defence mechanism.

The factors which contribute to increased risk of VAP in Trauma & Neurosurgery and Poisoning patients are:

i) increased risk of aspiration because of prolonged duration of ventilation and impaired cough reflux and increased gastric reflux due to use of muscle relaxants
ii) Prolonged sedation to avoid self extubation
iii) Higher incidence of reintubation due to failed weaning

iv) Post surgical stress.

ORGANSIMS CAUSING VAP:

In our study Acinetobacter species (28.46%), Pseudomonas aeruginosa (23.56%) and Klebsiella pneumoniae (16.54%) were the most common organism causing Early and Late onset VAP which is similar to Dey et al[39]. In the study by Dey et al Acinetobacter species and Pseudomonas aeruginosa accounted to 48.94% and 25.53% respectively.[39]

According to Joseph et al the most common causative agents of early-onset VAP were members of Enterobacteriaceae (25%) and Acinetobacter spp. (25%) while Pseudomonas spp. (39%) and Acinetobacter spp. (32%) were the most common pathogens causing late-onset VAP. [49,54]In a study by Rello et al Staphylococcus aureus (23.7%) and Pseudomonas species (19.7%) was the most common organism causing early and late onset VAP respectively[23,52].

According to Fagon et al the members of the Enterobacteriaceae accounted to 14% of infections which included Escherichia coli, Proteus species, Enterobacter species, and Klebsiella species and smaller numbers of Citrobacter and Hafnia species[15]. In our study 31.38% of the infections were caused by members of the family Enterobacteriaceae which included Klebsiella pneumonia (16.78%), Enterobacter species (6.5%), E.coli and Citrobacter diversus (2.91%) each respectively.

In our study among the Gram positive cocci, 25% of MSSA were associated with Early onset VAP while 43.75% of MRSA were associated with late onset VAP which is similar to a study by Joseph et al wherein 13% of MSSA were associated with early-onset VAP and 50% of MRSA were associated with late-onset VAP [49, 54] .

In our study among the fungal agents, 5.10% were non Albicans Candida and 2.18% were Candida albicans. Though Candida species are isolated commonly from endotracheal aspirates it usually represents colonization of the airways which could be due to prolonged exposure to steroids / antibiotics and chronic debilitating diseases. Treatment with antifungal agents is controversial and is rarely required.[17]

A variation in the microbial flora was noted at various centers. This variation is due to the diversity of the patient population studied, ecology of the organisms and

previous antibiotic exposure thus emphasizing the need for periodic surveillance. The microbial flora when studied in each setting can thus guide the clinician in more effective and rational use of anti-microbial agents and also helps to develop an antibiotic policy for empiric therapy in VAP patients.

ASSOCIATION OF ORGANISMS WITH CLINICAL DISEASE

In our study 28.20% of the Acinetobacter species were isolated from Head trauma and neurosurgical cases while only 2.05% was seen in Respiratory cases. This could be due to increased duration of ventilation and as Acinetobacter is an environmental pathogen it can survive on the hands of health care workers and environment. Most of the patients in our ICU were on Cephalosporin therapy. A review by Park et al[19,17] has shown that Acinetobacter species was associated with neurosurgery, ARDS, head trauma, in patients with prior cephalosporin theraphy and lack of hand hygiene .However in our study 47.82% of the respiratory infections was caused due to Klebsiella pneumoniae .

In our study only 32% of the Pseudomonas was isolated from Respiratory cases, majority being Klebsiella pneumoniae (47.82%). Infections caused due to Klebsiella pneumoniae can be attributed to its ability to form biofilms on the ET tubes and naso gastric tubes and presence of a capsule which evades phagocytosis. Park et al[19,17] have reported an association of Pseudomonas with COPD. [25] Pseudomonas has the ability to cause recurrent or persistent pneumonia due to toxin mediated damage to the alveolar epithelial cells and macrophages.

In our study among the Gram positive cocci, 42.85% of the MSSA was isolated from Head trauma and neurosurgery cases. While 44.44% of the MRSA was isolated from Respiratory diseases which is similar to the report by Park et al[19,17]. Staphylococcus aureus is endogenous in origin and colonizes the anterior nares, axilla and gluteal region presence of invasive devices could result in the spread of these MRSA strains .

ANALYSIS OF SENSITIVITY PATTERN AND OUTCOME:

In our study 32.5% of the isolates of the family Enterobacteriaceae were ESBL producers, 25% Amp C producers and 15% were Carbapenamse. Most of MDR strains were isolated from the Late onset VAP patients which could be due to prolonged

ventilation, increased duration of stay in the hospital and exposure to prior antibiotics.

ESBL producers: In our study 34.78% of Klebsiella pneumoniae and 50% of theE.coli were ESBL producers. 22.2% of Enterobacter species and 25% of the Citrobacter diversus were ESBL producers. Whereas Joseph et al showed 50% and 67% of E. coli and K. pneumoniae were ESBL producers respectively.[54]

Amp C: In our study Amp C β-lactamases were produced by 25% of the members of Enterobacteriaceae. Among the Amp C producers, 50% were Citrobacter diversus and E.coli followed by Enterobacter (33.3%). Amp C β-lactamases were produced by 33.3% of the members of Enterobacteriaceae as per Joseph et al.[54]

CARBAPENAMASES: Of the Carbapenamse producing strains 17.39% were Klebsiella pneumoniae and 22.2% were Enterobacter species.

NON FERMENTERS:

Among the non fermentors, 72% of Acinetobacter species and 79% of Pseudomonas species were sensitive to Carbapenems. None of the strains were resistant to Colistin and polymyxin. Sensitivity to Levofloxacin ranged from 75% to 80% while 80% of the strains were sensitive to Amikacin. Sensitivity to Cephalosporins ranged between 40 to 50%.

Four of the trauma and neurosurgery patients and one OP Poisoning patient who were infected with Carbapenamse producing strains were treated with Colistin of which 3 patients responded to therapy while one succumbed. One of the patients was treated with Tigecycline who responded initially to the treatment but later due to financial constrains the treatment was withdrawn and patient expired. One patient recovered with combination of levofloxacin and piperacillin-tazobactum.

GRAM POSITIVE COCCI:

53.33% of the isolates were MRSA while 46% were MSSA. 100% sensitivity to Vancomycin and Linezolid, 90% sensitivity to Chloramphenicol and 80% sensitivity to Cotrimoxazole was noted. Most of the MRSA were isolated from Late onset VAP. None of the six patients who were infected with MRSA survived inspite of treatment with

linezolid.

Analysis of the sensitivity pattern of all the organisms show that most of them were sensitive to Chloramphenicol, Cotrimoxazole and Amikacin as they were less commonly used. Hence antibiotic cycling with these drugs along with cephalosporins may prevent the emergence of drug resistant strains. Gruson and colleagues observed a reduction in the incidence of VAP after introducing an antimicrobial program that consisted of supervised rotation and restricted use of ceftazidime and ciprofloxacin[35]. However the toxic side effects of aminoglycosides must be considerd before administration of the drug.

OUTCOME

Mortality rate was less in the Early onset category accounting to (26.31%) due to early extubation, sensitive strains while the Mortality rate in the Late onset category was 48.07% which is similar to Rakisth et al and Joesph et al.[4,54]

VAP is polymicrobial in nature and is associated with increased mortality. Hence periodic surveillance of the organisms and their sensitivity pattern will guide the clinician in choosing appropriate antibiotics thereby preventing drug abuse and development of MDR strains. Use of appropriate preventive measures and good nursing care can reduce the incidence of VAP.

CONCLUSION

- Incidence of VAP in our ICU was 25.6/1000 ventilated days and polymicrobial in nature.
- Clinical criteria used, in combination with quantitative culture is helpful in the diagnosis of VAP.
- Late onset VAP was more common in our ICU set up and was associated with MDR strains.
- Acinetobacter species was the most common organism causing both early and late onset VAP and the incidence of VAP was most common in patients with underlying respiratory diseases.
- Klebsiella pneumoniae was the predominant Carbapenamase producing strain. Infection caused due to MRSA was associated with 100% mortality.
- A variation in the microbial flora was noted when compared to other studies. This variation is due to the diversity of the patient population studied, ecology of the organisms and previous antibiotic exposure thus emphasizing the need of timely surveillance.
- Antibiotic policy can be formulated in the ICU and empiric treatment can be initiated to decrease the mortality rate.
- Integrated approach involving the clinicians and microbiologists may be helpful to the patients in early diagnosis and institution of appropriate treatment.
- However, further studies are required to analyse the risk factors and the use of biomarkers for the early and rational treatment and prevent drug abuse.

SUMMARY

A prospective study was conducted to analyze the incidence of VAP, microbial spectrum and their antibiotic sensitivity pattern in the ICU at R.L.Jalappa Hospital and Research centre. Of the 100 clinically suspected patients, 71% were confirmed as VAP of which 26.76% were categorized as Early onset VAP and 73.3% as Late onset VAP. The incidence of VAP in our set up is 25.6/1000 patient ventilated days.

Late onset VAP was more common when compared to Early onset VAP accounting to 73.23%. VAP was commonly seen in patients with underlying respiratory diseases (36.6%) which included ARDS, Bronchopneumonia and COPD, followed by head trauma and neurosurgical cases (21.12%) and OP poisoning (19.71%).

In our study Acinetobacter species (28.46%), Pseudomonas aeruginosa (23.56%) and Klebsiella pneumoniae (16.54%) were the most common organism causing Early and Late onset VAP. About 5.10% of non albicans candida species was isolated. However its role as pathogens is controversial. 31.38% of the infections were caused by members of the family Enterobacteriaceae. A higher incidence of infections caused due MRSA was noted in the Late onset VAP patients.

32.5% of the isolates of the family Enterobacteriaceae were ESBL producers, 25% Amp C producers and 15% were Carbapenamse producers. Among the gram positive cocci .53.33% of the isolates were MRSA while 46% were MSSA. Among the non fermentors 72% and 79% of Acinetobacter species and Pseudomonas species were sensitive to Carbapenems.

Mortality rate was less in the Early onset category accounting to (26.31%) due to early extubation, sensitive strains while the Mortality rate in the Late onset category was 48.07%.

Therefore the microbial flora when studied in each setting can guide the clinician in more effective and rational use of anti-microbial agents and also helps to develop an antibiotic policy for empiric therapy in VAP patients

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APPENDIX A

PROFORMA

NAME -

AGE -

SEX -

HOSPITAL NO -

CLINICAL DIAGNOSIS -

VAP DAY- EARLY/ LATE

TREATMENT -

VAP SIGNS

TEMPERATURE -

WBC COUNT -

X RAY CHANGES : INFILTERATE/ CONSOLIDATION/ CAVITY

COUGH -

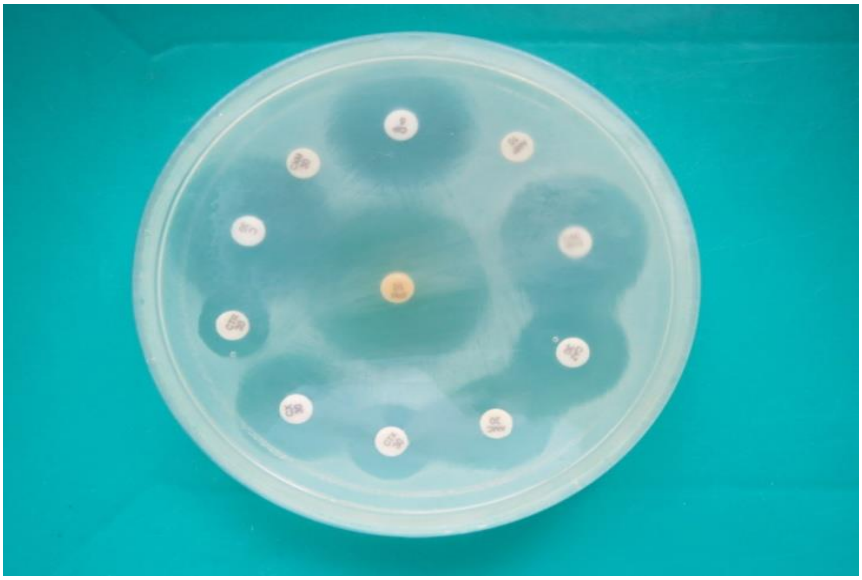
RALES -

ABG ANALYSIS: P02/FiO2

TRACHEOSTOMY- YES/NO

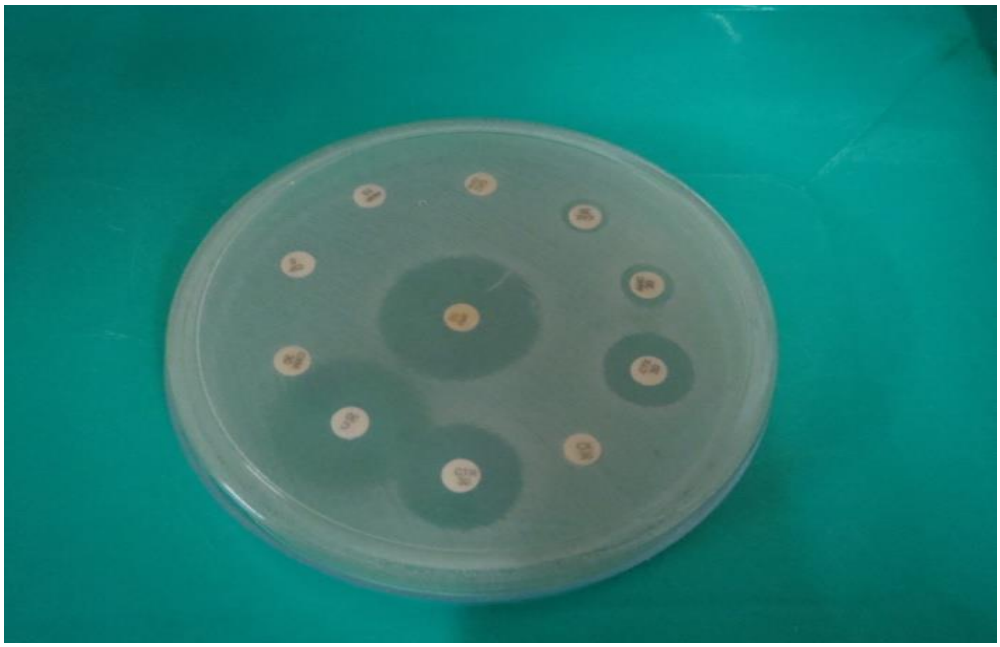
OUTCOME -

ESBL PRODUCTION



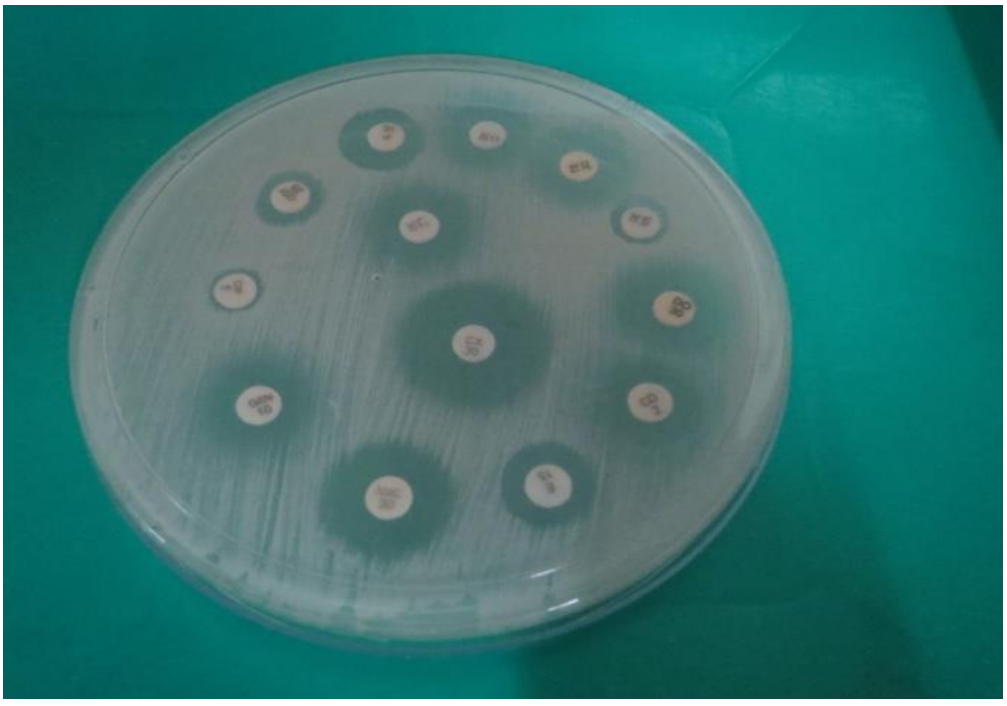
>5mm or more increase in the Zone of inhibition around Cefoxitin disc

Amp C PRODUCTION



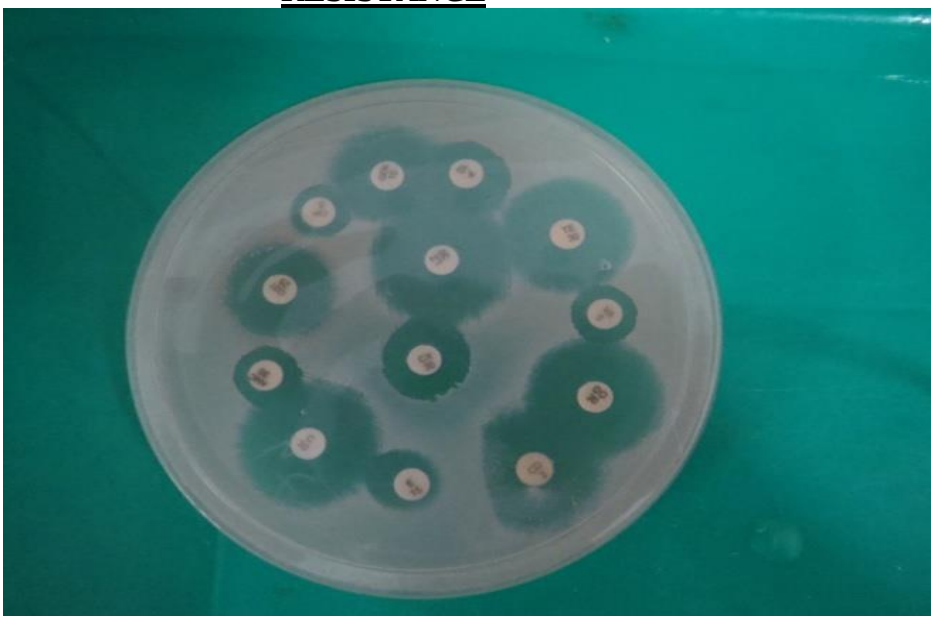
No zone of inhibition for Ceftazidime –Clavulanic acid when compared to Ceftazidime disc alone

DETECTION OF MSSA



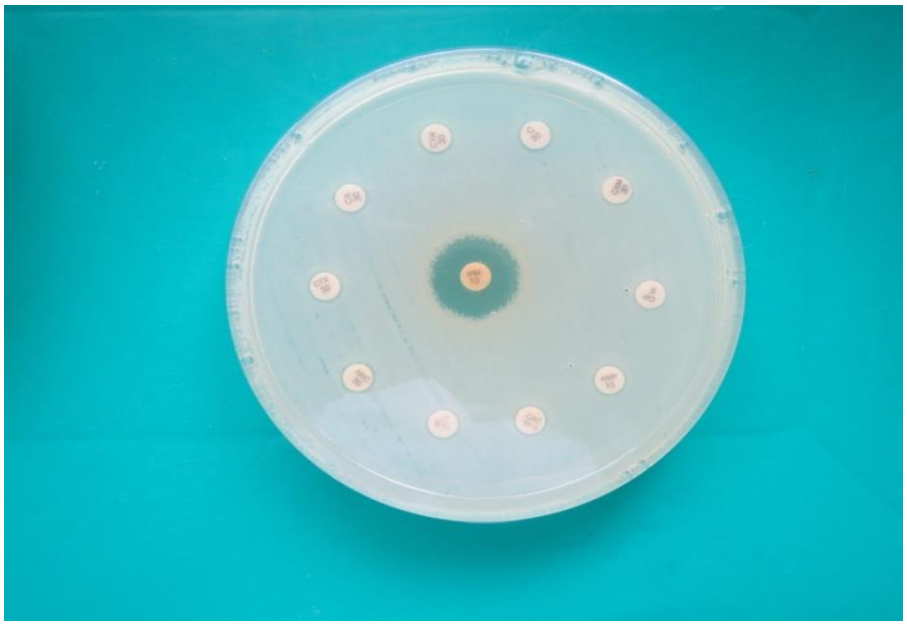
Zone of inhibition around cefoxitin disc > 22mm

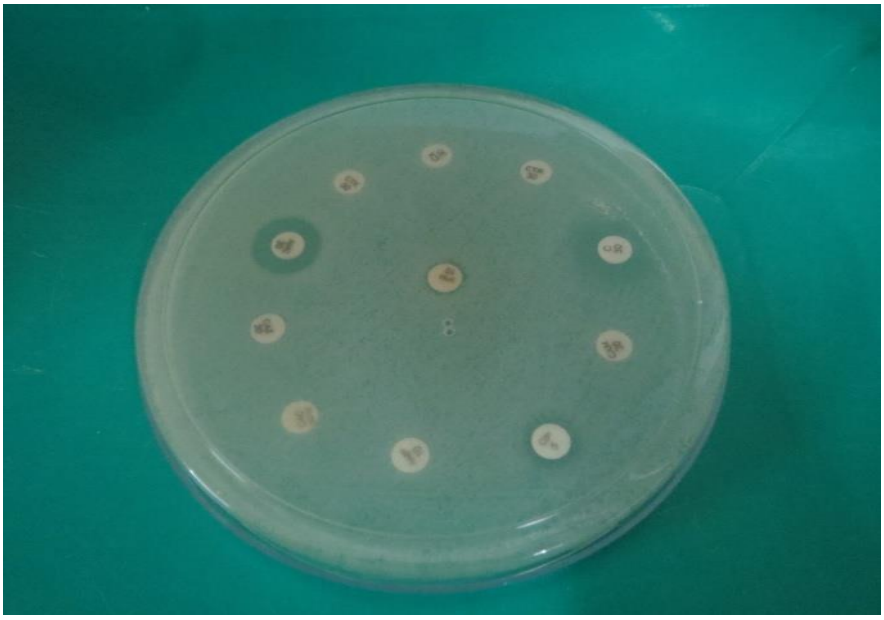
DETECTION OF MRSA WITH INDUCIBLE CLINDAMYCIN RESISTANCE



Zone of inhibition around cefoxitin disc <22mm. with D Zone around Clindamycin disc, when placed adjacent to Erythromycin

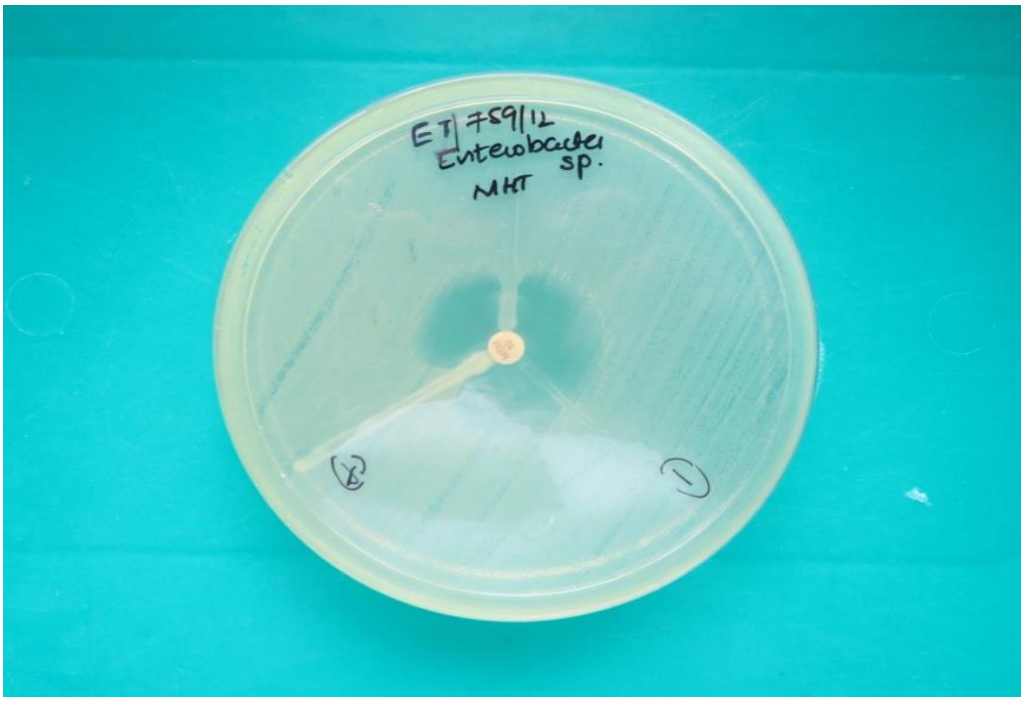
DETECTION OF CARBAPENAMASE PRODUCTION





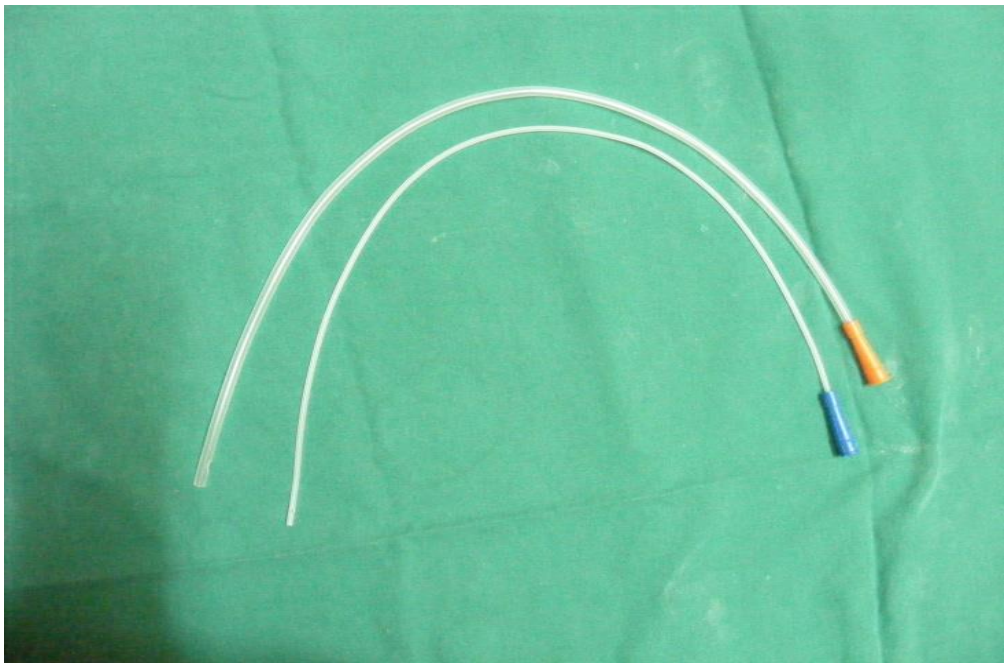
Zone of inhibition around Imipenem disc < 23 mm suggesting resistant to imipenem

MODIFIED HODGE TEST

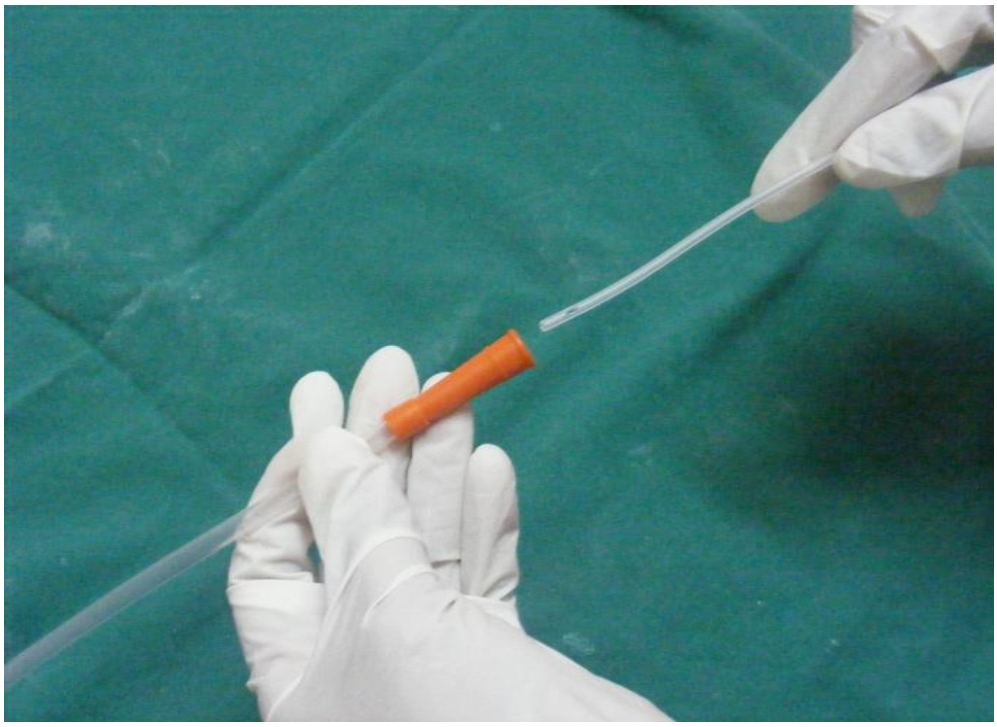


CLOVER leaf shaped zone of inhibition due to Carbapenemase production

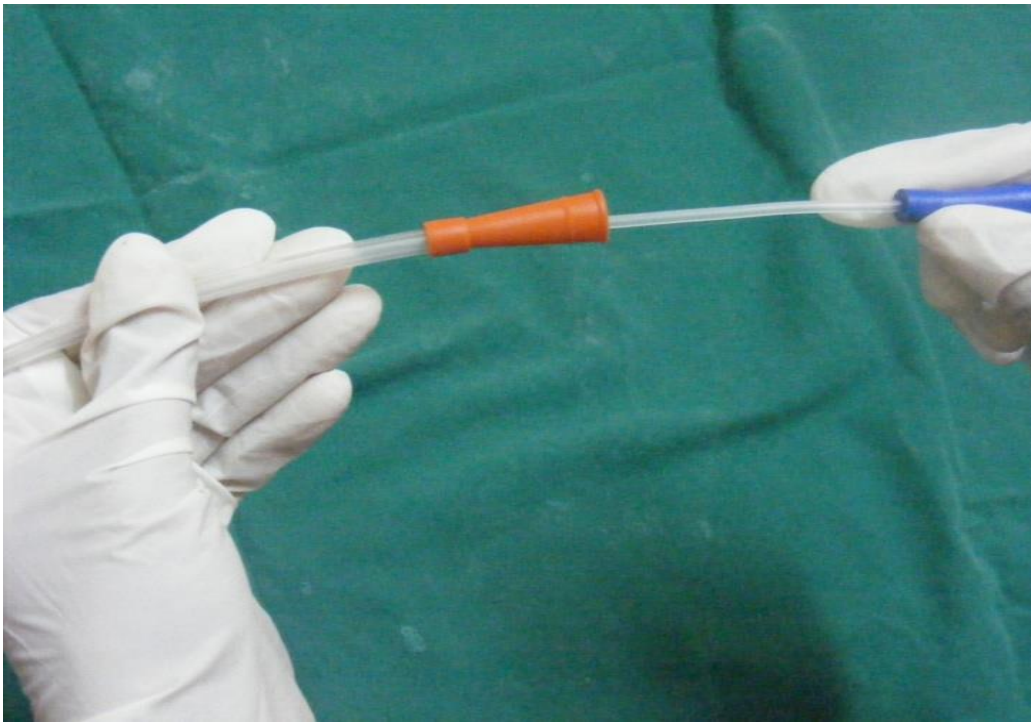
SAMPLE COLLECTION

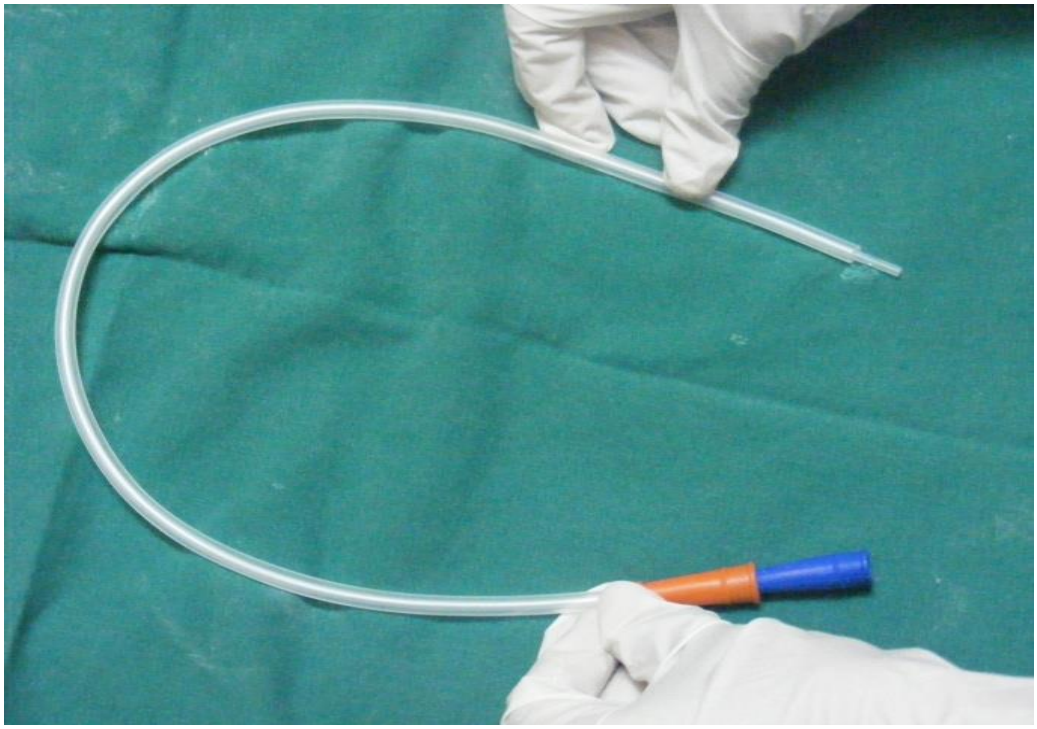


16 Fr (Orange) & 8 Fr (Blue) Ramson's Catheters



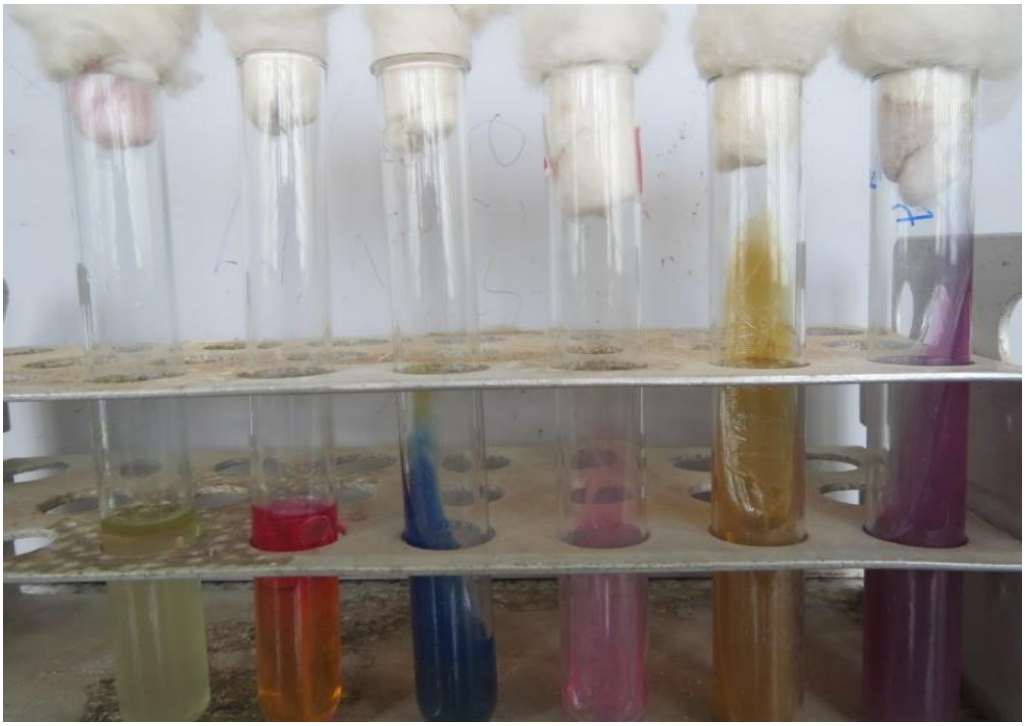
Telescoping 8 Fr into 16 Fr catheter





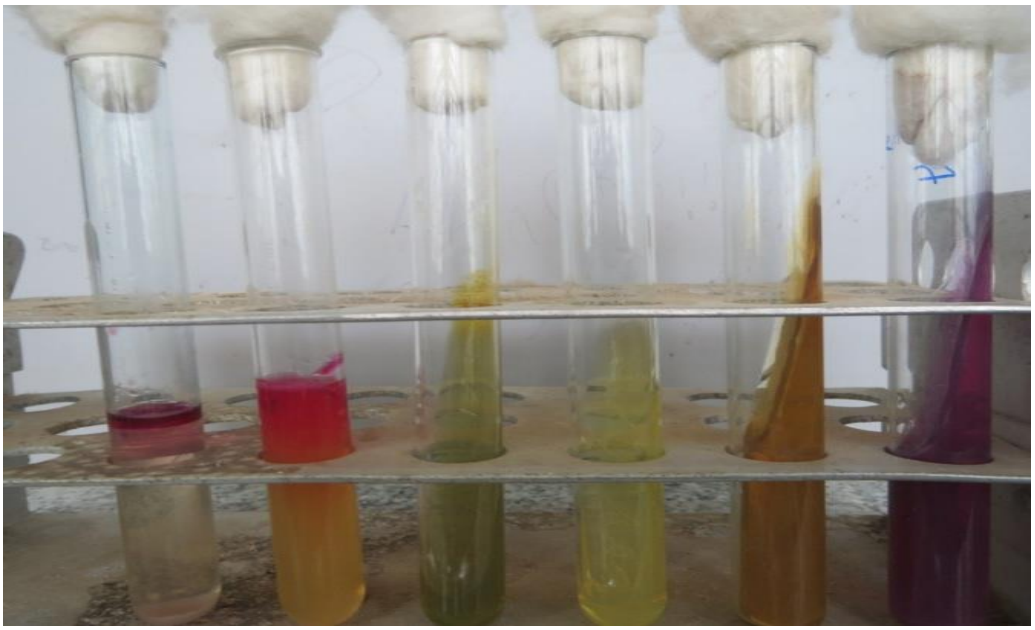
IDENTIFICATION OF ORGANISMS

(1) (2) (3) (4) (5) (6)

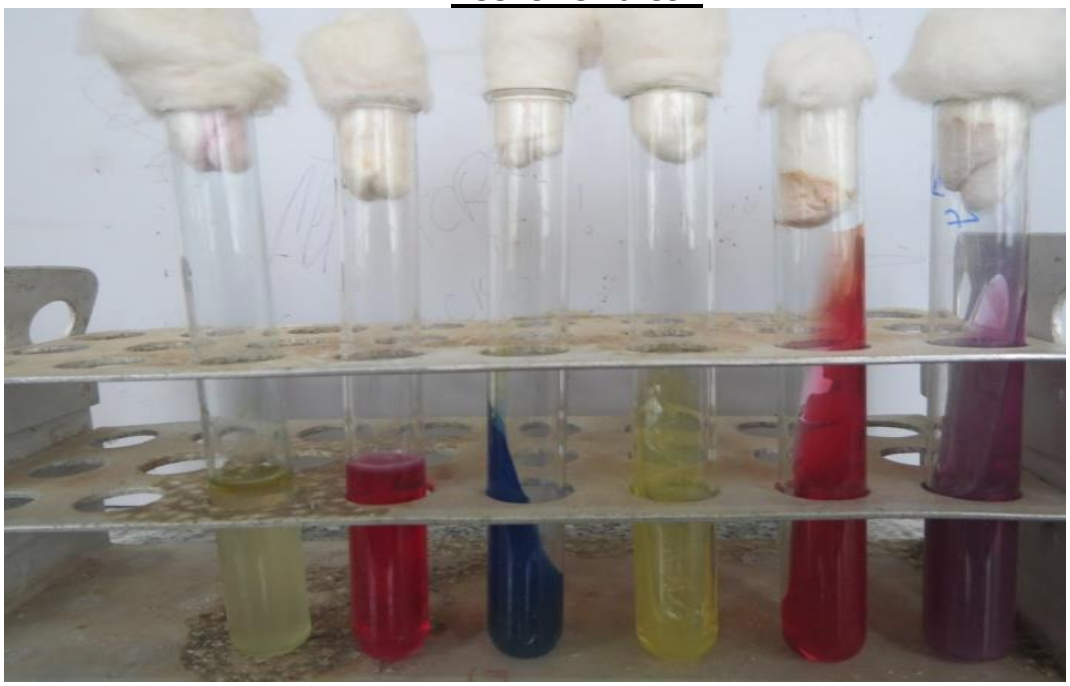


Klebsiella pneumonia

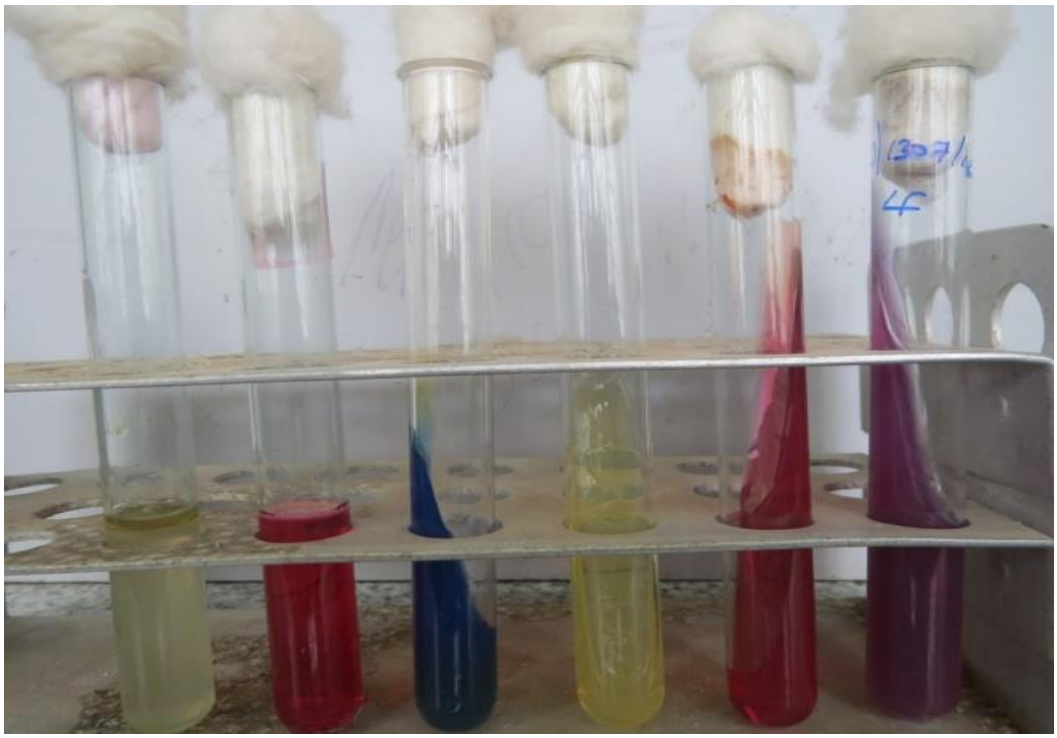
(1)-Indole test, (2)- Mannitol motility test, (3)- Citrate test, (4)-Urease test, (5)- Triple sugar iron agar test, (6)- Lysine iron agar test



Escherichia coli



Acinetobacter species



Pseudomonas aeruginosa