



ROLE OF LACTOSE NON FERMENTERS IN VENTILATOR ASSOCIATED PNEUMONIA (VAP) AND THEIR ANTIBIOGRAM

Microbiology

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ABSTRACT

INTRODUCTION: Ventilator associated pneumonia(VAP) is the serious hospital acquired infection, occurs in intensive care units(ICU) which is associated with high mortality rate. This prospective study was done to determine causative agent and their resistant mechanism.

AIM: To study antibiogram of Lactose non fermenters and phenotypic detection of carbapenamase producers among them.

METHOD: Total 170 sample of endotracheal aspirates were collected from different ICU of tertiary care hospital, Gujarat. Culture of endotracheal aspirates was done by Semi-quantitative method and Antibiotic susceptibility testing was done by Kirby Bauer method of disk diffusion. All gram-negative bacilli (GNB) were tested for MBL (Metallo-beta lactamase) production by a Combination disc method.

RESULT: Of the 170 patients, 59 (35%) developed VAP during their ICU stay. Most common isolates were *Pseudomonas* followed by *Acinetobacter* and other gram negative bacilli and gram positive cocci.

CONCLUSION: Amongst the Carbapenamase producers *Pseudomonas* was the most common at 33.3% followed by *Acinetobacter*.

KEYWORDS

Ventilator associated pneumonia, *Pseudomonas*, *Acinetobacter*, MBL.

INTRODUCTION

Ventilator associated pneumonia(VAP): Pneumonia occurring after 48 hours of endotracheal intubation and initiation of mechanical ventilation¹. Intubation and mechanical ventilation are associated with 6-21 fold increased risk of acquiring pneumonia in hospital settings.² The occurrence of VAP in Asian countries is much higher and ranges from 3.5 to 46 infections/1000 MV days.³ There is necessary to know the microbiological profile of VAP as it helps to clinicians to select antibiotic treatment and improving the outcomes. Common pathogens include *Pseudomonas* and *Acinetobacter* species and other gram negative and gram positive organisms. Now a days carbapenam resistant pathogen are emerging and deal with carbapenamase producers is a serious problem.³ so main purpose of the study to detect carbapenamase producers among *Pseudomonas* and *Acinetobacter* species.

MATERIAL AND METHOD

A prospective study was carried out at Department of Microbiology, tertiary Care hospital, Gujarat from January 2013 to March 2014. Total 170 Samples were collected. Samples of patients admitted in various intensive care units (ICU) of tertiary care hospital, on mechanical ventilation for >48 hours irrespective of age and gender are accepted and Patients already having pneumonia at the time of ICU admission or patient who developed pneumonia in the first 48 hours of mechanical ventilation or those with other causes of radiological infiltrates like pulmonary haemorrhage, pulmonary embolism, atelectasis, congestive cardiac failure and acute respiratory distress syndrome by C.T Scan and other diagnostic modalities are excluded. From each patient the following data were collected at ICU admission: name, age, gender, registration number, primary diagnosis, date of admission in hospital and ICU. The study patients were monitored for the development of VAP using clinical and microbiological criteria until either discharge or death. The relevant data were recorded from medical records, bedside flow sheets, radiographic reports, and reports of microbiological studies of the patients. This research study did not receive specific funding, but it was performed during residency period of Dr. P.Gandhi.

Criteria for diagnosing VAP

The patients fulfilling both the clinical and microbiological criteria were diagnosed to be suffering from VAP. Clinical criteria included modified clinical pulmonary infection score (CPIS) > 6 (Table 1),⁵ and microbiological criteria included positive Gram stain (>10 polymorphonuclear cells/low power field and ≥ 1 bacteria/oil immersion field with or without the presence of intracellular bacteria) and semi quantitative endotracheal aspirate culture showing ≥ 10⁶ CFU/ml.^{6,8}

Table 1: Modified Clinical Pulmonary Infection Score (CPIS)⁵

CPIS points	0	1	2
Temperature (°C)	≥ 36.5 and ≤ 38.4	≥ 38.5 and ≤ 38.9	≥ 39 and ≤ 36
Leucocyte count (per mm ³)	4000- 11000	< 4000 or > 11000	<4000 or > 11000 + band forms ≥ 500
Tracheal secretions	Rare	Abundant	Abundant + Purulent
PaO ₂ / FiO ₂ mm Hg	>240 or ARDS	-----	-----
Chest radiograph	No infiltrate	Diffuse infiltrate	≤ 240 and no ARDS
Culture of tracheal aspirate	Light growth or no growth	Moderate or heavy growth of pathogenic bacteria	Localized infiltrate Moderate or heavy growth of pathogenic bacteria and presence of the same bacteria in Gram stain

Pathogen identified based on standard bacteriological procedures including Gram's stain, colony morphology on blood agar and Mac Conkey agar, and biochemical reactions. Non-lactose-fermenting, motile, oxidase positive, nitrate reducing, Gram-negative bacilli, with a characteristic sweet grape-like odour and distinctive blue-green pigment were identified *Pseudomonas aeruginosa*. Non-lactose-fermenting, non-motile, oxidase negative, nitrate non-reducing, Gram-negative coccobacilli, producing acid from glucose oxidatively, were identified as *Acinetobacter baumannii*. Oxidase negative, catalase positive, nitrate reducing, non-spore forming, Gram-negative bacilli, fermenting glucose and other carbohydrates, were considered as members of *Enterobacteriaceae*. Catalase-positive, mannitol fermenting, coagulase producing, Gram-positive cocci in clusters, with characteristic golden yellow pigment and hemolysis, were identified as *Staphylococcus aureus*.⁹ Antimicrobial susceptibility of the clinical isolates to routinely used antibiotics was determined by Kirby- Bauer disk diffusion method and Combination disc method is used for detection of MBL producer.

Detection of Metallobeta lactamase (MBL) :

All gram- negative bacilli (GNB) were tested for MBL production by a Combination disc method. This relies on inhibition of MBL by EDTA. Following inoculation of the test isolate on Mueller-Hinton agar plate to produce a semi-confluent growth, two discs are placed separately. This method involves use of two discs; One imipenem (10ug) disc and

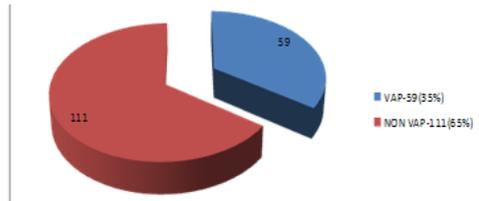
the other imipenem with an EDTA. An increase in the zone diameter around the imipenem with an EDTA by 4-5mm or above, indicate positive MBL production.



RESULT:

1. INCIDENCE

- Out of the 170 patients, 59 (35%) developed VAP during their ICU stay. The overall incidence of VAP was 26.97 per 1,000 ventilator days.(figure1)



(Figure 1: Incidence of VAP)

2. BACTERIAL ISOLATES FROM VAP

- Organisms were isolated in 59 cases of VAP. Gram positive cocci was seen in 3(5%) cases while 56(95%) cases showed gram negative bacilli. (figure 2)

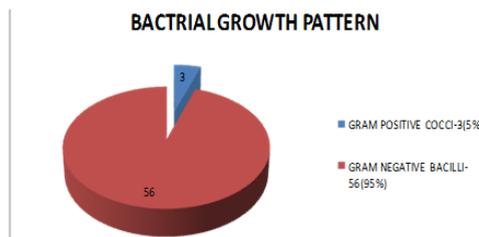
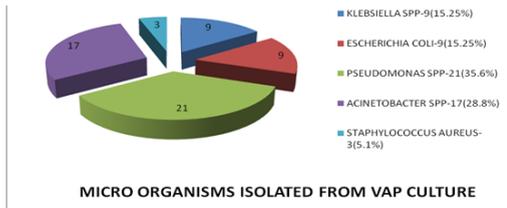


Figure 2: Bacterial Isolates In Diagnosed Cases Of VAP

3.MICRO- ORGANISMS ISOLATED FROM CULTURE

All patients who had evidence of VAP had microorganisms grown on culture.The most common organisms in VAP and non-VAP patients were *Pseudomonas spp.* followed by *Acinetobacter spp.*, *E.coli*, *Klebseilla spp.* and *Staphylococcus aureus*(figure 3).We have interpreted the results by detection of microorganisms in ET culture with clinical correlation like CPIS score.⁵⁻⁸



(Figure 3)

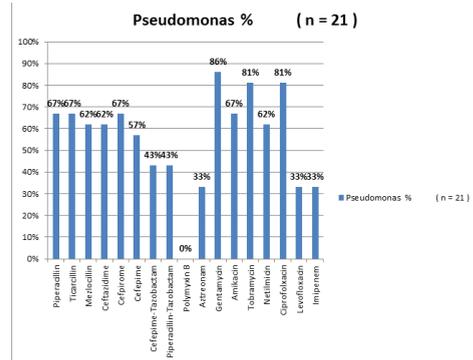
4 ANTIBIOTIC SENSITIVITY PATTERN AND RESISTANCE PATTERN

Study of antibiotic pattern for isolated bacteria is an important aspect for management and treatment. Treatment with sensitive antibiotic can change the course of disease and save life of at risk patient. Clinical microbiological correlation is important in our study. In our study antibiotic sensitive and resistant pattern is studied for all the groups of bacteria isolated.

Pseudomonas spp. Most sensitive antibiotic for *Pseudomonas* is Polymyxin B (100%) followed by Imipenem (71%), Aztreonam

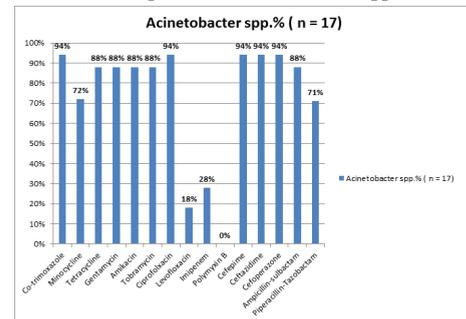
(67%), Levofloxacin (67%), Piperacillin-Tazobactam (57%) and Cefepime-Tazobactam(57%).

Antibiotic Resistance pattern of Pseudomonas spp



Acinetobacter spp. Most sensitive antibiotic for *Acinetobacter* is Polymyxin B (100%) followed by Levofloxacin (82%) and Imipenem (72%).

Antibiotic Resistance pattern of Acinetobacter spp.



5. INCIDENCE OF CARBAPENAMASE PRODUCER

Amongst the Carbapenamase producers *Pseudomonas* was the most common at 33.3% followed by *Acinetobacter*, *Klebsiella* and *E.coli*. (Table 2)

(Table :2Carbapenamase producer %)

Organisms	Carbapenamase producer %
<i>Pseudomonas spp.</i>	33.33%
<i>Acinetobacter spp.</i>	23.52%
<i>Escherichia coli</i>	11.11%
<i>Klebsiella spp.</i>	22.22%

DISCUSSION

Critically ill patients admitted to ICU benefit from close surveillance, cardiovascular monitoring and invasive devices such as mechanical ventilation, urinary bladder catheterisation and vascular access. Nosocomial infections are five times more common in ICU patients because of altered sensorium, presence of invasive devices and prior use of antibiotics in these patients.¹⁰The incidence of VAP (26.97 per 1,000 ventilator days) in our study was high, almost similar to another Indian study.¹¹ Studies from middle-income countries as Greece and from Middle East countries as Saudi Arabia and Lebanon, showed similar frequencies of VAP in multidisciplinary ICUs: 32–33.8% in the formers,¹² and 25.2–47% in the later.¹³ But in studies from other Asian countries the incidence rate is relatively less, ranging from 9 to 12 per 1,000 ventilator days.¹⁴⁻¹⁵ In our present study 59 out of 170 were VAP positive. 56(95%) out of 59 showed positive culture for gram negative organisms while 3(5%) out of 59 showed positive culture for gram positive cocci. Organism encountered was commonly *Pseudomonas spp.* 35.6% followed by *Acinetobacter Spp.* 28.8%, *E.Coli*15.25%, *Klebsiella* 15.25% and *S.aureus*5.1%. Our results were compared with few national and international studies as mentioned below (Table 3).

(Table 3: Results of different study)

Organisms	Fagon et al ¹⁷ (1984)	Torres et al (1990) ¹⁸	Rakshit P et al (2005) ¹¹	Sharma et al (2012) ¹⁶	Present Study
<i>Pseudomonas</i>	31%	28%	46%	31.48%	35.6%
<i>Acinetobacter Spp</i>	15%	24%	8%	1.85%	28.8%

<i>E.Coli</i>	8%	12%	12%	16.67%	15.25%
<i>Klebsiella</i>	4%	12%	29%	14.8%	15.25%
<i>Staph Aureus</i>	33%	20%	25%	9.26%	5.1%

Metallobetalactamases mediated resistance to carbapenems is an emerging threat in hospital isolates. Metallo beta lactamase enzymes belongs to Ambler class B. beta lactamase productivity is an important aspect of non-fermenters. It not only affects the treatment but mortality is also affected by it. In our study, among non-fermenters *Pseudomonas* is the most common metallobetalactamases producer which was statistically same as two different studies published.

(Table 4 : % of MBL detection)

Organisms	Ranjan N et al 2014 ¹⁹	Dey A et al 2007 ²⁰	PRESENT STUDY
<i>Acinetobacter</i>	20.83%	21.74%	23.52%
<i>Pseudomonas Spp</i>	27.18%	50%	33.33%

CONCLUSION

To conclude, VAP continues to be a major challenge to the critical care physicians in India. Causing pathogen and its antibiotic susceptibility pattern are essential for making antibiotic treatment choices available. Now a days carbapenems is an emerging threat in hospital isolates. It not only affects the treatment but mortality is also affected by it.

Funding source : This research study did not receive any specific funding, but it was performed during residency period (Dr.P.Gandhi.) as availability of sources in laboratory.

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