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Prevalence and antibacterial sensitivity pattern of bacterial isolates from endotracheal aspirate of ICU patient at tertiary care hospital

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Abstract
Introduction: Nosocomial infection is a most important health-care problem. Ventilator- associated pneumonia (VAP) remains the major cause of hospital morbidity and mortality in Intensive Care Unit (ICU) patients. During ventilation, the endotracheal tube (ETT) provides a direct channel for pathogenic bacteria to enter the lower respiratory tract and lung parenchyma. Several organisms are accounted for causing pneumonia and the study is aimed to see their antimicrobial susceptibility pattern.
Material & Methods: This study was done on all endotracheal aspirate and tube samples received in Department of microbiology, National Institute of Medical Sciences and Research, Jaipur over a period of 5 months from July 2018 to November 2018. Samples were received and processed as per the standard guidelines and antimicrobial susceptibility testing were done according to the standard CLSI guidelines.
Result: There were 52 endotracheal tube/aspirate Samples received in lab out of which 41(78.85%) samples showed significant growth with the rest samples either no growth or polymicrobial. There was major bacterial isolates Klebsiella pneumoniae (31.71 %), Escherichia coli (19.52%), Staphylococcus aureus (17.07%), Pseudomonas aeruginosa (17.07%), Citrobacter koseri (12.19%), Acinetobacter spp. (2.4%). Meropenem, Piperacillin/tazobactum, Vancomycin, Polymixin B, showed great sensitivity pattern while Cefepim, Ceftriaxone, Penicillin and Ampicillin showed high resistant pattern.
Conclusion: Current study will be very helpful in initiating proper Empirical treatment therapy of patients suffering from VAP and thus it will be helpful in reducing the morbidity rate and mortality rate.

Keywords: VAP, Klebsiella pneumoniae, meropenem, piperacillin/tazobactum, cefepime

Introduction

Nosocomial infection is a most important health-care problem. According to WHO 5-10% of hospitalized patients of developed countries and about 25% of developing countries were affected by nosocomial infection[1]. ventilator- associated pneumonia (VAP) remains the major cause of hospital morbidity and mortality in Intensive Care Unit (ICU) patients. VAP is the most frequent ICU acquired infection, occurring in 25% of patients intubated for longer than 48 h [2].

Early- onset VAP is usually less severe, associated with a better prognosis, and it caused by antibiotic- sensitive bacteria. Late- onset VAP is usually caused by multi- drug resistant (MDR) pathogens and is associated with increased morbidity and mortality [3].

During ventilation, the endotracheal tube (ETT) provides a direct channel for pathogenic bacteria to enter the lower respiratory tract and lung parenchyma. Where, soon after intubation of ETT bacteria can adhere and form a biofilm on the surface of the ETT Important procedure for life threatening conditions is Endotracheal intubation. The use of invasive therapeutic procedures has saved many lives but on the other hand it can also cause life threatening consequences due to severe persistent resistant infections and increase the Incidence of nosocomial infection, especially in ICU and CCU ward[4]. An Endotracheal Tube (ETT) is a flexible plastic tube, usually made of polyvinyl chloride (PVC), which is passed into trachea through the mouth (oral) or nose (nasal) to establish a patent airway and ensure adequate gaseous exchange or ventilation. It is also used to deliver or administer anesthetic gases to patients during surgery[5]. Endotracheal specimens or tracheal aspirates are usually done quantitatively in culture in order to determine the presence of pneumonia in ventilated patients admitted in most hospitals rather than bronchoalveolar lavage (BAL) or protected specimen brush (PSB) and are considered as reliable alternative compared to the latter specimens [6].

Several organisms are accounted for causing pneumonia. Common causative agents are that of Pseudomonas species, Acinetobacter species, Staphylococcus aureus, and Enterobacter including several endogenous bacteria. Up to 40% of these infections can be polymicrobial. The common problem however as noted in other studies is that bacterial pathogens from tracheal aspirates are multidrug-resistant type [7]. Antibiotic treatment using piperacillin-tazobactam has shown efficacy in lowering hospital mortality rate, so as with fluoroquinolones, amikacin, and carbapenems. Therefore, the local microbial flora causing VAP needs to be studied in each setting to guide more effective and rational utilization of antimicrobial agents [8].

Background information

Mainly Pneumonia is classified as in two types. They are community-acquired (CAP), healthcare-associated (HAP), or VAP. VAP is a sub-classification of HAP, if the patient is hospitalized during the period of mechanical ventilation. CAP is defined as pneumonia for which the first positive bacterial culture is obtained within 48 hours of admission to the hospital and the patient does not have risk factors for HAP. HCAP occurs when the patient's first positive bacterial

culture is obtained within 48 hours of admission [9].

An Endotracheal tube has inner and outer diameter. The inner diameter (ID) is the diameter of the tube lumen while the outer diameter (OD) measures the diameter of the lumen and the thickness of the tube. The size of the tube is determined by the inner diameter. For instance, if the inner diameter of a tube is 7.5mm, the tube is size 7.5. The normal length of ETT is different in oral and nasal rout for adult male is 23-26cm while that of adult female is 21-24cm [10].

Research hypotheses

1. Ventilator-Associated Pneumonia is common in patients presenting difficulties in breathing, having trauma and coma and those ones presenting severe febrile illness in NIMS Medical College and hospital, Jaipur.
2. Hospital Acquired Infections with the ability to resist several antibacterial agents are without doubt in the patients hospitalized in intensive care units of NIMS Medical college and hospital, Jaipur.

Material & methods

The present study was carried out in the department of microbiology of National Institute of Medical Sciences and Research, Shobha Nagar, Jaipur (Rajasthan). This was a cross-sectional observational study. The study was carried out during the period of July 2018 – November 2018, during this period, the samples was collected two times from all inserting ETT ventilated patients. First sample was collected admitted time and second sample was collected after 48 hours.

Endotracheal tube/aspirate samples were inoculated on Glucose broth, Blood agar and MacConkey agar plates (Hi Media labs Ltd.) and incubated at 37^0C for 24 hours using a nichrome wire loop. When no growth seen on culture plates after 24 hours but turbidity seen in the Glucose broth than again sub cultute on Blood agar and Mac Conkey agar plate from glucose broth. Fisrt sample was sterile and second sample was grown microbial growth on culture plates was considered significant and first and second samples different microbial growth was also considered significant. Polymicrobial growth was not considered. The culture isolates were further identified by their morphologies and biochemical characteristics.

Antibiotic susceptibility testing was done by Modified Kirby Bauer's disc diffusion method as per CLSI guidelines 2017 using commercially available discs. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains[11].

Results

A total of 52 endotracheal tube/aspirate samples were obtained from ICUs patients of NIMS Hospital and processed in National institute of medical sciences and research medical college. Among them 40 (76.93%) were males and 12 (23.07%) were female patients. Out of 52 samples 41(78.85%) samples showed significant growth and remaining 11(21.15%) samples were either sterile or polymicrobial growth. Patient developing VAP within 96 hours of mechanical ventilation were categorized as having "Early-onset VAP" and those who developed after 96 hours were classified as "Late-onset VAP". Out of the 41 VAP cases; 12(29.26%) were categorized under the early-onset group and the remaining 29(70.73%) under the late onset group. In the present study, major bacterial isolates was Klebsiella pneumoniae (31.71 %), followed by, Escherichia coli (19.52%), Staphylococcus aureus (17.07%), Pseudomonas aeruginosa (17.07%), Citrobacter koseri (12.19%), Acinetobacter spp. (2.4%).

Among the all bacterial isolates in endotracheal tube/aspirate Klebsiella pneumoniae showed highly sensitive to Meropenem (92.30%), piperacillin/tazobactum (92.30%). Followed by Gentamycin (69.23%), Imipenem (69.23%), ceftriaxone (53.84%), (46.16%) Amikacin, azteronam(38.47%), Cefexime (30.77%), Ciprofloxacin(15.39%), and cefepime (15.39%) showed lesser sensitivity for Klebsiella pneumoniae.

Escherichia coli were found (62.50%) sensitivity to Amikacin, Meropenem (87.50%), Gentamycin (62.50%), Imipenem (69.23%), piperacillin/tazobactum (92.30%). Escherichia coli was highly sensitive to piperacillin/tazobactum (92.30%) and lesser sensitive was seen to azteronam (37.50%), Cefexime (25%), Ciprofloxacin (25%), cefepime (25%), ceftriaxone (12.50%).

Staphylococcus aureus were found (16.67%) sensitivity to Amikacin, Ampicilin(33.33%), Chloramphenicol (83.33%), cifoxitin(33.33%), Clindamycin(66.67%), Erythromycin(16.67%), Linezolid(100%), Vancomycin(100%), Penicillin G (0%), Gentamycin(50%), Tetracycline(33.33%).

Pseudomonas aeruginosa were found (50%) sensitivity to Amikacin, azteronam(75%), Polymixin B(100%), Colisitin (100%), Ciprofloxacin (25%), cefepime (25%), Meropenem (50%), Ofloxacin (0%), Caftazidime(25%), Gentamycin (50%), Imipenem (25%), piperacillin(50%), piperacillin/tazobactum (75%), Tobramycin (75%), Cefotaxime(0%), Levofloxacin (50%), Chloramphenicol (75%).

Citrobacter koseri were found highly sensitivity to Gentamycin (80%), piperacillin/tazobactum (80%), Meropenem (80%), followed by (40%) Amikacin, azteronam(40%), Imipenem (40%), Ciprofloxacin(20%), cefepime (20%), Cefexime (40%), ceftriaxone (20%).

Acinetobacter spp. were found highly sensitivity to piperacillin/tazobactum (80%),followed by Imipenem (60%), Amikacin (40%), Gentamycin (40%), Meropenem (40%). and lowest sensitive to azteronam(20%), cefepime (20%), Cefexime (20%), ceftriaxone (20%), Ciprofloxacin(0%).

Table 1: Distribution of samples by sex, sterile, polymicrobial and significant growth.

Sex	Significant	Polymicrobial	Sterile
Male	34	4	2
Female	7	3	2
Total	41	7	4

Table 2: Onset of VAP.

Onset	Number (n=41)	Persentage
Early	12	29.26%
Late	29	70.74%

Table 3: Frequency of bacterial isolets in endotracheal tube/aspirate.

Isolates	Number of isolates in positive samples. (N=41)	Percentage
Klebsiella pneumoniae	13	31.71%
Escherichia coli	8	19.51%
Staphylococcus aureus	6	14.63%
Pseudomonas aeruginosa	4	9.75%
Citrobacter koseri	5	12.20%
Acinetobacter spp.	5	12.20%

Discussion

In this study, all the positive colonies obtained from the endotracheal tube/aspirate were considered. In this study, the incidence of positivity was 78.84% (41 out of 52 cultures). In the study done by Ghosh B *et al*. presence of positivity was 50.09% (271 positive case out of 541 total cases) [12]. Simoni *et al*[13]. showed that 100% of samples from airway prosthesis are positive in culture; however, other studies have reported a positive culture rate between 0% and 33% in obtained samples from airway tubes. The variation could be explained by the technique of intubation, clinical and individual characteristics of study population, colonization during intubation or lack of sufficient precautions for intubation due to the high work load in an emergency setting.

In this study incidence of positivity in males was 82.93% which was significant as compared to females (17.07%), which was similar to Ghosh B *et al*. [12]

In this study out of the 41 cases of VAP, 29.26% were early-onset and 70.73 were categrorised as late onset. Similar result were obtained by Mukhopadhyay *et al*. [203] with 38% being early-onset and 62% late-onset VAP [14].

In this study, 85.36% Gram negative bacteria and 14.63% Gram positive bacteria were isolated. Incidence of prevalent bacteria in this study were Klebsiella pneumoniae (31.71 %), Escherichia coli (19.52%), Staphylococcus aureus (17.07%), Pseudomonas aeruginosa (17.07%), Citrobacter koseri (12.19%), Acinetobacter spp. (2.4%) So incidence of Klebsiella pneumoniae was highest followed by Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. Which is similar to Ghosh *et al*. [12]

In this study, Klebsiella were highly sensitive to Piperacillin/tazobactum and Meropenem (92.30%) and Ciprofloxacin and cefepime (15.29%) highly resistant which was similar to the study done by Geetanjali Panda *et al*. [15]

In this study, Staphylococcus aureus was highly sensitive to Vancomycin and Linezolid (100%) and moderately sensitive to Chloramphenicol 83.33% & Clindamycin 66.67% but 50% sensitive to Gentamycin. While Amikacin 83.33%, Erythromycin 83.33% and Penicillin-G 100% were resistant in present study which is similar to Geetanjali Panda *et al*[15].

In this study E.coli were showing highly sensitive to piperacillin-tazobactam (92.30%), Meropenem (87.50%) and moderate sensitive to Gentamycin (62.50%), Imipenem (69.23%) while Ciprofloxacin, Cefexime and Cefepime (75%) were resistant in present study, When it was compared with Haque L *et al* (2013)[16] the result was similar, E coli were highly sensitive to Gentamycin, piperacillin-tazobactam, Meropenem.

In this study, Acinetobacter species was highly sensitive to piperacillin-tazobactam (80%) and moderately sensitive to Imipenem (60%), but 20% sensitive to Azetronem and Cifepime and 40% to Gentamycin. While Amikacin 60%, Ceftriaxone 80% and Ciprofloxacin 100% were resistant in present study when compared by the study of Hoque *et al* (2013)[16], Acinetobacter was 100% sensitive to colistin but 100% resistant ceftriaxone and Amikacin were similar.

Conclusion

This study provides a baseline data of current scenario of VAP in our set up which can be utilized to formulate infection control strategies. An ongoing study would be beneficial to maintain a track of the VAP rates. There is also a need for many more hospital based prospective studies in our country to prevent these infections in intensive care settings.

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