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Original Article

Antibiotic Resistance Pattern among Bacteria causing Ventilator Associated Pneumonia in An Intensive Care Unit of Bangladesh

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Abstract

Background : *Ventilator-associated pneumonia (VAP) is the most common type of nosocomial infection in critical care practice with high morbidity and mortality. Microorganisms responsible for VAP vary from place to place. So, identification of causative organism and knowledge of their resistance pattern is very important for empirical choice of antibiotic in managing VAP. The aim of this survey was to evaluate the quantitative cultures of endotracheal aspirates to determine the microorganisms responsible for VAP and to study their antibiotic resistance pattern.*

Materials and Methods: *This cross sectional study was performed over a period of six month starting from November, 2015 to April, 2016 in the Intensive Care Unit (ICU) of BIRDEM General Hospital. Patients with a clinical and radiological diagnosis of VAP were included in this study.*

Result: *A total of 51 patients with a clinical diagnosis of VAP were included in this study. Growth was obtained in100% of the samples yielding 88 organisms. Gram-negative organisms were the mostly isolated organism (76.13%), followed by fungi (17.04%) and gram-positive cocci (6.81%). The most common pathogen was Acinetobacter sp. followed by Klebsiella sp., Candida sp. and Pseudomonas sp. respectively. Among the gram negative organisms, Acinetobacter sp., Klebsiella sp. and Pseudomonas sp. were highly resistant (>80%) to third generation cephalosporins and fluoroquinolones. Resistance to aminoglycosides (>68%) and imipenem (>60%) was also high. Resistance of Pseudomonas sp. to piperacillin-tazobactum was lower (18.2%) in comparison to Acinetobacter sp. and Klebsiella sp. All the Gram-negative organisms were 100% sensitive to colistin except proteus. Regarding gram-positive cocci,Staphylococcus aureus is 100% sensitive to netilmycin and vancomycin with variable resistance pattern to other antibiotics.*

Conclusion: *Emergence of drug resistance against the microorganism causing VAP is a serious concern in most of the ICUs. A knowledge of antibiotic susceptibility pattern will avoid its irrational use in order to control the spread of infection and for proper management of VAP.*

Key Words: *multidrug-resistant organisms, ventilator-associated pneumonia, Intensive care unit*

Introduction:

Although intensive care units (ICUs) account for fewer than ten percent of total beds in most hospitals, more than 20 percent of all nosocomial infections are acquired in ICUs.[1] The incidence of nosocomial infections in ICUs is showing a rising trend mainly because of invasive procedures performed in ICU. Ventilator-associated pneumonia (VAP) is the pneumonia occurring in patients 48 hours after endotracheal intubation and mechanical ventilation. It is the most common cause of hospital-acquired infections among patients admitted in ICU.[2] The incidence of VAP ranges from 6.8% to 44% and its occurrence is associated with increased length of hospital stay, mortality, and financial burden.[3] The pathogenesis of VAP is related to the number and virulence of microorganisms entering the lower respiratory tract and the response of the host. VAP may be caused by a wide variety of pathogens including multidrug resistant (MDR) organisms and can be polymicrobial. The pattern of microorganisms especially MDR pathogens varies among hospitals, specific hospital units, and patient populations including those with recent exposure to antibiotics. Most common bacterial agents of lower respiratory tract infection (LRTI) in the ICU are *Pseudomonas*, *Acinetobacter*, *Klebsiella*, *Citrobacter*, and *Escherichia coli*.[4-6] In almost all cases, there is a need to initiate empirical antimicrobial treatment before obtaining the microbial results, but the situation is further complicated by the emergence of multiple beta lactamase producers and multidrug resistant pathogens. In a recent report, Infectious Disease Society of America (IDSA), specifically addressed three categories of gram negative bacilli (GNB), namely extended spectrum beta lactamase (ESBL) producing *Escherichia coli*, and *Klebsiella* spp., Multidrug resistant (MDR) *Pseudomonas*, and carbapenem resistant *Acinetobacter* spp., as high priority bacterial pathogens.[7] So early and appropriate diagnosis is very important to reduce the incidence of VAP particularly to reduce the frequency of MDR pathogen. An awareness of the susceptibility patterns of the nosocomial pathogens within a given healthcare setting is also important for appropriate empiric antimicrobial therapy.

Materials and Methods:

This cross sectional study was performed over a period of six month starting from November, 2015 to April, 2016 in the ICU of Department of Critical Care Medicine, BIRDEM General Hospital which is a tertiary care hospital.

Mechanically ventilated patients who developed VAP during the study period were included in the study. VAP is defined as pneumonia that occurs 48 to 72 hours or thereafter following endotracheal intubation, characterized by the presence of a new or progressive infiltrate, signs of systemic infection, changes in sputum characteristics, and detection of a causative agent.[8] The diagnosis of VAP was established on the basis of clinical and radiological parameters as per Centers of Disease Control and Prevention (CDC) guidelines.[9] Patients who did not fulfill the criteria of VAP were excluded from the study.

Endotracheal aspirate (ETA) was collected as per the method described by Deyet al.[10] An acceptable ETA had <10 squamous epithelial cells/low power field or organisms seen under oil immersion (1000x) in the Gram stain. Presence of 25 or more polymorphonuclear leucocytes per 100x field, together with few squamous epithelial cells implied an excellent specimen.[11] Cultures were carried out by semi-quantitative method.[12] Bacterial isolates were identified by conventional standard technique.[13] Colony Count $\geq 10^5$cfu/ml was consistent with the definitive diagnosis of VAP and $< 10^5$cfu/ml due to colonisation or contamination. Antibiotic sensitivity was performed by Kirby Bauer's disc diffusion method as per Clinical and Laboratory Standards Institute (CLSI) guidelines 2015.[14] Extended spectrum beta lactamase (ESBL) testing was performed by combination disc method. All test data were analyzed by the WHONET 5 software.

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Results:

A total of 51 patients with a clinical and radiological diagnosis of VAP were included in this study. Total of 51 samples of tracheal aspirates were analyzed. Growth was obtained in100% of the samples yielding 88 organisms as there was growth of more than one organism in some samples. Gram-negative organisms were the mostly isolated organism (76.13%), followed by fungi (17.04%) and gram-positive cocci (6.81%). Among them, the most common pathogen was *Acinetobacter sp.* followed by *Klebsiella sp., Candida sp.* and *Pseudomonas sp.* respectively which is shown in figure 1. All the fungal isolates were found in conjunction with growth of bacteria

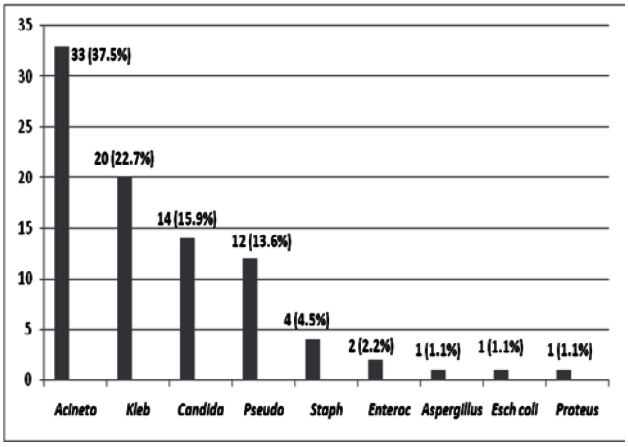

Fig 1: Pattern of organisms causing VAP (% showed in bracket)

Bacteria abbreviation: *Acineto= Acinetobacter, Enteroc= Enterococci, Esch coli= Eschericia coli, Kleb= Klebsiella, Pseudo= Pseudomonas, Staph=Staphylococus aureus*

The antibiograms of major isolates are shown in Table I and II. Among the gram negative organisms, *Acinetobacter, Klebsiella* and *Pseudomonas* were highly resistant to third generation cephalosporins (>80%) and fluoroquinolones (>80%). They were also resistant to aminoglycosides in >68% cases. Resistance of *Acinetobacter, Klebsiella* and *Pseudomonas* to Imipenem were 87.9%, 60% and 90% respectively. Resistance of *Pseudomonas* to piperacillin-tazobactum was lower (18.2%) in comparison to *Acinetobacter* and *Klebsiella* which were higher. *Eschericia coli* was 100% sensitive to cephalosporin, aminoglycosides, imipenem, piperacillin-tazobactum and colistin but was 100% resistant to quinolone group. All the Gram-negative organisms were 100% sensitive to colistin except Proteus. Though there was no ESBL positive *Eschericia coli,* 25% of isolated *Klebsiella* were ESBL positive.

Regarding gram-positive cocci, *Staphylococcus aureus* was 100% sensitive to netilmicin and vancomycin with variable resistance pattern to other antibiotics (Table II). 25% of the isolated *Staphylococcus aureus* were methicillin resistant.

Table I: Antibiotic Resistance of Isolated Gram Negative Organisms (shown in %)

	Acinetobacter (n=33)	*Klebsiella* (n=20)	*Pseudomonas* (n=12)	*Esch coli* (n=1)	*Proteus* (n=1)
Amikacin	90.9	70	81.8	0	100
Gentamicin	90.9	75	90.9	0	100
Netilmicin	83.9	68.4	81.8	0	-
Aztreonam	96.6	100	71.4	0	0
Cefixime	96.9	100	100	0	0
Cefotaxime	97	100	100	0	0
Ceftriaxone	97	100	100	0	0
Ceftazidime	97	100	81.8	0	0
Ciprofloxacin	93.5	94.4	81.8	100	100
Cotrimoxazole	90.9	80	90.9	100	100
Imipenem	87.9	60	90.9	0	100
Piperacillin +Tazobactum	84.8	63.2	18.2	0	100
Colistin	0	0	0	0	100
Tigecycline	30.8	33.3	-	0	

-Bacterial abbreviation: *Esch coli= Eschericia coli*

Table II: Antibiotic Resistance of Major Gram Positive Organisms (shown in %)

	Staphylococcus aureus **(n=4)**	***Enterococci*** **(n=2)**
Amoxicillin	33.3	-
Oxacillin	25	-
Erythromycin	50	-
Amikacin	25	100
Gentamicin	25	100
Netilmicin	0	100
Ceftazidim	-	100
Ciprofloxacin	100	100
Levofloxacin	33.3	-
Cotrimoxazole	25	100
Rifampicin	25	-
Vancomycin	0	0

Discussion:

ICU is the place where specialized care is given to critically ill patients. Most of the critically ill patients who have a number of comorbidities often require endotracheal intubation and mechanical ventilation. This bypasses natural barriers of respiratory tract and allow microorganism to enter the respiratory system causing infection particularly VAP.

In our study 51 tracheal aspirates from 51 patients with a clinical diagnosis of VAP were analyzed and a total of 88 pathogenic microbial strains were isolated from these samples (fig 1). The frequency of pathogens causing VAP may vary by hospital, patient population, exposure to antibiotics, types of ICU patient, and changes over time. This emphasizes the need for timely and routinely local surveillance data.[8]

Predominant organisms isolated were gram-negative organisms (76.13%), followed by fungi (17.04%) and gram-positive cocci (6.81%). Most common pathogen isolated was *Acinetobacter* (37.5%) followed by *Klebsiella, Candida* and *Pseudomonas* respectively which is shown in Table I. Ali S et al conducted a study which showed major pathogenic bacteria causing VAP were gram negative (74%).[15] In his study, *Eschericia coli, Pseudomonas, Klebsiella* and *Acinetobacter* were the commonest organisms. The predominant bacterial isolates reported in the Jordanian, Indian and European studies were similar to our results.[16-18] The type of organism causing VAP depends on the duration of mechanical ventilation. Early-onset VAP which develops within 2 to 5 days of mechanical ventilation, is usually caused by *Streptococcus pneumoniae, Hemophilus influenza,* methicillin-sensitive *Staphylococus aureus* (MSSA), antibiotic sensitive *Eschericia coli, Klebsiella pneumoniae, Enterobacter sp, Proteus sp.* and *Serratia marcescens.*[19] Late-onset VAP develops after 5 to 7 days of mechanical ventilation. Culprits of late VAP are methicillin-resistant *Staphylococcus aureus* (MRSA)*, Acinetobacter sp, Pseudomonas aeruginosa,* extended-spectrum beta-lactamase producing bacteria (ESBL).[20] However this is by no means a rule.[19] In the current study, we did sample analysis from VAP patients. They were not categorized as early-onset or late-onset VAP. Findings of this study suggested that most of the isolates were bacteria causing late VAP.

Respiratory samples can be obtained using several techniques like non-invasive ETA, broncho-alveolar lavage (BAL) with bronchoscopic guidance, mini-BAL without bronchoscopy, and protected specimen brush (PSB). IDSA and ATS recommend the non-invasive sampling with semi-quantitative cultures to diagnose VAP, rather than invasive sampling.[21] This was the reason for us to do ETA sampling for VAP patients in our ICU.

Antimicrobial resistance is an increasingly emerging problem worldwide, especially in ICUs. In this study, we found 97%, 100%, and 81.8% resistance of ceftazidime to *Acinetobacter sp, Klebsiella sp* and *Pseudomonas sp* respectively. Goel N et al. in his study noticed 100%, 96.9%, and 68.4% resistance to ceftazidime against *A. baumannii*, *Klebsiella sp* and *P. aeruginosa* respectively.[22] Similar observations were made by other investigators that reported 96 to 100% resistance.[23,24] High rate of resistance at our center might be due to the injudicious use of third generation cephalosporins.

Carbapenems are frequently used drug in treating serious

infections caused by GNB. In our study, 87.9% isolates of *Acinetobacter sp*, 90.9% isolates of *Pseudomonas sp.* and 60% isolates of *Klebsiella sp*., were resistant to imipenem in contrast to another study, where imipenem resistance was found in 14.2% isolates of *A*. *baumannii* and 12 to 42.5% isolates of *P*. *aeruginosa*, respectively.[25,26] Another study reported 100% sensitivity to carbapenem against *Klebsiella sp*.[27] The reason behind this high resistance in our study is extensive use of carbapenem. This finding suggests that carbapenem should be used judiciously in ventilated patients to prevent any further increase in resistance to carbapenem.

Another important observation of our study is all the gram-negative organisms were 100% sensitive to colistin except *Proteus sp*; and resistance of *Acinetobacter sp* and *Klebsiella sp* to tigecycline was 30.8% and 33.3% respectively. Present study also showed that *Staphylococcus aureus* was 100% sensitive to netilmicin and vancomycin. This high sensitivity for tigecycline and colistin against gram-negative isolates, and vancomycin and netilmicin for *Staphylococcus aureus* may be so because these drugs are reserved as second-line of antibiotic therapy.

Our data showed that the proportion of fungal isolates was high (17.04%) in our ICU and took the third position before *Pseudomonas sp* and gram positive cocci. *Candida sp* is the main fungal isolates in ETA of mechanically ventilated patients in our ICU and this might be due to presence of diabetes mellitus and the use of steroids and broad spectrum antibiotics. Growth of *Candida sp* from respiratory secretions usually indicate colonization and rarely required treatment.[28]

Our limitation was that we did not categorize the patients as early-onset and late-onset VAP. Further large scale study may be done with categorization of the patients suffering from VAP, their risk factors assessment and outcome of the patients suffering from VAP.

Conclusions:

The emergence of antibiotic resistance against many microorganisms causing VAP is a matter of serious concern in this study. This high rate of resistance also demonstrates the need for antibiotic stewardship protocol to be set up in health facilities. Regular surveillance of antibiotic susceptibility patterns is very important to prevent multi-resistant bacterial infections. A knowledge of the antibiotic susceptibility of the organisms isolated in the ICU helps to formulate an antibiotic policy for the ICU. This will also avoid unnecessary use of broad spectrum antibiotics and will guide the clinicians in choosing empirical therapy of infected patients and prevent emergence of drug resistant bacterial strains.

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