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Original Research Article

Detection and Antimicrobial Susceptibility Pattern of Bacteria Isolated from Ventilator Associated Pneumonia in Intensive Care Unit Patients in Rajshahi Medical College Hospital

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Abstract: ***Background*:** Ventilator associated pneumonia is a major cause of higher morbidity and mortality among hospitalized patients especially in intensive care unit despite of recent advances in diagnosis and treatment. It occurs mainly among Gram negative pathogens. Most of the bacteria are multidrug resistant bacteria including penicillin's, 3rd generation of cephalosporins and carbapenems. ***Objective:*** To isolate and identify bacteria from ventilator associated pneumonia in intensive care unit patients with their antibiogram in rajshahi medical college hospital. ***Materials Method:*** A Cross-sectional type of descriptive study was done during the period of July 2017 to June 2018. Endotracheal aspirates were collected from VAP patients in intensive care units of Rajshahi Medical College Hospital. The specimens were inoculated in blood agar, nutrient agar and MacConkey's agar media and incubated aerobically at 370 C for 24 hours. The isolated bacteria were identified by their colony morphology, pigment production, hemolysis on blood agar plate, motility test, Gram staining and relevant biochemical tests. Susceptibility tests of the bacterial isolates were done by using the modified Kirby Bauer disk diffusion method on Mueller Hinton agar media. ***Results:*** Out of a total 80 samples, Culture yielded growth were 71(88.75%) and 09(11.25%) had yielded no growth. Among the culture positive isolates, Gram negative organisms were higher 57(80.30%) than gram positive 14(19.70%). P. aeruginosa 24(33.8%) was the predominant organism followed by S. aureus 14(19.7%), Klebsiella spp. 11(15.5%), Acinetobacter spp. 10(14.1%) and E. coli 8(11.3%). Among 71 isolates, 41(57.8%) were MDR pathogens. ***Conclusion:*** It may be concluded that, most of the isolated bacteria isolated from VAP are multidrug resistant and causes complicated life-threatening infections. Due to the increasing incidence of multidrug resistant bacteria in ICU, early and correct diagnosis of VAP is an urgent challenge for an optimal antibiotic treatment.

Keywords: Ventilator Associated Pneumonia, Endotracheal Aspirates, Intensive Care Unit, Antimicrobial Susceptibility Pattern, Multidrug Resistant Bacteria.

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Introduction

Ventilator associated pneumonia is defined as pneumonia that arise more than 48 hours after initiation of mechanical ventilation by tracheostomy or endotracheal intubation. Ventilator associated pneumonia is the most common nosocomial infection in the ICU contributes disproportionately to both poor outcomes and high cost of care in critically ill patients.

VAP is estimated to occur in 9-27% of all mechanically ventilated patients with the highest risk being early in the course of hospitalization and mortality ranges between 20-50% and may reach more than 70% when the infection is caused by multidrug resistant and invasive pathogens. Timely diagnosis and prognostic assessment of VAP remain major challenges in critical care.[1, 2]

The incidence rates of VAP ranges in Bangladesh 10-40%%, in India 11-25%, in Italy 4-16%, and in USA 2-16% respectively.[3, 4] The high rate of respiratory infection due to gram negative bacilli occurs in most VAP cases. Several studies have reported that more than 60% of VAP is caused by aerobic gram-negative bacilli (Joseph). Pathogens causing VAP and their percentage are Pseudomonas aeruginosa (24.4%), Staphylococcus aureus (20.4%), Enterobacteriaceae (34.1%), Acinetobacter spp. (9.8%) and others (11.3%). In another study organisms associated with VAP include the following Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter spp. and Enterobacteriaceae.[5, 6] Regarding the antimicrobial resistance rates of VAP patients of ICU in Bangladesh to third generation cephalosporins 80%-100%, to fluroquinolones, aminoglycosides, monobactam 60%-80% and to carbapenem 10%-30% respectively.[7] Therefore, the local bacterial agents causing VAP needs to be studied in ICU to guide more effective and rational utilization of antimicrobial agents. To produce an effective empirical antibiotic protocol on VAP in ICU, knowledge of common organisms and their sensitivity pattern is essential.[8]

Methods

Antimicrobial susceptibility of 71 bacterial isolates of endotracheal aspirates was collected from VAP patients in intensive care units were analysed in the present study. Aerobic culture and sensitivity tests were done in the Microbiology department of Rajshahi Medical College. All the specimens were inoculated in blood agar, nutrient agar and MacConkey's agar media and incubated aerobically at 370 C overnight. If culture plates showed the growth of bacteria, then it was identified by their colony morphology, pigment production, hemolysis on blood agar plate, motility test, Gram staining and relevant biochemical tests. The identified bacteria were sub cultured and processed for drug sensitivity test. Susceptibility tests of the bacterial isolates with different antimicrobials were done by using the modified Kirby Bauer disk diffusion method on Mueller Hinton agar media by commercially available antimicrobial disks.[9]

Results

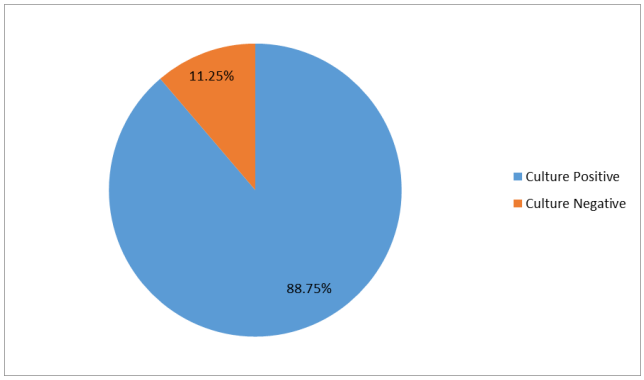

Figure 1: Frequency of Culture Positive and Negative Cases (N=80)

Figure 1 shows culture positivity of isolated organisms. Out of 80 samples, 71(88.75%) samples were culture positive while 09(11.25%) samples were culture negative.

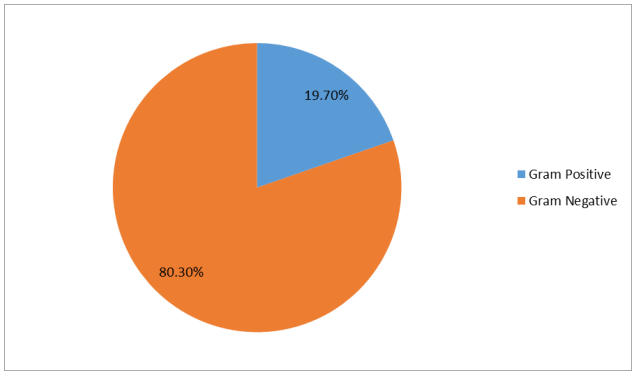

Figure 2: Frequency of Gram Positive and Gram-Negative Bacteria (N=71)

Figure 2 shows the distribution of gram-positive and gram-negative isolate among culture positive cases. Among the total 71 isolates, Gram negative bacteria predominated 57(80.3%) and gram-positive bacteria were 14(19.7%).

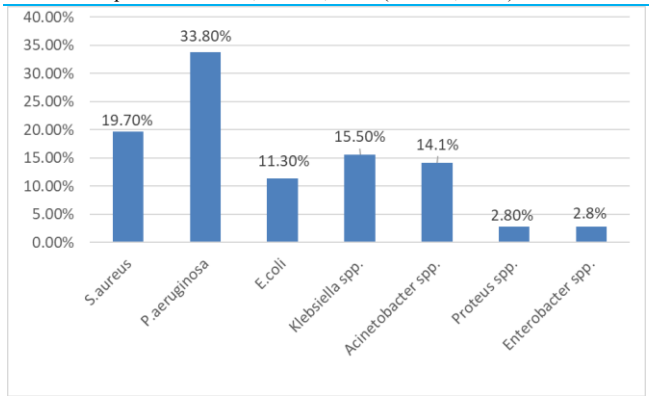

Figure 3: Pattern of Bacteria Isolated from VAP (N=71)

Figure 3 shows the identified species of bacteria from endotracheal aspirates. Out of 80 samples, total 71 bacteria were identified. P. aeruginosa was 24(33.8%) followed by S. aureus was 14(19.7%), Acinetobacter spp. was 10(14.1%), Klebsiella spp. was 11 (15.5%) and E. coli was 8(11.3%).

Table 1: Antimicrobial Resistance Pattern Pseudomonas Aeruginosa, Enterobacteriaceae, Acinetobacter Spp. and S. Aureus.

Antimicrobial agents	**Pseudomonas aeruginosa (n=24)**	**Enterobacteriaceae (n=23)**	**Acinetobacter spp. (n=10)**	**S.aureus (n=14)**
Imipenem	04(16.7%)	04(17.4%)	2(20%)	2(14.2%)
Azithromycin	18(75%)	19(82.6%)	9(90%)	12(85.7%)
Ciprofloxacin	16(66.7%)	13(56.5%)	7(70%)	9(64.3%)
Ceftriaxone	22(91.6%)	22(95.6%)	10(100%)	12(85.7%)
Cefepime	13(54.2%)	14(60.9%)	9(90%)	
Piperacillin/tazobactam	9(37.5%)	11(47.8%)	7(70%)	
Meropenem	8(33.3%)	9(39.1%)	4(40%)	4(28.4%)
Aztreonam	12(50%)	10(43.5%)	8(80%)	
Amikacin	14(58.3%)	12(52.2%)	6(60%)	7(50%)
Cefuroxime	23(95.8%)	22(95.6%)	10(100%)	12(85.7%)
Colistin	02(8.3%)	02(8.7%)	2(20%)	
Levofloxacin	9(37.5%)	8(34.8%)	5(50%)	5(35.7%)
Amoxiclav	17(70.8%)	16(69.6%)	8(80%)	10(71.4%)
Linezolid				2(14.2%)
Vancomycin				00

Table 1 shows the antimicrobial resistance pattern among Pseudomonas aeruginosa, Enterobacteriaceae, Acinetobacter spp. and S. aureus. Gram negative bacteria were highly resistant against ceftriaxone, cefuroxime, azithromycin, amoxiclav and ciprofloxacin. Colistin, imipenem and meropenem were showed lower resistance against gram negative bacteria. S. aureus was highly resistant against ceftriaxone, cefuroxime, azithromycin, amoxiclav and ciprofloxacin. Vancomycin, Linezolid and imipenem were showed lower resistance against S. aureus.

Table 2: Frequency of Multidrug Resistant Bacteria (N=71).

Organisms	**Total isolates**	**MDR isolates (%)**
Gram negative bacteria	57	33(57.9%)
Staphylococcus aureus	14	08(57.1%)

Table 2 shows multi drug resistance pattern among isolated gram-negative bacteria and *Staphylococcus aureus.* Among 57 isolated gram-negative bacteria

33(57.9%) and among 14 isolated *Staphylococcus aureus* 08(57.1%) were MDR pathogens.

Discussion

Out of 80 endotracheal aspirates samples obtained in the Microbiology lab from ICU department of RMCH, Rajshahi for aerobic culture and sensitivity, 88.75% yielded positive culture whereas 11.25% yielded no growth. Study is nearly similar with the study of Nusrat *et al*. and De *et al*. Study is nearly dissimilar with the study of Shohid *et al*. and Sarkar *et al*.[7, 10-12] The incidence of nosocomial infections in ICUs is showing a rising trend mainly because of invasive procedures performed in ICU, cross infection, inadequate measures for prevention of the spread of resistant pathogen in hospital environment. In this study, out of a total 80 samples, gram negative bacteria accounted for higher isolation rate (Gram-negative 80.3% and Gram- positive 19.7%) than gram positive bacteria. This study is nearly similar with the study of Shohid *et al*. and Saha *et al*. Study is nearly dissimilar with the study of Sarkar *et al*. and Kanipakam *et al*.[7, 12-14] The cause of predominant isolation rate of the gram-negative organism among hospitalized patient might be due to selective pressure of broad-spectrum antibiotics causing persistent of drug resistance genes/ plasmids. Another possible explanation of the predominance of gram-negative bacteria is that asymptomatic colonization of patients, the contaminated environment or both can serve as reservoirs for these pathogens, which are then transmitted by the hands of health care workers.

In this study, P. aeruginosa is the most frequent isolates 24(33.8%). Study is similar with the study of Shohid *et al*. and Sohal *et al*.[7, 15] Finding is dissimilar with Ahsan *et al*. and Selina *et al*.[2, 6] The high prevalence of P. aeruginosa may be because it is an opportunistic pathogen and one of the leading causes of nosocomial infection. It causes severe airway infections and while colonizing the human airways P. aeruginosa could acquire genetic mutations that often lead to its better adaptability to the host environment and develops resistant to commonly used antimicrobial agents. Antimicrobial resistance pattern among Pseudomonas aeruginosa, Enterobacteriaceae, Acinetobacter spp. and S. aureus. Gram negative bacteria were highly resistant against ceftriaxone, cefuroxime, azithromycin, amoxiclav and ciprofloxacin. Colistin, imipenem and meropenem were showed lower resistance against gram negative bacteria. This study is nearly similar with Shohid *et al*. and Saha *et al*.[7, 13] Study is nearly dissimilar with the study of Ahsan *et al*. and Kanipakam *et al*.[6, 14] S. aureus was highly resistant against ceftriaxone, cefuroxime, azithromycin, amoxiclav and ciprofloxacin. Vancomycin, Linezolid and imipenem were showed lower resistance against S.aureus. This study is nearly similar with Shohid *et al*. and Ahmed *et al*.7, 16 Study is nearly dissimilar with the study of Ahsan *et al.* and Saha *et al*.[6, 13] The overall MDR among gram positive and gram-negative bacterial isolates were 57.1% and 57.9% respectively. In this study, among 57 isolated gram-negative bacteria 33(57.9%) were identified as MDR bacteria. This study was nearly similar with the study of Badhon *et al*. and De *et al*. but nearly dissimilar with the study of Shohid *et al*. and Mathai *et al*.[7, 11, 17, 18] Among 14 isolated gram-positive bacteria 08(57.1%) were identified as MDR bacteria. This study was nearly similar with the study of Selina *et al*. and Krishnamurthy *et al*. but nearly dissimilar with the study of Shohid *et al*. and Joseph *et al*.[2, 7, 19, 20] This variation may be due to multifactorial causes such as geographical location, presence of many systemic diseases, longer stay in hospital and duration of ventilation. Due to arbitrary use of antibiotics common pathogen develops resistance against frequently used drugs. In ICU use of third generation antibiotics to treat the severely ill patient. VAP patients are developing resistance against these third-generation antibiotics which is quiet alarming. The rational use of antibiotics to defeat this emerging situation and to use specific drug for the VAP patients according to the microbiological report.

Ethical Approval: Ethical clearance for the study was taken from the Instutional Review Board and concerned authority, Rajshahi Medical College & Hospital.

Conflict of Interest: None declared.
Consent: Informed written consent was taken from each patient's attendant.
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