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Detection of Carbapenemase Producing Drug-Resistant Gram-Negative Bacteria of Ventilator Associated Pneumonia Patients in Intensive Care Unit in Rajshahi Medical College Hospital

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ABSTRACT: ***Background:*** Carbapenem resistance is a major and on-going public health problem globally and locally. 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. ***Objective:*** To isolate and identify Carbapenemase producing drug resistance gram negative bacteria with their antibiogram of VAP patients in Intensive care unit in Rajshahi medical college hospital. Materials and ***Method:*** A Cross-sectional type of descriptive study was done during the period of July 2017 to June 2018. Endotracheal aspirates were collected of VAP patients in intensive care unit of Rajshahi Medical College Hospital. The specimens were inoculated in blood agar, nutrient agar and MacConkey's agar media and incubated aerobically at 37° C for 24 hours. The isolated bacteria were identified by their colony morphology, pigment production, haemolysis 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. Carbapenemase producing bacteria were identified phenotypically by modified Hodge test. ***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 57 isolated gram-negative bacteria 27 (49.7%) were carbapenemase producers. ***Conclusion:*** It may be concluded that, most of the isolated bacteria isolated from VAP are multidrug resistant and causes complicated life-threatening infections. Identification of the risk factors associated with carbapenem resistant bacterial infections is necessary to guide appropriate empirical antibiotic therapy, thus, reducing unfavorable outcomes and morbidity.

Keywords: Carbapenemase Producing Bacteria, Ventilator Associated Pneumonia, Intensive Care Unit, Modified Hodge Test.

Article at a glance:
Study Purpose*: To contribute to existing knowledge or propose new ideas.*
Key findings: *Among 57 gram-negative bacteria, 27 (49.7%) were identified as carbapenemase producers of VAP patients in ICU.*
Newer findings: *Prevalence of Carbapenemase producing gram-negative bacteria of VAP patients in ICU were higher from previous study (33%) than this study (49.7%).*
Abbreviations: *ICU: Intensive Care Unit, MHT: Modified Hodge Test, P. aeruginosa: Pseudomonas aeruginosa.*

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 cause 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 carbapenemase producing gram negative bacteria in ICU in Bangladesh to third generation cephalosporins 80%-100%, to fluroquinolones, aminoglycosides, monobactum 60%-80%, to carbapenem 10%-30% respectively.[7, 8] The emergence of antibiotic resistance against many VAPs related to bacteria is a matter of serious concern. This high rate of resistance also demonstrates the need for antibiotic stewardship protocol to be set up in health facilities. It is of utmost importance to do regular surveillance of antibiotic susceptibility patterns for preventing multidrug resistant bacterial infections. The knowledge gathered in this study will be helpful to formulate an antibiotic policy for the management of VAP patients in ICUs.[7]

METHOD

Antimicrobial susceptibility of 71 bacterial isolates of endotracheal aspirates 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 37° C overnight. If culture plates showed the growth of bacteria, then it was identified by their colony morphology, pigment production, haemolysis 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. Carbapenemase producing bacteria were identified phenotypically by modified Hodge test.[9]

Screening for Carbapenemase

The 2013 recommendations of Clinical and Laboratory Standard Institute (CLSI), isolates with reduced susceptibility to meropenem and imipenem (diameters of zones of inhibition ≤13 mm) by disk diffusion method were screened to produce carbapenemase.[10]

RESULTS

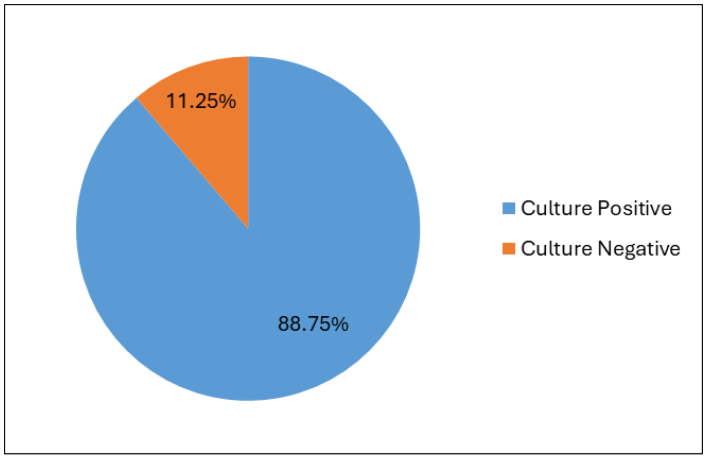

Figure 1: Frequency of Culture Positive and Negative Cases (N=80)

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.

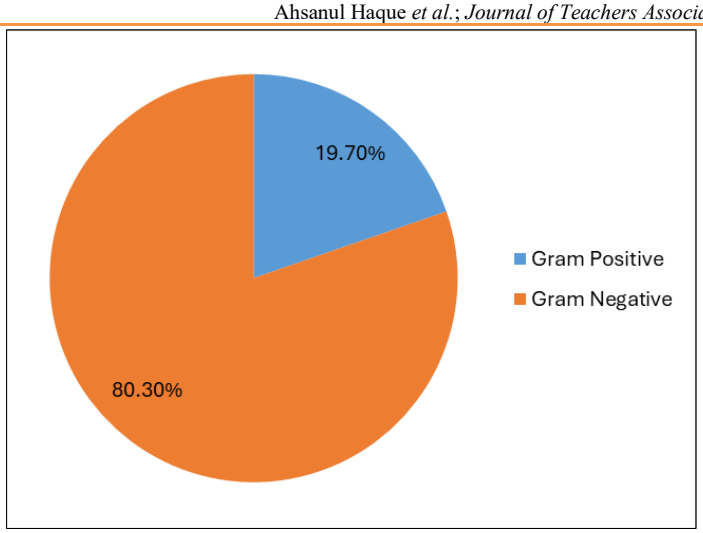

Figure 2: Frequency of Gram Positive and Gram-Negative Bacteria (N=71)

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

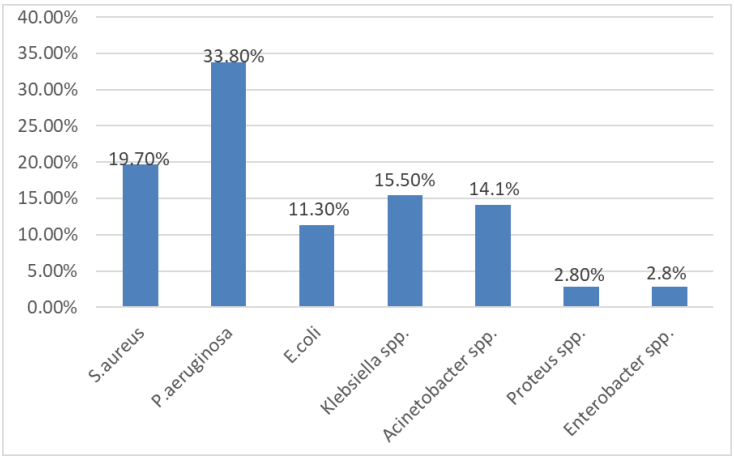

Figure 3: Pattern of Bacteria Isolated from VAP (N=71)

Figure 3 shows the identified species of bacteria from endotracheal aspirates. Out of 80 samples, a total of 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: Frequency of Carbapenemase Producing Bacteria

Isolates	**Tested Org.**	**No. of positive org. confirmed by phenotypic method (%)**
Carbapenem resistant *Pseudomonas aeruginosa*	24	12 (50%)
Carbapenem resistant *Acinetobacter spp.*	10	06 (60%)
Carbapenem resistant *Enterobacteriaceae*	23	09 (39.1%)
Total	57	27 (49.7%)

Table 1 shows the distribution of Carbapenemase producing bacteria from endotracheal aspirates. Among 24 isolated *Pseudomonas aeruginosa* 12 (50%), among 10 isolated *Acinetobacter spp.* 06 (60%) and among 23 isolated *Enterobacteriaceae* 09(39.1%) were phenotypically confirmed by modified hodge test as carbapenem resistant bacteria.

Table 2: Antimicrobial Resistance Pattern Among the Carbapenemase Producing *Pseudomonas Aeruginosa, Acinetobacter Spp.* and *Enterobacteriaceae*.

Antimicrobial agents	***Pseudomonas aeruginosa* (N=24)**	***Acinetobacter spp.* (N=10)**	***Enterobacteriaceae* (N=23)**
Imipenem	12(50%)	6(60%)	09(39.1%)
Azithromycin	18(75%)	9(90%)	19(82.6%)
Ciprofloxacin	16(66.7%)	7(70%)	13(56.5%)
Ceftriaxone	22(91.6%)	10(100%)	22(95.6%)
Cefepime	13(54.2%)	9(90%)	14(60.9%)
Piperacillin/tazobactam	9(37.5%)	7(70%)	11(47.8%)
Meropenem	14(58/.3%)	7(70%)	11(47.8%)
Aztreonam	12(50%)	8(80%)	10(43.5%)
Amikacin	14(58.3%)	6(60%)	12(52.2%)
Cefuroxime	23(95.8%)	10(100%)	22(95.6%)
Colistin	02(8.3%)	2(20%)	02(8.7%)
Levofloxacin	9(37.5%)	5(50%)	8(34.8%)
Amoxiclav	17(70.8%)	8(80%)	16(69.6%)
Tigecycline	04(16.7%)	3(30%)	04(17.4%)

Table 2 shows the antimicrobial resistance pattern among carbapenemase producing *Pseudomonas aeruginosa*, Enterobacteriaceae and *Acinetobacter spp.* Gram negative bacteria were highly resistant against ceftriaxone, cefuroxime, azithromycin, amoxiclav and ciprofloxacin. Colistin, tigecycline and imipenem were showed lower resistance against carbapenemase producing gram negative bacteria.

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*.[11, 12] Study is nearly dissimilar with the study of Shohid *et al.* and Sarkar *et al*. [13, 14] 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 of 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*.[13, 15] Study is nearly dissimilar with the study of Sarkar *et al.* and Kanipakam *et al.*[14, 16] The cause of predominant isolation rate of the gram-negative organism among hospitalized patients might be due to selective pressure of broad-spectrum antibiotics causing persistence 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 isolate 24(33.8%). Study is similar with the study of Shohid *et al.* and Sohal *et al.*[13, 17] 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 develop resistance to commonly used antimicrobial agents. Among 57 isolated gram-negative bacteria 27 (49.7%) were carbapenemase producers. This study was nearly similar with the study of Nusrat et al., De *et al.*

and Sharma *et al*. but nearly dissimilar with the study of Uddin *et al*. and Dwived *et al.*[8, 11, 12, 18, 19] 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. Antimicrobial resistance pattern among carbapenemase producing *Pseudomonas aeruginosa, Enterobacteriaceae* and Acinetobacter *spp*. Gram negative bacteria were highly resistant against ceftriaxone, cefuroxime, azithromycin, amoxiclav and ciprofloxacin. Colistin, tigecycline and imipenem were showed lower resistance against gram negative bacteria. This study is nearly similar with Hoque *et al*. and Sharma *et al.*[7, 8] Study is nearly dissimilar with the study of Haque *et al.*, Saha *et al.* and De *et al.*[12, 15, 20-35] Due to arbitrary use of antibiotics common pathogen develops resistance against frequently used drugs. In ICU, third generation antibiotics are used to treat severely ill patients. VAP patients are developing resistance against these third-generation antibiotics which is quite 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.

Declarations

I, hereby, declare that the submitted Research Paper is my original work and no part of it has been published anywhere else in the past.

Ethical Approval

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

Conflict of interest: None declared.

Consent for Publication: Informed written consent was taken from each patient's attendant.

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