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Original Article

Bacteria isolated from endotracheal aspirates and their sensitivity pattern in patients suspected of ventilator associated pneumonia in a tertiary care hospital

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ABSTRACT:
Background: Mechanically ventilated patients are at high risk of acquiring Ventilator Associated Pneumonia (VAP) due to complex interplay between the endotracheal tube, host immunity and virulence of invading bacteria. Knowledge about antimicrobial sensitivity patterns of VAP related microorganisms helps to start prompt and judicious antimicrobial treatment to combat the fatal consequences of VAP.
Aim: To identify the antibiotic sensitivity patterns of the bacteria isolated from endotracheal aspirates of suspected VAP patients in ICU of a tertiary care hospital.
Materials and Methods: It was a prospective observational study conducted in the adult ICU of Combined Military Hospital, Dhaka from January 2017 to December 2017. This study included the tracheal aspirates of 590 suspected VAP patients. Isolation of bacteria and their antibiotic sensitivity tests were done at Armed Forces Institute of Pathology (AFIP) according to CLSI guidelines.
Results: Out of 590 samples, 198 showed significant growth that included 12 polymicrobial infections. Among 198 culture positive samples Klebsiella were the most predominant bacteria (41.90%) followed by Acinetobacter (35.71%), Pseudomonas (16.20%), Proteus (2.86%), *Staphylococcus aureus* (1.90%) and *E. coli* (1.43%). Colistin and tigecycline were highly effective against Klebsiella and Acinetobacter, but these isolates showed higher resistance against third and fourth generation cephalosporins and meropenem. Pseudomonas isolates were highly sensitive to carbenicillin, ticarcillin, piperacillin - tazobactam combination and colistin. Proteus isolates were fully sensitive to tigecycline and meropenem, but 100% resistant to colistin.
Conclusion: Acinetobacter and Pseudomonas isolates showed more resistance pattern than other bacterial isolates. Colistin and tigecycline were found most effective antibiotics whereas commonly used antibiotics showed marked resistance pattern. The findings of this study will help in framing the appropriate antibiotic policy of the critical care centres of hospitals regarding the VAP related patients.

Keywords: Endotracheal aspirate, Mechanical ventilation, Ventilator associated pneumonia

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INTRODUCTION

Ventilator associated pneumonia (VAP) occurs within 48 hours or more after establishment of endotracheal intubation and mechanical ventilation. It has become one of the major Intensive Care Unit (ICU)-acquired infections worldwide. Accurate and rapid diagnostic methods are key to initiate appropriate antimicrobial treatment and to reduce healthcare costs and mortality. It has also an indirect effect on the emergence of bacterial resistance.[1]

In case of intubated patients, colonization in the respiratory tract is most common.[2] Mechanical ventilation (MV) is responsible for 6 to 10 fold increase in the risk of respiratory tract infections. In this case

tracheal colonization of bacterial isolates may be responsible for added or super infections and at the same time, increases the risk of mortality. Again, due to inadvertent and irrational use of antibiotics, there are increasing emergence of drug resistant bacteria. So, obviously, it is a new challenge for critical care physicians to treat these patients. These drug resistant bacteria are gram negative bacteria prevalent all over the world.[3]

The worldwide incidence of VAP ranges from 6.8% to 44% and its occurrence is associated with increased mortality and financial burden. 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. VAP can be polymicrobial as well. The pattern of microorganisms especially MDR pathogens varies among hospitals, specific hospital units, and patients 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*.[4,5,6]

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. So early and appropriate diagnosis is very important to reduce the incidence of VAP particularly to reduce the frequency of MDR pathogen.[7] Bronchoalveolar lavage and Bronchial brushing have been reported to have high sensitivity and specificity for the diagnosis of VAP, but these methods are invasive and difficult to perform.[8] Endotracheal aspirate is relatively noninvasive method that can be easily performed.[9] The selection of proper antimicrobial agents early initiation of therapy are important determinants for reducing morbidity and mortality VAP patients.[10]

MATERIALS AND METHODS

It was a prospective observational study conducted at the Department of Microbiology of Armed Forces Institute of Pathology, Dhaka Cantonment. Study period was 12 months, from January 2017 to December 2017. A total 590 patients, admitted at the ICU of Combined Military Hospital, Dhaka were selected as study population. All these patients were suspected cases of VAP.

Selection criteria

Inclusion criteria: Patients under mechanical ventilation for more than 48 hours in the ICU.

Exclusion criteria; Patients having pneumonia prior to mechanical ventilation, patients having pulmonary oedema or Acute Respiratory Distress Syndrome (ARDS).

The endotracheal aspirates were collected by nonbronchoscopic method with a 22-inch Ramson's 12-F suction catheter. It was gently introduced through the endotracheal tube for a distance of approximately 25-26 cm and gentle aspiration was performed. After the withdrawal of the catheter, 2 ml of sterile 0.9% normal saline was injected into it with a sterile syringe to flush the exudates into a sterile container. It was then transported to microbiology laboratory.[11]

Endotracheal aspirate samples were immediately inoculated in various culture media (MacConkey's agar media, Blood agar media, Chocolate agar media) and Gram's staining of aspirates were performed. Incubation was done under standard temperature (37ºC), time (48 hours) and optimum condition. Isolated microorganisms were identified by their colony morphology, Gram's staining and relevant biochemical tests. Gram's staining was performed by primary staining with crystal violet, mordanting with Lugol's iodine, decolourizing with acetone and lastly counter-staining by carbol fuchsin. Antibiotic sensitivity tests for isolates were performed by Modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates according to CLSI guidelines.[11]

RESULTS

Out of 590 samples, 198 showed significant bacterial growth (33.56%) which include 12 polymicrobial infections. These culture positive cases include 69.19% male and 30.81% female. Maximum numbers of positive cultures were found in 61-70 years age group (30.30%) (Table-1). Among all culture positive samples, Klebsiella were found in 88 samples (41.90%), Acinetobacter in 75 (35.71%), Pseudomonas in 34 (16.20%), Proteus in 06 (2.86%), *Staphylococcus aureus* in 04 (1.90%) and *E. coli* in 03 samples (1.43%) (Figure-1).

Table1. Age distribution of VAP positive patients (n=198).

Age in years	No of isolates (%) (n=198)
100-81	27 (13.64%)
80-71	48 (24.24%)
70-61	60 (30.30%)
60-51	39 (19.70%)
< 50	24 (12.12%)
Total	198 (100%)

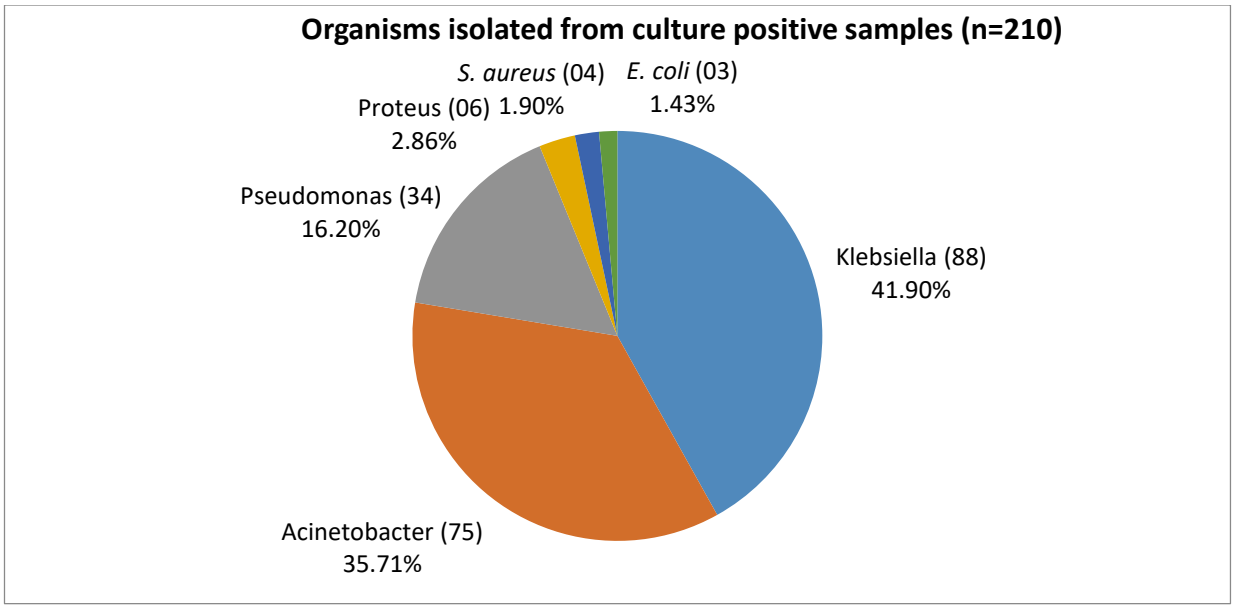

Figure 1. Organisms isolated from culture positive samples (n=210).

Table 2. Sensitivity pattern of isolated bacteria of VAP against commonly used antibiotics.

Antibiotics	Klebsiella (n=88)	Acinetobacter (n=75)	Pseudomonas (n=34)	Proteus (n=06)	*S. aureus* (n=04)	*E. coli* (n=03)
AZM	25(28.4%)	8 (10.7%)	13 (38.2%)	0 (0%)	0 (0%)	1 (33.3%)
CIP	38(43.2%)	8 (10.7%)	15 (44.1%)	3 (50.0%)	3 (75.0%)	1(33.3%)
LE	42(47.7%)	20 (26.7%)	19 (55.9%)	3 (50.0%)	4 (100%)	2 (66.6%)
AMC	53(60.2%)	8 (10.7%)	9 (26.5%)	3 (50.0%)	2 (50.0%)	1 (33.3%)
COT	5 (5.7%)	8 (10.7%)	6 (17.6%)	0 (0%)	-	0 (0%)
CXM	7 (7.9%)	6 (8.0%)	6 (17.6%)	0 (0%)	-	0 (0%)
CAZ	13(14.8%)	3 (4.0%)	19 (55.9%)	0 (0%)	-	1(33.3%)
CFM	18 (20.5%)	3 (4.0%)	7 (20.6%)	6 (100%)	3 (75.0%)	3 (100%)
CTR	18 (20.5%)	6 (8.0%)	17 (50.0%)	3 (50.0%)	3 (75.0%)	1 (33.3%)
AK	55 (62.5%)	27 (36.0%)	-	3 (50.0%)	-	3 (100%)
GEN	53 (60.2%)	27 (36.0%)	10 (29.4%)	3 (50.0%)	-	3 (100%)
NET	50 (56.8%)	35 (46.7%)	10 (29.4%)	6 (100%)	-	3 (100%)
CB	-	-	32 (94.1%)	-	-	-
TIC	-	-	29 (85.3%)	-	-	-
AT	7 (7.9%)	10 (13.3 %)		0 (0%)	-	0 (0%)
TGC	52 (59.1%)	45 (60.0%)	20 (58.8%)	6 (100%)	-	1 (33.3%)
MER	30 (34.1%)	6 (8.0%)	23 (67.6%)	6 (100%)	-	2 (66.6%)
TZP	35 (39.8%)	10 (13.3%)	24 (70.6%)	3 (50.0%)	-	1 (33.3%)
CPM	10 (11.4%)	3 (4.0%)	8 (23.5%)	3 (50.0%)	-	1 (33.3%)
CL	65 (73.9%)	60 (80.0%)	28 (82.4%)	0 (0%)	-	3(100%)

*[AZM: Azithromycin, CIP: Ciprofloxacin, LE: Levofloxacin, AMC: Amoxycillin + Clavulanic acid, COT: Cotrimoxazole, CXM: Cefuroxime, CAZ: Ceftazidime, CFM: Cefixime, CTR: Ceftriaxone, AK: Amikacin, GEN: Gentamycin, NET: Netilmicin, CB: Carbenicillin, TIC: Ticarcillin, AT: Aztreonam, TG: Tigecycline. MER: Meropenem, TZP: Piperacilin +Tazobactam, CPM: Cefipime, CL: Colistin]
**[In case of *S. aureus* isolates flucloxacillin, vancomycin, and linezolid were used.]

Klebsiella was the most predominant organism which was found highly sensitive to colistin (73.9%), amikacin (62.5%), gentamycin (60.2%), amoxyclav (60.2%), tigecycline (59.1%) and netilmicin (56.8%), but showed high resistance to co-trimoxazole (94.3%), cefuroxime (92.1%), cefipime (88.6%), ceftazidime (85.2%), cefixime (79.5%), ceftriaxone (79.5%), meropenem (65.9%) and piperacillin + tazobactam (60.2%) (Table: 2). Acinetobacter were the second most predominant bacteria which were highly sensitive to colistin (87.0%) and tigecycline (64.5%), but showed high resistance to all other commonly used antibiotics, such as ceftazidime (96.0%), cefipime (96.0%), cefixime (92.0%), ceftriaxone (92.0%), meropenem (92.0%), amoxyclav (89.3%) and gentamycin (64.0%) (Table: 2). Pseudomonas isolates were highly sensitive to antipseudomonal penicillins, such as carbenicillin (94.1%), ticarcillin (85.3%) and piperacillin + tazobactem (70.6%). It was also highly sensitive to colistin (82.4%) and meropenem (67.6%), whereas

resistant to other commonly used antibiotics (Table: 2). Proteus isolates were 100% sensitive to netilmicin, tigecycline and meropenem, but fully resistant to azithromycin, cefuroxime, ceftazidime and colistin (Table: 2). Gram positive bacteria *Staphylococcus aureus* were 100% sensitive to levofloxacin, linezolid, vancomycin and cefexime and highly (75%) sensitive to ceftriaxone, flucloxacillin and meropenem. Only 01 (25%) isolate was methicillin resistant (MRSA) (Table: 2). *E. coli* isolates were 100% sensitive to cefixime, aminoglycosides and colistin, whereas 100% resistance was shown to cefuroxime and aztreonam (Table: 2). Comparative sensitivity pattern of isolated bacteria against colistin and tigecycline has been shown in Figure-2. Most of the Klebsiella, Acinetobacter and Pseudomonas isolates were highly sensitive against colistin and tigecycline. All the Proteus isolates were sensitive against tigecycline, whereas 100% resistant to colistin. In case of *E. coli* isolates, less sensitivity was found against tigecycline (Figure-2).

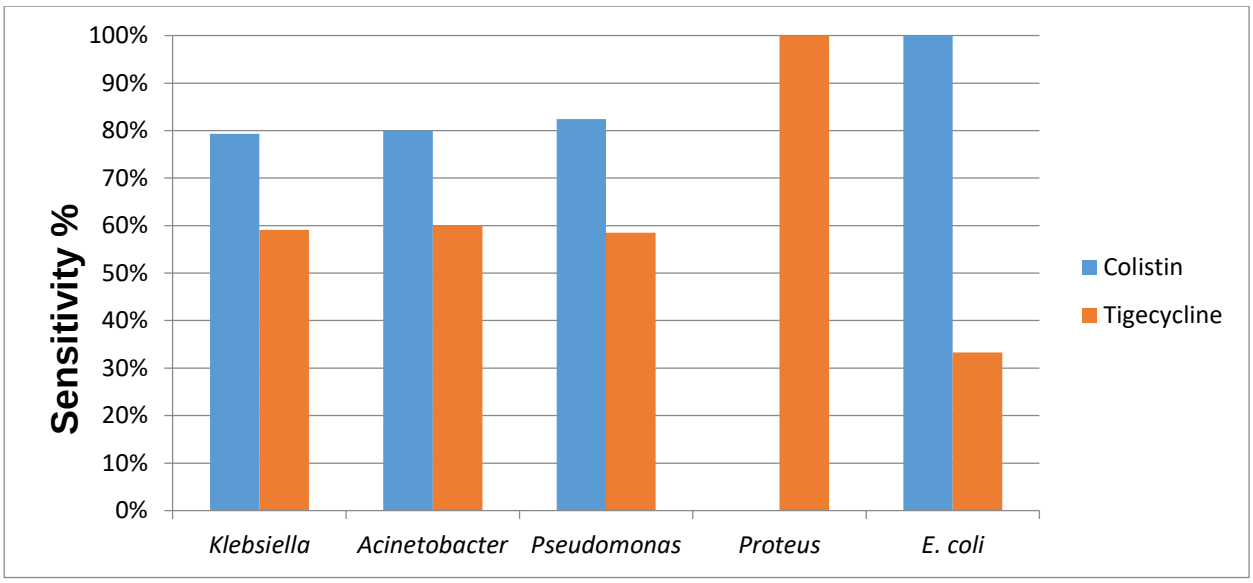

Figure 2. Sensitivity pattern of colistin and tigecycline against bacterial isolates.

DISCUSSION

In the ICUs most of the critically ill patients 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.[7] The rate of culture positivity in our study was 33.56%, whereas the rate was 22.55% in the study done by Ishtiaq et al. in Pakistan.[12] Another study done by Sarkar M et al. in Bangladesh found the rate of culture positivity 52.29%.[13] Similar higher rate of positivity (59.26%) was found in an Indian study done by Saha AK et al.[3] This variation of the rates in different settings is mainly due to the wide spectrum of causative agents of ventilator associated pneumonia and difference in the standards of maintenance of hospital infection control policies.

Our study revealed 70.85% positivity in males, which was higher as compared to females (29.15%). Panda G et al. found the similar high rate of positivity in males (69.17%).[2] We found higher rate of gram negative isolates (98.1%) which is similar to the results of the study done by Rahbar et al. in Iran.[14]

Klebsiella (41.90%) was the most predominant bacteria isolated in our study. A study done by Ghosh et al. also revealed the Klebsiella (36%) as the most predominant bacteria.[15] But Saha AK et al. found the Acinetobacter as most predominant bacteria in India.[3] Amini et al. found Staphylococcus aureus as the commonest isolate in their study, which differs from our findings.[16] In this study we observed that Klebsiella isolates were highly sensitive to colistin (73.9%), amikacin (62.5%) and tigecycline (59.1%), but highly resistant to meropenem and third generation cephalosporins. Similar findings were revealed by a study in Bangladesh done by Ahsan ASM et al.[7]

Acinetobacter was the second most predominant bacteria (35.71%) in our study. But another two studies revealed the Acinetobacter as the most frequent bacteria.[7,13] Acinetobacter isolates were highly sensitive to colistin (87.0%) and tigecycline (64.5%), but showed high resistance to all other antibiotics commonly used for Acinetobacter infection, such as ceftazidime (96.0%), cefipime (96.0%), ceftriaxone (92.0%) and meropenem (92.0%). A study done by Hoque et al. found the Acinetobacter isolates 100% sensitive to colistin and fully resistant to third generation cephalosporins.[17] From these findings it is obvious that Acinetobacter is a rapidly emerging multidrug resistant bacteria as a predominant causative agent of VAP.

Pseudomonas isolates were highly sensitive to colistin (82.4%), meropenem (67.6%) and anti-pseudomonal penicillins, whereas resistant to aminoglycosides, third and fourth generation cephalosporins in our study. But

100% sensitivity to colistin was found in the studies done by Salma KB et al and Vincent JL et al.[18,19] *Staphylococcus aureus* isolates were fully sensitive to levofloxacin, linezolid, vancomycin, cefexime, and ceftriaxone. Only 01 (25%) isolate was methicillin resistant (MRSA). Siddique et al. found 62.5% MRSA in their study.[20]

We found *E. coli* isolates were highly sensitive to cefixime (100%), aminoglycosides (100%) and colistin (100%). This is almost similar to the findings of the study done by Hoque L et al.[17] Our study was conducted in a resource-limited setting with only small number of suspected VAP patients from a single centre. We did not perform any molecular study and bacterial gene sequencing. These could be considered as limitations of our study.

CONCLUSION

The emergence of antibiotic resistance against many VAP related bacteria 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. It is of utmost importance to do regular surveillance of antibiotic susceptibility patterns for preventing multidrug resistant bacterial infections. The knowledge we gathered in this study will definitely be helpful to formulate an antibiotic policy for the management of VAP patients in ICUs.

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