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ORIGINAL RESEARCH

The study of antibiotic susceptibility pattern of isolates from tracheal and endotracheal aspirates

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ABSTRACT

Background: Critically ill patients on mechanicalventilation are always at a higher risk of acquiring ventilator-associated respiratory infections. The current study was intended to determine the antibiotic-resistance pattern of bacteria recovered from the tracheal and endotracheal (ET) specimens of ventilated patients. **Aims & objectives**: To isolate and identify bacteria from tracheal and endotracheal aspirates and to determine the antimicrobial susceptibility of organisms isolated from tracheal and endotracheal aspirate specimens. **Methodology**: A descriptive study was conducted in the Department of Microbiology, Guru Gobind Singh Medical College, Faridkot for a period of 6 months. All the tracheal and endotracheal specimens collected were inoculated directly on blood and MacConkey's agar and incubated at 37°C for 24-48 hours. **Results**: Maximum samples 18(36%) obtained were from the age group of 0-10 years. the common isolate was *Acinetobacter spp.* and all isolates were resistant to ampicillin and third generation cephalosporins. The isolates of *P. aeruginosa* showed resistance to ampicillin and third generation cephalosporins (ceftazidime 66.6%,cefepime 33.3%) followed by amikacin(66.6%), *E.coli* isolates were resistant to third generation cephalosporins,,ciprofloxacin and ampicillin(100% each). Colistin was found to be most effective antimicrobial against these gram negative isolates in our study. **Conclusion**: Gram-negative organisms were the most common isolates from the secretions. All these isolates showed high levelsof resistance to commonly used antimicrobial agents. This poses a serious therapeutic challenge as it limits the therapeutic options available.

Keywords: antibiotic susceptibility, tracheal and endotracheal aspirates

INTRODUCTION

Respiratory infections are associated with high morbidity and mortality, especially in critically ill patients (1). According to the World Health Organization (WHO) highest percentage of nosocomial infections occur in intensive care units (ICUs) which are five to seven folds higher as compared to other infections (2). Critically ill patients associated with respiratory tract infection are usually maintained using invasive devices (catheters, endotracheal tube, mechanical ventilators, etc.) which themselves are a major reservoir for hospital-acquired infections (3). About 15% of hospital acquired infections (HAIs) are caused by ventilator-associated pneumonia (VAP) which is the second-most-common HAI having the highest morbidity and mortality (1).

Mechanical ventilation is a life-saving procedure for patients in the ICU, but it is associated with a high risk of acquiring respiratory infections especially in serious patients(4). Insertion of ETT promotes the accumulation of tracheobronchial secretions by impairing the mucocilliary clearance and disturbing the cough reflex. (2).

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The collection procedure of ET aspirate is a simple, minimally invasive and inexpensive procedure which can easily be performed at the bedside of the patient (5). ET culture is an additive diagnostic tool along with other routine tests in detection of pneumonia causing organisms (6). Within 24 hours of insertion of ETT, organisms lead to formation of biofilm.These biofilms are generally polymicrobial in nature and show multidrug-resistance patterns, leading to high mortality rates (7).

Gram negative bacteria (*Pseudomonas* species, *Acinetobacter* species, and members of the *Enterobacteriaceae* family) and *Staphylococcus aureus* have been reported to be the most common agents of HAIs (8,9).

Knowledge of local antimicrobial resistance patterns are important to initiate the empirical antimicrobial therapy. This will help in preventing overuse and misuse of antimicrobial agents which has arisen as a serious challenge to clinicians in choosing the most effective therapeutic option.

Thus, the present study was conducted to isolate and identify the bacteria from tracheal and endotracheal aspirates and determine their antimicrobial susceptibility pattern from tracheal and endotracheal aspirate specimens.

AIMS AND OBJECTIVES

1. To isolate and identify bacteria from tracheal and endotracheal aspirates.
2. To determine antimicrobial susceptibility of organisms isolated from tracheal and endotracheal aspirate specimens

MATERIAL AND METHODS

The study was conducted in the department of Microbiology of Guru Gobind Singh Medical College and Hospital, Faridkot for a period of 6 months. Keeping view of the availability and feasibility of the participants purposive sampling was used. Hence, total of 50 tracheal and endotracheal specimens were collected from patients admitted in the hospital. The samples were inoculated directly on blood and MacConkey's agar and then incubated at 37°C for 24-48 hours. Also, Complete biodata of patients was recorded.

Collection of sample

Tracheal aspirate (10): The specimen was collected under aseptic conditions from the ventilated patients. Percutaneuos tracheal aspirates were obtained by inserting a small plastic catheter into the trachea via a needle previously inserted through skin and crico-thyroid membrane. **Endotracheal secretions (10):** The sample was taken from patients who were intubated and having large volume of secretions and not recovering even after taking antibiotics. The sample was collected aseptically by sucking the secretions by inserting the suction tube into endotracheal tube (already intubated patients). If case of any delay, the specimen was refrigerated. The specimens were sent to the microbiology department in sterile containers for culture and sensitivity.

OBSERVATIONS AND RESULTS

Table 1: Sex wise distibution of patients (n=50)

S.no.	Sex of patients	No. Of patients (n=50)	Percentage(%)
1.	Male	35	70%
2.	Female	15	30%
	Total	50	100%

Table 1 shows that there were predominance of males (70%) over the female (30%) patients with the ratio of (female to male) 0.43:1 and the difference was statistically significant (p value <0.00001)

Table 2: Age wise distribution of patients (n=50)

S.no.	Age in years	No. Of patients (n=50)	Percentage(%)
1	0-10 years	18	36%
2	11-20 years	7	14%
3	21-30 years	6	12%
4	31-40 years	6	12%
5	41-50 years	4	8%
6	51-60 years	2	4%
7	>60 years	7	14%
	Total	50	100%

Table 2 shows that, out of the 50 patients enrolled in the study, the maximum 18(36%) were from the age group of 0-10 years, followed by 7(14%) were from age group of 11-20 years and >60years each.

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Table 3: Bacteria isolated from tracheal and endo-tracheal aspirate (ETA) culture (n=50)

S.no.	Organism	No. Of isolates	Percentage(%)
1.	Gram negative organisms	48	96%
2.	Gram positive organisms	2	4%
	Total	50	100%

Table 3 shows that out Of 50 samples, 48(96%) showed growth of gram negative bacteria and 2(4%) had growth of gram positive bacteria.

Table 4: Distribution of gram negative bacteria isolated from tracheal and ETA specimens (n=50)

Bacterial isolates	Number of isolates	Percentage (%)
Acinetobacter baumannii complex	21	42
Klebsiella pneumoniae	11	22
Pseudomonas aeruginosa	9	18
E.coli	4	8
Citrobacter freundii	3	6
Staphylococcus aureus	2	4
TOTAL	50	100

Table 4 shows that, among the GNB, *Acinetobacter baumannii* complex was the common isolate (42%) followed by *Klebsiella pneumonia* (22%), *Pseudomonas aeruginosa* (18%), *E.coli* (8%) and *Citrobacter freundii*(6%). Gram positive isolate was *Staphylococcus aureus* (4%).

Figure 1: Distribution of gram negative bacteria isolated from tracheal and ETA specimens (n=50)

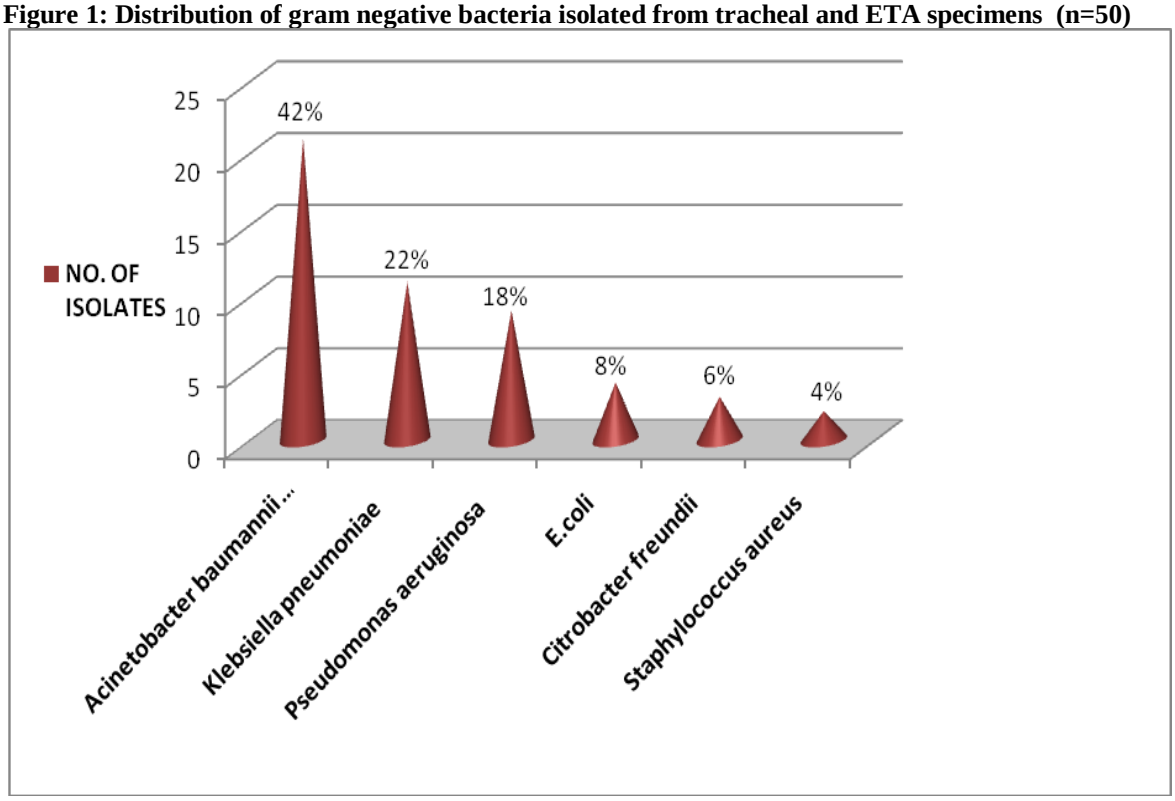

Figure 1 shows that, among the GNB, *Acinetobacter baumannii* complex was the common isolate (42%) followed by *Klebsiella pneumonia* (22%), *Pseudomonas aeruginosa* (18%), *E.coli* (8%) and *Citrobacter freundii*(6%). Gram positive isolate was *Staphylococcus aureus* (4%).

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Figure 2: Antimicrobial susceptibility pattern of *acinetobacter baumannii* complex (n=21)

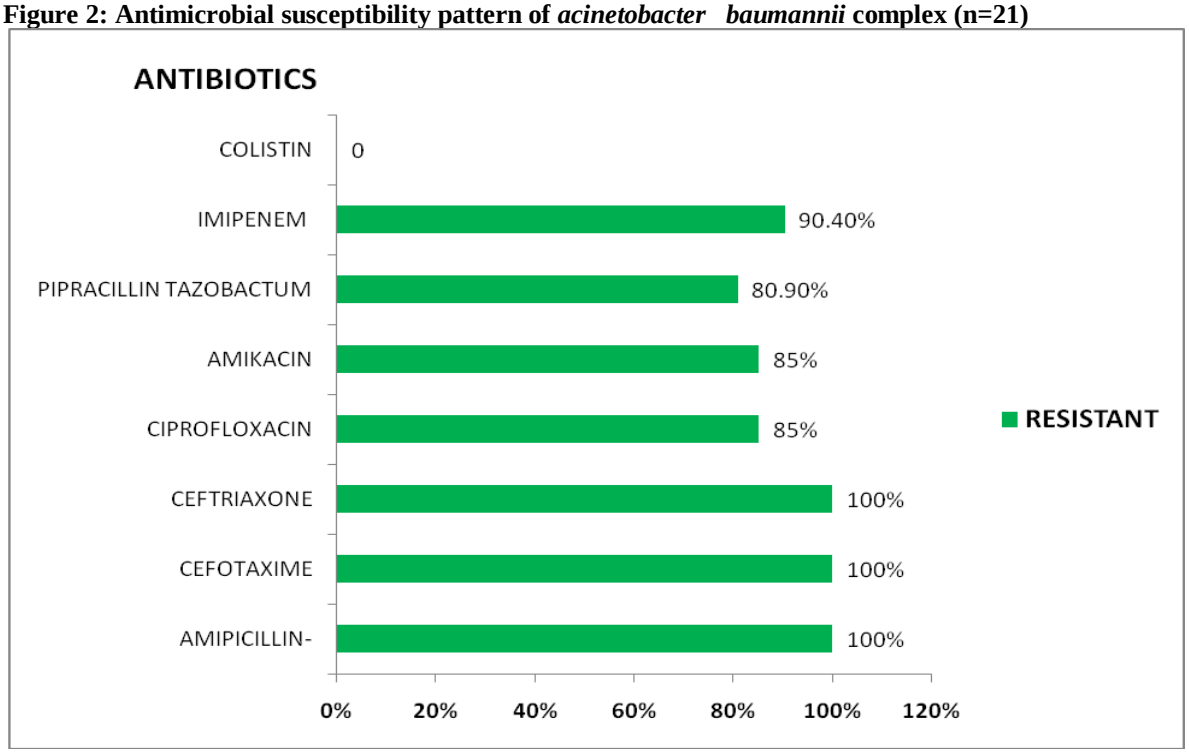

Figure 2 shows antimicrobial susceptibility pattern of *Acinetobacter baummanii* complex. All isolates showed 100% resistance to ampicillin, cefotaxime and ceftriaxone followed by imipenem(90.4%) and ciprofloxacin and amikacin(85% each) and piperacillin-tazobactum(80.96%). No resistance was observed against colistin.

Figure 3: Antimicrobial susceptibility pattern of *pseudomonas aeruginosa* (n=9)

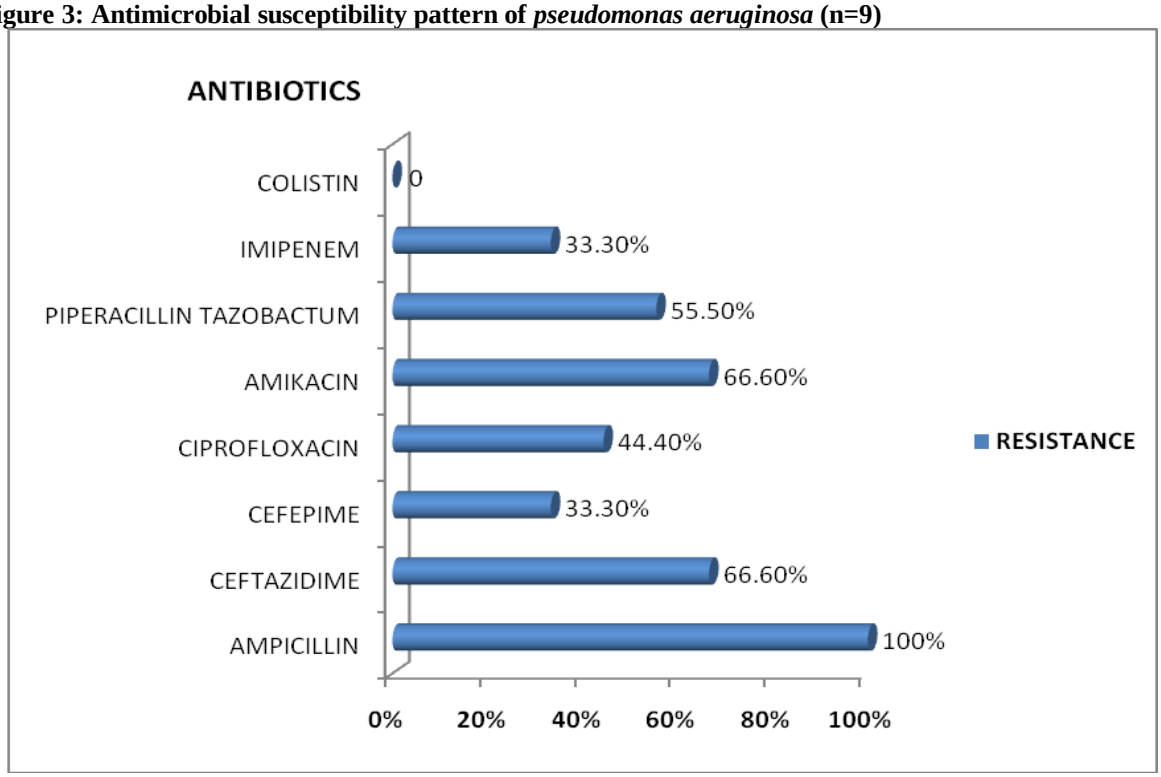

Figure 3 shows antimicrobial susceptibility pattern of *Pseudomonas aeuroginosa*. All isolates showed resistance to ampicillin (100%), third generation cephalosporins (ceftazidime 66.6%, cefepime 33.3%) followed by amikacin (66.6%), piperacillin/tazobactum (55.5%), ciprofloxacin (44.4%), imipenem (33.3%). No resistance was observed against colistin.

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Figure 4: Antimicrobial susceptibility pattern of other gram negative bacilli of family *enterobacteriaceae* isolated

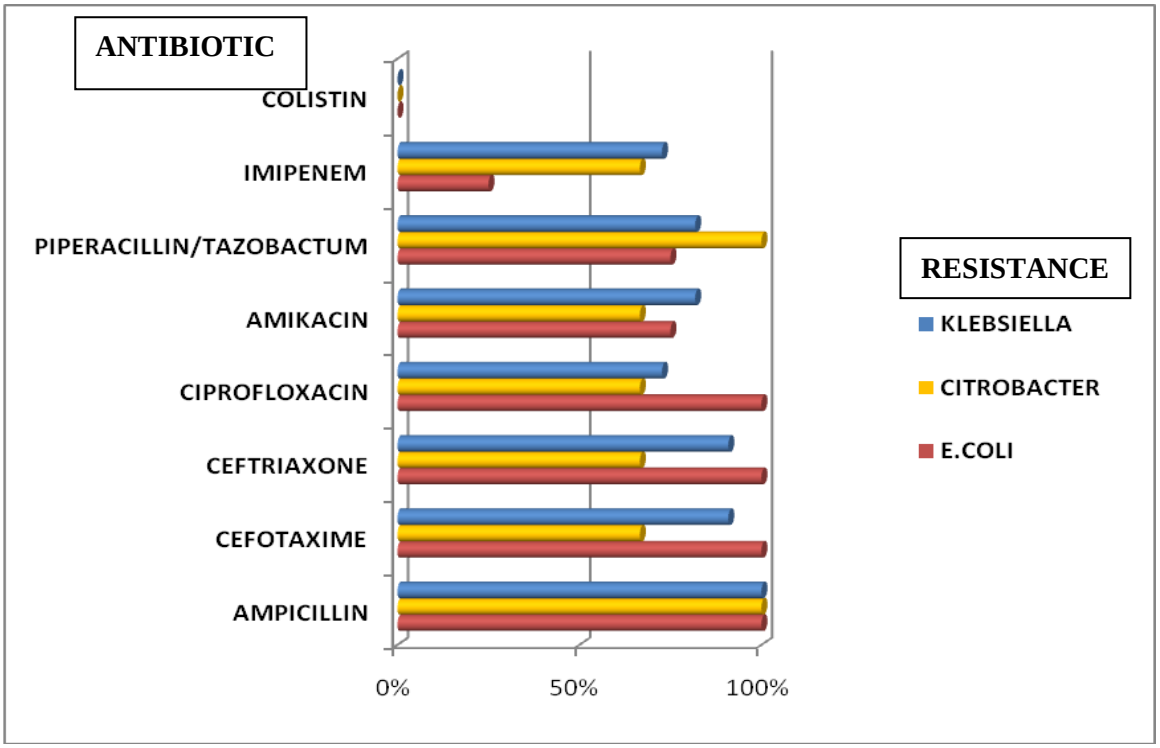

Figure 4 shows AST pattern of Gram Negative isolates. All the isolates were resistant to Ampicilin(100%) and all the isolates were sensitive to colistin.The isolates of *K. pneumoniae* showed high level of drug resistance to third generation cephalosporins (90.9%) followed by piperacillin-tazobactum, amikacin (81.8% each), imipenem and ciprofloxacin (72.7% each). *E.coli* isolates also showed 100% resistance to third generation cephalosporins and ciprofloxacin followed by amikacin and piperacillin/tazobactum (75% each) and imipenem (25%). *Citrobacter* spp. isolated showed 100% resistance to piperacillin/tazobactum followed by third generation cephalosporins, ciprofloxacin, amikacin and imipenem (66.6% each).

Figure 5: Antimicrobial susceptibility pattern of *staphylococcus aureus* (n=2)

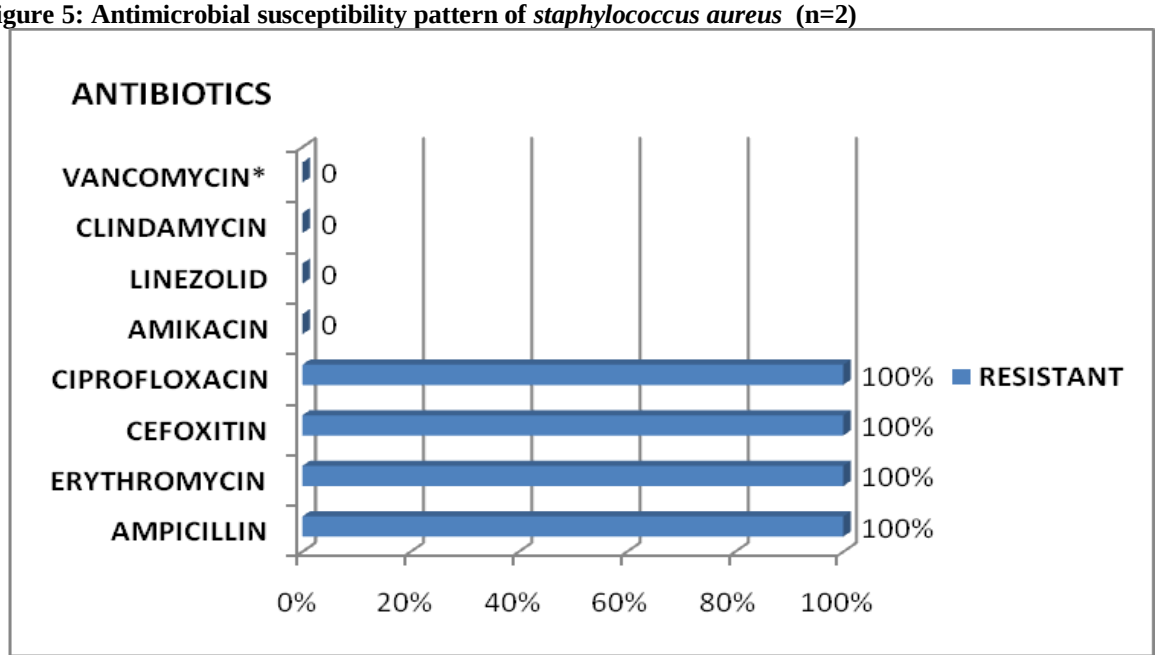

Figure 5 shows Antibiotics susceptibility testing of *Staphylococcus aureus.* Allisolates were resistant to ampicillin and cefoxitin (100%). All the isolates were labelled as Methicillin resistant Staphylococcus aureus (MRSA). Both the isolates were 100% susceptible to vancomycin and linezolid. Vancomycin screen agar supplemented with 6 µg/ml of vancomycin (VSA) was considered to interpret susceptibility/resistance to vancomycin as per CLSI guidelines. No growth was seen on VSA.

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DISCUSSION

Out of 50 isolates, 35 (70%) were from males and 15 (30%) were from females. The male to female ratio was 0.43:1. This is in accordance with study conducted by Panda et al., Santaram et al., Khoshfetrat et al., and Soni et al., where number of males were more as compared to females (11-14).

Age wise distribution of the 50 isolates showed that the maximum isolates 18(36%) were from the age group of 0-10 years, followed by 7(14%) each from age group of 11-20 years and >60 years. However, in a study conducted by Panda et al., most of the specimens were from age group of >70years (11).

In the present study, among the isolated organisms, majority of isolates were of gram negative bacteria (96%) followed by gram positive bacteria (4%). This is in accordance with the studies conducted by Panda et al., Khatun et al., and Soni et al., who have also reported predominance of gram negative organisms over gram positive organisms (11,15,14).

In the present study antimicrobial susceptibility testing of *Acinetobacter* spp. isolates showed resistance to ampicillin (100%) cephalosporins(100%) followed by imipenem(90.4%), ciprofloxacin(85%), amikacin(85%), piperacillin-tazobactum (80.9%). All the isolates of *Acinetobacter* spp. were found to be susceptible to colistin. Khatun et al., have also reported similar findings with high level resistance to various antibiotics among their clinical isolates (15).

Out of the 50 isolates, 90.4% isolates were resistant to imipenem however Rathod et al., have reported only 19% resistance to imipenem (16). The high resistance to imipenem in our study is a warning sign regarding its use by the clinicians.

In the present study, *Pseudomaonasaeuroginosa* isolates showed 100% resistance to ampicillin, high level of drug resistance to third generation cephalosporins followed by amikacin (66.6%), All the *Pseudomonas* isolates were found to be susceptible to colistin. Similarly in a study conducted by Panda et al., all the Pseudomonas isolates were sensitive to colistin.

In the present study 33.33% isolates of *Pseudomonas* were found to be resistant to imipenem. This is in accordance with the study conducted by Rajkumari et al., and Juayang AC et al., whose *Pseudomonas* isolates showed 25% resistance to imipenem. (17,18).

Of the 11 (22%) isolates of *Klebsiella***,**90.9% isolates showed resistance to cephalosporins followed by 81.8% resistance to piperacillin-tazobactam and amikacin, followed by 72.7% resistance to ciprofloxacin and imipenem. Panda et al., have also reported high levels of resistance to ceftriaxone, cefotaxime and ceftazidime (11).

Of the 4 (8%) isolates of *E coli***,** 100% resistance was observed to 3 rd generation cephalosporins and ciprofloxacin followed by 75% resistance to piperacillin-tazobactam and amikacin. In the present study, 3 (6%) isolates of *Citrobacterspp***.** 66.66%resistance was observed against to cephalosporins, ciprofloxacin, amikacin and imipenem. This is similar to study conducted by Panda et al. However, Bhat et al., have reported 100% resistance against amikacin, gentamicin, ciprofloxacin, imipenem and meropenem(11,19).

Both the Staphylococcus aureus isolates in the present study were resistant to cefoxitin i.e methicillin resistant *Staphylococcus aureus* (MRSA) and these isolates showed 100% resistance against ampicillin, erythromycin and ciprofloxacillin. These findings are consistent with similar studies conducted by Rajkumari et al., and Rathore et al., who have reported that all of their isolates of *S. aureus* were resistant to methicillin but sensitive to vancomycin. Bhat et al., also reported 100% susceptility to vancomycin and linezold among their clinical isolates (18,16,19).

CONCLUSION

Gram-negative organisms were the most common isolates from the secretions. All these isolates showed high level resistance to commonly used antimicrobial agents. This poses a serious therapeutic challenge as it limits the therapeutic options available.

Routine surveillance of antimicrobial profile and susceptibility pattern should be conducted so that rational antibiotic policy could be adopted and it should be reviewed at regular intervals. This would serve as guide for the clinicians for the empirical and definitive therapy while treating the patients. Also it would help reduce morbidity and mortality associated with multidrug resistant organisms.

The study highlights the need for stringent hospital infection control practices. Implementation of good infection control practices can play a major role in the prevention of infection and spread of multi-drug resistant pathogens.

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