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Original Research Article

Antibiotic sensitivity pattern of bacteria isolated from respiratory samples of intensive care units in a tertiary care hospital in north India

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ABSTRACT

Background: The burden of life-threatening conditions requiring mechanical ventilation (MV) in the intensive care unit has grown in the last couple of years, so understanding the pattern of bacteria isolated and their antibiogram has become necessary in all tertiary care hospitals. The present study was designed to know the bacterial profile and their antimicrobial sensitivity pattern from respiratory samples among patients admitted in the ICU of the institute.
Methods: This was a 2-year retrospective study (from January 2022 to December 2023) in a Punjab tertiary care hospital and endotracheal and tracheal aspirates using standard microbiological methods were analyzed.
Results: A total of 602 respiratory samples were collected from six ICUs, with 274 (46%) culture-positive. Among these, 81% were Gram-negative bacilli, 18% Gram-positive cocci, and 2% yeast. The most common isolates were *Klebsiella spp*. (61%), followed by *Staphylococcus aureus* (17%), and *Pseudomonas spp*. (15%). Multidrug resistance was observed in 33% of *Klebsiella spp*., 24% of *Pseudomonas spp*., and 32% of *Staphylococcus aureus* were methicillin-resistant (MRSA).
Conclusions: Gram negative bacteria are more prevalent in patients in intensive care units with a decrease in sensitivity patterns to the antibiotics commonly available; which calls for an alarm to the healthcare workers.

Keywords: Antimicrobial sensitivity, ICU, Mechanical ventilation

INTRODUCTION

The burden of life-threatening conditions requiring mechanical ventilation (MV) in the intensive care unit has grown significantly in the last couple of years because of the emerging pandemic, urbanization, and hospital expansions.[1] Lower respiratory tract infection (LRTI) is common in an intensive care unit (ICU), with an increase in incidence from 10% to 25%, and mortality from 22% to 71%.[2] The species of microorganisms isolated from the respiratory samples from the ICU patients vary greatly from country to country and from ICU to ICU. However, the most common microorganisms causing LRTI are Acinetobacter *baumannii*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*.[3]

Epidemiological studies revealed that about 70% of patients admitted to the intensive care unit (ICU) require mechanical ventilation at some point during their stay in ICU due to various medical conditions. Recent surveillance information from the national nosocomial infection surveillance system of the Centers for Disease Control of USA showed that hospital-acquired pneumonia (HAP) or commonly known as 'nosocomial pneumonia' is the most typical infection in the ICUs.[4] HAP is the most common nosocomial infection in mechanically ventilated patients in ICU, labelled as ventilator-associated pneumonia (VAP). Several studies found that mortality rate of VAP ranged from 24% to 80%.[5] Patients who are mechanically ventilated are at higher risk of acquiring respiratory infections due to complex interplay between the Endotracheal tube (ETT), host immunity, and the virulence of invading bacteria. Although the exact mechanism of VAP is not understood but it is believed that its strong link exists with the microbial consortium on the ETT.[6] Our study focuses on the bacteria isolated from the respiratory samples received in our laboratory and further detection of their antibiotic susceptibility pattern which was used to guide the physicians, intensivists to initiate the empirical therapy. This also helped in establishing the importance of Regular screening of respiratory secretions for early diagnosis of the microorganisms linked to VAP that can have a positive impact on patient treatment and survival. The present study was designed to know the bacterial profile of respiratory samples and to determine their antimicrobial sensitive pattern among patients admitted to the intensive care units of the institute.

METHODS

A retrospective cross-sectional laboratory-based study was carried out for a period of 2 years from January 2022- December 2023. All endotracheal and tracheal samples were received from intensive care units of our hospital. Samples received from units other than intensive care units were excluded from the study. A total of 602 endotracheal aspirates and tracheal aspirates were cultured and analysed in the clinical microbiology laboratory of the hospital. Bacterial species were identified by standard microbiological methods and antibiotic sensitivity of the bacteria isolated was done by the modified Kirby Bauer disc diffusion method according to CLSI guidelines.

For Sample collection, endotracheal aspiration was done using a sterile 22 inch, 12F suction catheter. The catheter was introduced through the endotracheal tube for at least 30 cm. Gentle aspiration was then performed without instilling saline solution. The first aspirate was discarded. The second aspirate was collected after tracheal instillation of 5 ml saline in a mucus collection tube. The specimens were sent to laboratory and were cultured within 1 hour of collection.

After routine examinations, endotracheal aspirates and tracheal aspirates were processed for a semi-quantitative estimation. A standardized wire loop of 1.2 mm diameter (1 µl volume) was used for inoculation. The inoculation was done initially in Blood agar and then in MacConkey agar and incubated aerobically at 37°C for 24 hours. After 24 hours. agar plates were examined for any growth of bacteria. A colony forming unit (CFU) count of 105/ml (i.e. 100 colonies) or 104/ml (i.e. 10 colonies) respectively, was considered significant.

Identification of bacteria was done by identifying the morphology characteristics, Gram stain, and biochemical tests interpretation from the isolated bacterial colonies.

Antibiotic susceptibility testing of the bacteria isolated was done by the modified Kirby Bauer disc diffusion method and interpretation of the result was done according to CLSI guidelines.

The results were entered in MS Excel sheet and data was analysed and plotted in pie charts, bar diagrams, and tables.

RESULTS

Total of 602 respiratory samples were received from six intensive care units situated in the hospital, out of which 328 (54%) of the isolates were sterile and 274 (46%) were culture-positive. Among positive cultures, 221(81%) were Gram negative bacilli followed by Gram-positive cocci 48 (18%), yeast 5 (2%), as shown in (Figure 1).

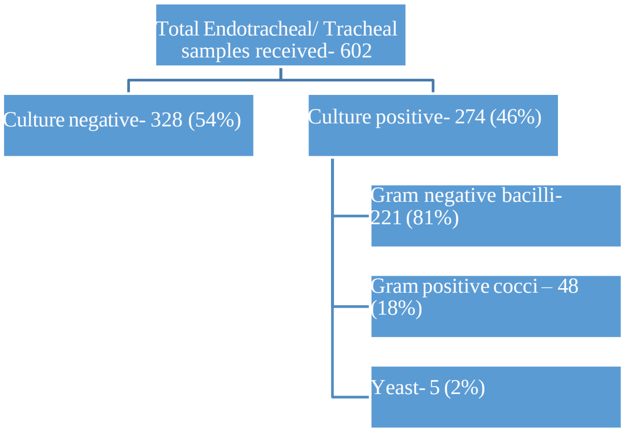

Figure 1: Total numbers of samples received, analyzed and plotted in pie chart depending upon growth and bacteria isolated.

Among the positive cultures, *Klebsiella spp*. 166 (61%) was the most frequent bacteria isolated followed by *Staphylococcus aureus* 46 (17%), *Pseudomonas spp*. 42 (15%), *Acinetobacter spp*. 8 (3%), *Escherichia coli* 3 (1%), *Proteus spp*. 2 (0.7%) and *Streptococcus spp*. 2 (0.7%) (Figure 2).

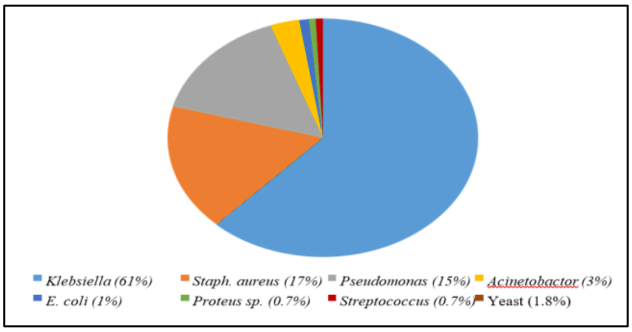

Figure 2: Percentage of various microorganisms detected in positive samples.

The sensitivity pattern of the three most frequent bacteria isolated was analysed.

Among the samples positively isolated for *Klebsiella spp*., all of them (100%) showed sensitivity to colistin followed by 97% showing sensitivity to tigecycline. furthermore, 67% showed sensitivity to cotrimoxazole, 58% to piperacillin tazobactam and carbapenems (imipenem and meropenem), 46% to cefepime, 44% to third generation cephalosporins (ceftriaxone, cefotaxime, ceftazidime). Lesser percentage showed sensitivity to aminoglycoside 35%. Least sensitivity was seen to fluoroquinolones (30%). This has been shown as a bar diagram (Figure 3).

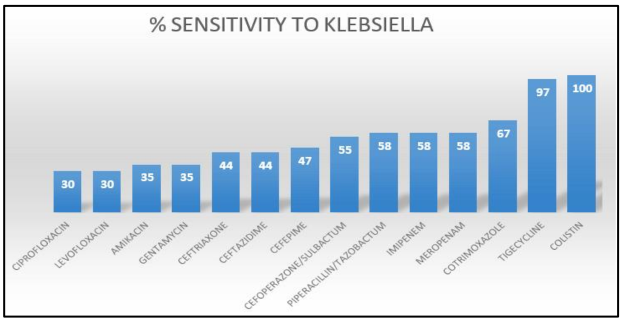

Figure 3: Percentage of antibiotic sensitivity detected in *Klebsiella* species.

Staphylococcus aureus isolated from the culture positive samples showed 100% sensitivity to linezolid and vancomycin followed by 80% sensitivity to chloramphenicol. Clindamycin and cefoxitin showed almost same percentage of sensitivity (69% and 68% respectively). Staphylococcus aureus isolated showed 64% sensitivity to gentamycin, erythromycin and azithromycin, 63% of isolates showed sensitivity to fluoroquinolones, cefazolin. Least sensitivity was seen to cotrimoxazole (54%) (Figure 4).

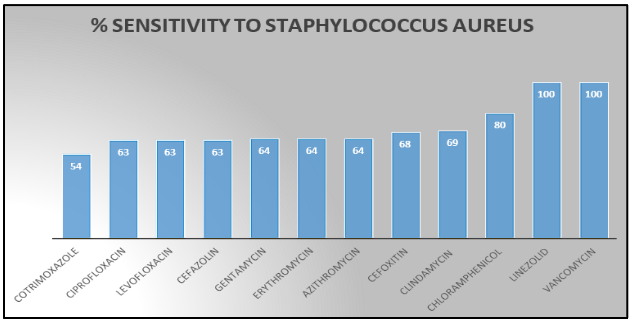

Figure 4: Percentage of antibiotic sensitivity detected in *Staphylococcus aureus.*

Culture positive isolates of *Pseudomonas spp*. showed 100% sensitivity to tigecycline and colistin, followed by 76% showing sensitivity to piperacillin tazobactam, and 75% to carbapenems. Lesser sensitivity was seen to aztreonam (42%), ceftazidime and cefepime (38%), aminoglycosides (31%). Least sensitivity was seen to fluoroquinolones (26%) This is depicted in Figure 5.

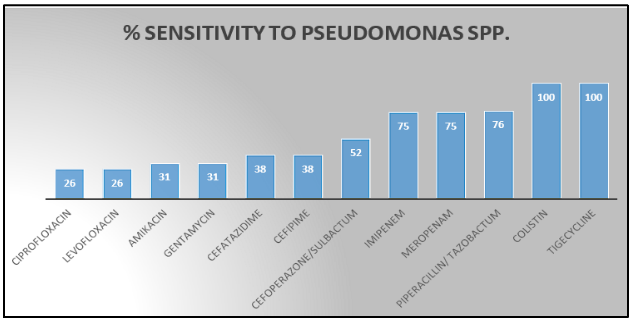

Figure 5: Percentage of antibiotic sensitivity detected in *Pseudomonas* species.

Table 1: Antibiotic susceptibility test of three most common isolated Gram negative bacteria from respiratory samples.

	Klebsiella spp. (n=166)				*Pseudomonas spp.* (n=42)				*Acinetobacter spp.* (n=8)			
	S	%	R	%	S	%	R	%	S	%	R	%
Amikacin	58	35	107	64	13	31	29	69	2	25	6	75
Gentamycin	58	35	107	64	13	31	29	69	2	25	5	75
Ciprofloxacin	49	30	115	69	11	26	31	74	2	25	6	75
Levofloxacin	49	30	115	69	11	26	31	74	2	29	6	71
Ceftriaxone	73	45	92	55	-	-	-		1	13	7	87
Ceftazidime	73	45	88	53	16	38	26	62	2	25	6	75
Cefepime	77	47	88	53	16	38	26	62	1	13	7	87
Piperacillin/tazobactam	96	58	70	42	32	76	10	24	2	25	6	75
Cefoperazone/sulbactam	92	56	72	43	11	52	10	48	1	17	5	75
Imipenem	96	58	69	42	30	75	10	25	2	25	6	75
Meropenam	96	58	69	42	30	75	10	25	2	25	6	75
Colistin	166	100	0	0	42	100	0	0	8	100	0	0
Tigecycline	161	97	5	3	42	100	0	0	8	100	0	0
Cotrimoxazole	112	67	36	22	-		-		-			-

Overall, 33% of *Klebsiella spp*. and 24% *Pseudomonas spp*. were multidrug resistant and about 32% were methicillin resistant *Staphylococcus aureus* (MRSA).

DISCUSSION

Our study showed that the most frequent bacteria isolated from respiratory samples was *Klebsiella spp.* followed by *Staphylococcus aureus* and *Pseudomonas spp.* Di Pasquale et al in their ICU-acquired pneumonia and few other studies have shown similar findings of *Klebsiella spp., Staphylococcus aureus, Pseudomonas spp*., and *Acinetobacter spp.* as the most common bacteria isolated from tracheal or endotracheal samples of critically ill patients admitted in ICU.[7-9] A study done on VAP patients by Rose et al showed that the most common bacteria isolated from the respiratory samples was *Klebsiella spp.*, which is in line with our study.[10] However, some studies like Kohbodi et al and Kabak et al showed that Gram-positive cocci *Staphylococcus aureus* was the most common pathogen isolated from the endotracheal aspirates from the patients admitted in ICU and on mechanical ventilation.[11]

In our study, it was also observed that Gram negative bacilli [221/274 (81%)] were more prevalent than the Gram positive cocci [48/274 (18%)] among the culture-positive which is in line with the study done by Mahendra, et al on patients admitted in respiratory ICU.[12] Paterson et al and Sydnor et al in their epidemiological studies suggest that the possible cause of higher prevalence of Gram negative bacterial infection in ICU setting is that Gram negative bacteria like *Klebsiella spp., Pseudomonas, Escherichia coli* are present in urinary catheters, equipment, and other contaminated fluids.[13] These bacteria can survive in inanimate surfaces for months and can live longer compared to Gram positive bacteria.[13,14] *Pseudomonas spp*. can also be present on objects like aerators, sinks, respiratory equipment, and any other water sources.[14] Together with *Acinetobacter species*, these bacteria can be passed on from person to person or by environmental contamination.[15] The overall antibiogram of our study showed that Gram-negative bacilli show less than 50% sensitivity to Aminoglycosides, fluoroquinolones, third-generation cephalosporins. Fair sensitivity was seen to Piperacillin tazobactam and carbapenems (58-75%) and the highest sensitivity was observed to colistin and tigecycline (100%). The sensitivity to aminoglycosides and cephalosporins in our study was similar to Behera et al study on antimicrobial susceptibility patterns of bacteria in ICU patients with lower respiratory tract infection.[2] However, the sensitivity of our study to colistin and tigecycline did not match with that study. The factors that lead to the development of rapidly emerging highly resistant Gram-negative bacilli include longer duration of stay in ICU, hospitalization for more than 48 hours within the previous three months, ages over 65, use of mechanical devices and inadvertent use of broad-spectrum antibiotics like fluoroquinolone, carbapenem, third-generation cephalosporins, antimicrobial therapy within the last three months, a high prevalence of antibiotic resistance in the community are risk factors.[16,17]

Klebsiella spp. belongs to the Enterobacteriaceae family. They can be commensals in various environments like soil, water, and various plants, insects, birds and animals and is widely distributed in human and animal mouth, skin, respiratory tract, urogenital tract, and intestine.[18] In our study *Klebsiella spp.* was highly sensitive to colistin and tigecycline followed by cotrimoxazole (67%) and then carbapenems and beta-lactam/beta-lactamase inhibitor combination (58%). Our study also showed that third and fourth generation cephalosporins show sensitivity of 44-46% and sensitivity to aminoglycosides, and fluoroquinolones was 30-33% only. *Klebsiella spp.* is prone to form biofilms, which have osmotic barrier properties and are resistant to antimicrobial agents reducing the sensitivity to gentamicin, ampicillin, and ciprofloxacin.[19,20] In our study about 33% of the *Klebsiella spp.* were multidrug resistant. In a study by Asri et al, nosocomial multidrug resistant *Klebsiella spp.* was 32.8% which is in consistent with our study. The range of Multidrug resistant *Klebsiella spp.* showed the lowest value of 12.9% in North America and a maximum of 82% in Bangladesh.[21,22] The mechanisms responsible for antibiotic resistance in *Klebsiella spp*. include enzymatic antibiotic inactivation and modification, antibiotic target alteration, porin loss and mutation, increased efflux pump expression of the antibiotic, and biofilm formation.[23,24]

In our study, the prevalence of *Pseudomonas spp* is 15%, which is within the range given by Vincent et al, in their study on prevalence and outcomes of infection among patients in ICU, suggest that the prevalence of *Pseudomonas spp.* in patients with intensive care unit (ICU)-acquired infections can reach up to 23%.[25] *Pseudomonas spp* is inherently resistant to several antibiotics such as ampicillin, amoxycillin clavulanate, ceftriaxone, cefotaxime, tetracyclines, nitrofurantoin, ertapenem, cotrimoxazole and chloramphenicol mainly due to the synergy between multi-drug efflux system or a type 1 AmpC β-lactamase and low outer membrane permeability.[2] A multicenteric study performed in Spain showed Multidrug resistant *Pseudomonas spp.* to be 26% which is similar to our study, which found 24% Multidrug resistance in *Pseudomonas spp.*[28] The prevalence of resistant *P. aeruginosa* can reach 48.7% in the ICU.[29]

S. aureus is found in the environment and in normal human flora, located on the skin and mucous membranes (most often the nasal area) of healthy individuals.[30] The main mechanism underlying the infection is the capacity of *S. aureus* to adhere to the devices, plastic material as well as to the matrix molecules that cover the devices soon after insertion, and to form a biofilm on the device.[31] In our study *Staphylococcus aureus* shows maximum sensitivity to vancomycin and linezolid 100%, followed by chloramphenicol at 80%, macrolides and lincosamides 64% and 69% respectively, cefoxitin 68%, gentamycin 64% and fluoroquinolones 63%. Around 32% of *Staphylococcus aureus* were methicillin resistant *Staphylococcus aureus* (MRSA). Infections by MRSA are accompanied by increased mortality, morbidity, and hospital stay, as compared to those caused by methicillin-sensitive *S. aureus* (MSSA).[32] In our study, the prevalence of MRSA is about 32%, which is within the range found by Patil et al in their meta-analysis on the prevalence of MRSA in India (25-55%) After analyzing our data (Table 1), based on the observations of sensitivity pattern in Gram negative bacilli isolated from respiratory samples from patients in ICU we recommend that beta lactam/beta lactamase inhibitor combination like piperacillin tazobactam or carbapenems in combination with aminoglycosides should be considered for empirical therapy in ICUpatients with respiratory distress.[33]

Our study data is limited to one tertiary care hospital and does not represent patterns across other hospitals or regions, reducing generalizability. Also, our study does not account for patient factors such as age, underlying conditions or previous antibiotic exposure. This could influence resistance patterns observed. Also, our study does not include resistance patterns in non-ICU patients or other hospital departments. The accuracy of bacterial identification and sensitivity testing can also be affected by the use of diagnostic methods. The method used in our study is culture based which is a conventional technique whereas now automation is also available which can alter the results.

CONCLUSION

In our study, Gram negative bacteria are most prevalent in patients in Intensive care units and have high resistance to the antibiotics commonly available. This observation calls for an alarm to the healthcare workers. With the emergence of multi-drug resistant bacteria and Pan drug-resistant bacteria, new antibiotics are being invented, and new drug combinations are being tested and used in ICUs, but there is still a need to emphasize the rational use of antimicrobials and strict adherence to the concept of “reserve drugs” to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential for ICU monitoring of the resistance patterns.

An appropriate antibiotic policy is an urgent need in all the ICUs to stop the rapid emergence and spread of multi drug resistance. The future of prevention of infections in ICUs will likely be based on our ability to adapt to policies and emerging technologies to specific risk profiles.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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