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Bacteriological profile and their antibiogram from Endotracheal aspirate and Bronchoalveolar lavage fluid samples from intensive care unit patients

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ABSTRACT

Background: Bacterial respiratory infections are most commonly causes of illness for all age group patients in ICU. Most of the patients suffer from urosepsis, postoperative disease and lower respiratory infection when admitted in ICU's. The main purpose of this study is control in ICU's infection & determine their antibiotic sensitivity pattern for better treatment of patients.

Aim & Objective: Bacteriological profile and their antibiogram from endotracheal aspirate and bronchoalveolar lavage fluid samples from intensive care unit patients.

Materials and Methods: We performed manual culture of Endotracheal aspirate & BAL fluid specimens collected from ICU's patients. Samples were cultured on Blood agar and Mac-Conkey agar then incubated at 37°C for 24 hours. Any growth were further confirmed by Gram stain and appropriate biochemical tests, and then Antibiotic sensitivity test was done.

Result: Among the 123 mechanically ventilated patients and 68 of them developed positive samples. In this study 55.3% (68) pathogenic isolates, 4.1% (5) were non pathogenic isolates and 40.6 (50) were no growth isolates in BAL and endotracheal aspirate samples. In which 67.6% (46) male and 32.4% (22) female patients. Older population falling in the 40-50 years of age group contributes the majority (27.64%) of the infection undergoing treatment for LRT infection in ICU's patients in TMMC & RC, Moradabad, and in 70-80 years of age group (4.06%) was less contributes of this infection. Among the causative agents, *Pseudomonas*, *Acinetobacter*, *Klebsiella*, *E.coli*, *Enterobacter*, *Citrobacter* were most common among gram negatives and *Staphylococcus aureus* was common among the gram positive isolates. In our study more resistance gram positive antibiotics were CIP, E, LE, and most sensitive antibiotics were VA, LZ, GEN and TEL. And gram negative bacteria most resistant antibiotics were OF, CPM, CAZ, CIP, CTR, MRP, LE, IPM, PIT and most sensitive antibiotics were AK, GEN, CL, TGC. In this study we conclude that Gram-negative bacilli were predominant in BAL fluid and endotracheal aspirates of ICU patients. Out of 68 multidrug resistance isolates, 1.47% were MRSA, 41.17% were ESBL and 57.36% were MBL isolates higher rate of ESBL production seen in *Pseudomonas* spp., and higher rate of MBL production in *Acinetobacter* spp.

Conclusion: High incidence of VAP and the potential MDR pathogens are a real threat in our ICU's. Combined approach of judicious antibiotic usage and training programs to health care personnel might be of help in combatting high incidence of antibiotic resistance in our institute.

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1. Introduction

Bacterial respiratory infections are most commonly causes of illness for all age group patients, which cured with

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invasive mechanical ventilation in ICU.¹ It is systemic pathway via which body acquired fresh oxygen and remove carbon dioxide. The respiratory tract system are classified in the following parts upper and lower respiratory tracts.²

Upper respiratory tract structure consist of nose, nasal passages, paranasal sinuses, the pharynx and the part of larynx above the vocal folds. The commonly occurring URTI are common cold, tonsillitis, pharyngitis, laryngitis, rhino sinusitis and otitis media.³

Lower respiratory tract structure includes larynx, vocal folds, trachea, bronchi, bronchioles and alveoli. The most commonly occurring respiratory tract infection are tracheitis, bronchiolitis and pneumonia. It can have severe effects which may lead to hospitalization and loss of life.⁴

In ICU's nosocomial infection have been included as a severe problem among intubated patients leading to major mortality and morbidity. Most of the patients suffer from urosepsis, postoperative disease and lower respiratory infection when admitted in ICU's.⁵

Respiratory tract infection are generally caused by *Acinetobacter baumannii*, *P. aeruginosa*, *K. pneumoniae*, *Citrobacter koseri*, *Enterobacter spp.*, *Staph. aureus*, *Staph. epidermidis*, *E. coli*, *Proteus spp.*, *Serratia*, *Burkholderia* are the most common organisms isolated from BAL and endotracheal sample in the patients of ICU who were affected by lower respiratory infection.⁶

2. Materials and Methods

The study was carried out in the department of microbiology, in TMU hospital Moradabad. Total numbers of 123 clinical samples were processed in which 68 pathogenic isolates were found. The samples were collected properly taken in clean, leak proof sterile container & tubes from various ICU's in our hospital. All sample received in the microbiology lab, smears were examined after performing gram staining to see the character of exudates, number and type of organisms. Culture of ET secretion, BAL fluid & trans-tracheal aspirates was done on Blood agar plates and Mac-Conkey agar plates and incubated at 37°C for overnight in the incubator. After 12 hours colonies appear (if growth) were processed for subsequent identification. Colonies have been identified on the basis of colony morphology and culture characteristics and After gram's staining speciation and do biochemical's & Antimicrobial Sensitivity pattern was by Kirby-Bauer disc diffusion method.^{7,8}

2.1. Identification & antimicrobial sensitivity testing

The Kirby-Bauer disc diffusion method used in this research, according with the CLSI guidelines, using antibiotic discs, all isolates were tested for antimicrobial susceptibility in Muller Hinton agar.^{9,10}

2.2. Screening and confirmation for MRSA, ESBL, MBL producers

CLSI has developed disk diffusion tests for the possible MRSA, ESBL, MBL production. CLSI has proposed disk screening for MRSA production by *Staphylococcus aureus* and for ESBL, MBL, production by *E.coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Enterobacter spp.*, *Citrobacter spp.*, *Acinetobacter spp.*^{10,11}

Confirmatory test for MRSA All microorganisms were isolated and tested for methicillin resistance using a Cefoxitin disc (30mcg) and incubated overnight at 35°C according to the CLSI guidelines. The zone of inhibition was studied under reflected light on a non-reflected backdrop on Petri plates. According to CLSI guidelines isolated organisms demonstration an inhibition of <14 mm were considered MRSA.^{9–12}

Confirmatory test for ESBL production all microorganisms were isolated and using a Cephalosporins/clavulanate combination Disk. The CLSI advocates the use of Ceftazidime (30mcg) disks with or without clavulanate (10mcg) for confirmation of the presence of ESBLs. A difference of >5 mm between the zone diameters of either of the Ceftazidime disks and their respective Ceftazidime/clavulanate disk is taken to be phenotypic confirmation of ESBL production, and were using to imipenem (10mcg) disk for testing MBL production.^{10,12}

3. Results

The samples were obtained from the patients admitted in ICU's of our hospital. Out of 123 total suspected cases of BAL fluid and endotracheal aspirate were included in this study among in which 68 (55.3%) positive samples were found.

Table 1: Showing sex wise distribution, Out of 123 total samples size.

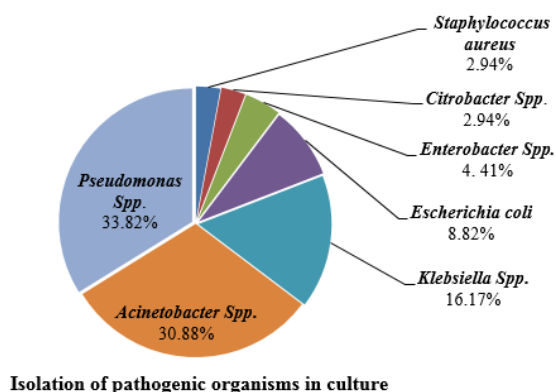
Gender	Number	Percentage
Male	79	64.2%
Female	44	35.8%
Total	123	100%

Table 2: Showing isolated organisms out of 123 samples.

Organisms isolated	Number	Percentage
Pathogenic isolates	68	55.3%
Non pathogenic isolates	5	4.1%
No growth	50	40.6%
Total	123	100%

Table 3: Showing antibiotic sensitivity pattern of isolated organisms.

		Sensitive antibiotics (Number and percentage)					
		<i>Citrobacter Spp.</i> (n=2)	<i>Klebsiella Spp.</i> (n=11)	<i>Enterobacter Spp.</i> (n=3)	<i>Pseudomonas Spp.</i> (n=23)	<i>Acinetobacter Spp.</i> (n=21)	
Staphylococcus aureus (n=2)							
Vancomycin (n=2)	100%			Amikacin (n=2)	Imipenam (n=19)	Levofloxacin (n=5)	
Linezolid (n=2)	100%			66.6%	82.6%	23.8%	
				Ceftazidime (n=2)	Cefoparazone/ Sulbactam (n=19)	Gentamicin (n=5)	
				66.6%	82.6%	23.8%	
E.coli (n=6)							
Tigecycline	100%						
(n=6)	100%						
Amikacin	83.4%						
(n=5)	83.4%						
Acinetobacter Spp. (n=21)							
Levofloxacin	83.4%						
(n=5)	83.4%						
Ticarcillin/ Clavuliniacid	83.4%						
(n=5)	83.4%						
Enterobacter Spp. (n=3)							
Meropenem	66.6%						
(n=2)	66.6%						
Colistin	66.6%						
(n=2)	66.6%						
Pseudomonas Spp. (n=23)							
Ticarcillin/ Claculinicacid	65.3%						
(n=15)	65.3%						
Colistin	60.8%						
(n=14)	60.8%						
Acinetobacter Spp. (n=21)							
Amikacin	60.8%						
(n=14)	60.8%						
Klebsiella Spp. (n=11)							
Colistin	45.5%						
(n=6)	45.5%						
Gentamicin	45.5%						
(n=5)	45.5%						
Enterobacter Spp. (n=3)							
Imipenem	66.6%						
(n=2)	66.6%						
Ciprofloxacin	66.6%						
(n=2)	66.6%						
Acinetobacter Spp. (n=23)							
Meropenem	69.5%						
(n=16)	69.5%						
Ticarcillin/ Claculinicacid	65.3%						
(n=15)	65.3%						
Colistin	60.8%						
(n=14)	60.8%						
Acinetobacter Spp. (n=21)							
Amikacin	60.8%						
(n=14)	60.8%						
Klebsiella Spp. (n=11)							
Colistin	66.6%						
(n=2)	66.6%						
Enterobacter Spp. (n=3)							
Tigecycline	66.6%						
(n=2)	66.6%						



Isolation of pathogenic organisms in culture

Fig. 1: Pie chart showing Isolation of pathogenic organism shows most prevalent organism *Pseudomonas* 23(33.82%), *Acinetobacter* 21(30.88%), *Klebsiella* 11(16.17%), *E.coli* 6(8.82%), *Enterobacter* 3(4.41%), *Citrobacter* 2(2.94%), *Staphylococcus aureus* 2(2.94%).

Table 4: Showing out of 68 positive isolates in which 1.47% were MRSA, 77.94% were ESBL, and 55.88 % were MBL.

Multidrug resistant organisms	Number	Percentage
MRSA	1	1.47%
ESBL	53	77.94%
MBL	38	55.88%

4. Discussion

The current study done in TMMC & RC, Moradabad, U.P. Respiratory tract disease are one of the major public health issues, affecting both children and adults; they appear to be more serious when located in the LRT. The most common infections seen in the community and among hospitalized patients remains pneumonia. Out of the 123 endotracheal and BAL fluid sample were collected after receiving written consent from the patient 64.22% (79) constitutes male population, the remaining 35.78% (44) being the female population.

In our study 55.28% (68/123) organisms were isolated in BAL and endotracheal aspirate samples. In which 67.6% (46) male and 32.4% (22) female patients. Older population falling in the 40-50 years of age group contributes the majority (27.64%) of the infection undergoing treatment for LRT infection in ICU patients in TMMC & RC, Moradabad. And in 70-80 years of age group (4.06%) was less contributes of this infection. A study conducted in Siriraj hospital from Thailand was highly comparable to our study. In this study done by Werarak P and Kiratisin P et al.¹³ who reported 24.7% HAP and 75.3% VAP patients. In this study 70% constitute male population and 30% female population with the mean age of 70 years respectively. The study was also based on parameters of age and gender. It was found that in our study both in male were more affected

due to various reasons.

Out of the total 68 positive samples was Analysis of our study *Pseudomonas* 33.82% as the most common pathogen then *Acinetobacter* 30.88%, *Klebsiella* 16.17%, *E.coli* 8.82%, *Enterobacter* 4.41%, *Citrobacter* 2.94% and *Staphylococcus aureus* 2.94%. A resembling study was done by Khatun Mst and Shamsuzzaman S.M. et al.⁵ percentage of *Acinetobacter baumannii* were 34.21%, *Pseudomonas aeruginosa* 15.79%, *Klebsiella spp.* 23.68%, *Citrobacter spp.* 2.63%, *Enterobacter spp.* 2.63%, *Staphylococcus aureus* 7.89%, *Staphylococcus epidermidis* 2.63%, *Moraxella catarrhalis* 5.26%, *Proteus spp.* 5.26%.

Our study also revealed about the antibiogram that in lower respiratory tract infection in ICU's patients. The most resistant antibiotics in gram positive bacteria Ciprofloxacin, Erythromycin, Levofloxacin and more sensitive antibiotics were Vancomycin, Linezolid, Teicoplanin, and Gentamicin, and the gram negative bacteria most resistant antibiotics Ofloxacin, Cefepime, Cefoperazone, Ciprofloxacin, Ceftriaxone, Meropenem, Levofloxacin, Piperacillin/tazobactam and more sensitive antibiotics Amikacin, Gentamicin, Colistin, Tigecycline A correlated with the study done by Shahrokhi E. et al.¹⁴ having similar results alike Piperacillin/tazobactam was resistant antibiotic and Ceftazidime, Amikacin being the most sensitive antibiotics.

In this study we conclude that Gram-negative bacilli were the predominant of BAL fluid and endotracheal aspirates of ICU patients. Out of 68 multidrug resistance isolates, 1.47% were MRSA, 77.94% were ESBL and 55.88% were MBL isolates. Higher rate of ESBL production seen in *Pseudomonas spp.*, and higher rate of MBL production in *Acinetobacter spp.* A correlated with the done by Santosh khalal et al.¹⁵ having similar results alike 5.4% were MRSA, 41.1% were ESBL and 53.4% were MBL isolates. Higher rate of ESBL production was seen in *Pseudomonas spp.*, and higher rate of MBL production in *Acinetobacter spp.*

5. Conclusion

The goal of our study was bacteriological profile and their antibiogram from endotracheal aspirate and BAL fluid samples in our hospital. In our study LRTI are the most common causes of nosocomial pathogens and that are responsible for causing variety of human illness. The emergence of multi-drug resistant organisms is more common in the current age of antibiotic resistance.

In our study total 123 patients included in which pathogenic isolates were obtained in 55.3% of cases. Most common pathogenic organism found to be *Pseudomonas*, *Acinetobacter*, *Klebsiella*, *E.coli* and *Enterobacter*, *Citrobacter*, *Staphylococcus aureus*. Since bacterial isolation may vary in different geographical regions and even over time in the same location and population. In this study we found 40.6% were no growth and 4.1% were non

pathogenic isolates (may be *S. pneumoniae*, *Candida spp.*) in ICU patients. Higher prevalence of LRTIs observed in the age group 41-50 years, as well as LRTIs still a major health threat in old age group.

In our study most common antibiotic sensitivity pattern for Gram positive cocci more sensitive were Gentamicin, Vancomycin, Teicoplanin, Linezolid and for Gram negative bacteria more sensitive were Amikacin, Gentamicine, Colistin, Tigecycline.

In this study we conclude that Gram-negative bacilli were predominant in BAL fluid and endotracheal aspirates of ICU patients. Out of 68 multidrug resistance isolates, 1.47% were MRSA, 41.17% were ESBL and 57.36% were MBL isolates. Higher rate of ESBL production was seen in *Pseudomonas spp.*, and higher rate of MBL production in *Acinetobacter spp.* In the main purpose of this study is control in ICU infections, to determine the common bacterial isolates and their antibiotic sensitivity pattern for better treatment of patients.

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7. Conflicts of Interest

The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this article.

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