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Bacterial isolates from Tracheal Aspirate of Patients in Intensive Care Unit and Their Antimicrobial Susceptibility Pattern in Tertiary Care Hospital in Dhaka, Bangladesh

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Abstract

Nosocomial infections are a critical issue among patients in the intensive care unit, particularly among intubated patients, leading to significant mortality and morbidity. So, accurate and timely identification and microorganism and their antimicrobial susceptibility pattern will help in the selection of proper antibiotics and prevent misuse in ventilated patients. The aim of this retrospective study was to determine the bacterial profile and susceptibility pattern of bacteria isolated from endotracheal aspirates of ventilator-associated pneumonia. This retrospective study was conducted in the department of microbiology and immunology, Bangladesh Medical University, Dhaka, from June 2023 to July 2024 for a period of one year. Written consent was taken from the authority. A total of 542 samples were collected from tracheal aspirates of the patients who were admitted to the hospital in the intensive care unit. A total of 542 tracheal aspirate samples were processed and cultured following standard techniques used in the medical microbiology laboratory. The isolated bacteria were identified by colony morphology, Gram staining, and biochemical reactions. Antibiotic susceptibility testing of the detected isolates was performed by Kirby-Bauer disc diffusion techniques as per guidelines set by the Clinical Laboratory Standards Institute (CLSI). Microsoft Excel software was used for data analysis.

About 542 Tracheal aspirate samples were collected, of which 371(68.45%) showed bacterial growth. Out of 371 bacterial growths, the majority (44.28%) of culture-positive cases were in the age group 56- 65 years, and 67.15% were male. Of the 371culture growth, 371(100%) were gram-negative bacteria. *Klebsiella spp.* (45.83%) was the prevailing isolate, followed by *Acinetobacter spp.* (38.27%), *Pseudomonas spp.* (14.56%), *Proteus spp.* (0.81%), *Enterobacter spp.* (0.26*%) and Morganella morganii* (0.26%). Among *Klebsiella spp*. were resistance to ceftriaxone (95.29%), followed by cefotaxime (91.17%), ciprofloxacin (90.0%), same to cotrimoxazole (89.41%), amoxicillin, cefuroxime (91.17%), and Ceftazidime, tazobactam-piperacillin (88.23%). The highest sensitivity was exhibited for colistin, which demonstrated 1.77% resistance among *Klebsiella spp.*, and the least resistant to amikacin (69.41%), meropenem (70.58%), and gentamicin (71.77%). Isolated *Acinetobacter spp.* were mostly resistant to cotrimoxazole and ciprofloxacin, ceftriaxone, ceftazidime, cefotaxime (95.07%), followed by gentamicin, amikacin (88.2%), meropenem, tazobactam-piperacillin (87.32%), but least resistance to colistin, which was 0.71%. Among *Pseudomonas spp.* showed the highest sensitivity, 98.14 % to colistin, followed by tazobactam-piperacillin (40.75%) and meropenem, and cefepime (35.18%), but higher resistant to ciprofloxacin (92.60%), followed by gentamicin (81.48%), ceftazidime (72.22%) and amikacin (70.37%). Most *proteus spp.* were resistant to amoxicillin, cotrimoxazole, ciprofloxacin, gentamicin and cefotaxime

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(100.0%). But higher sensitive to meropenem (66.66%), followed by ceftriaxone (33.33%). All the isolates were resistant to colistin, which is intrinsically resistant to it. Among *Enterobacter spp.* higher sensitive to colistin and cefepime, which was 100%. But other common drugs are resistant to it. In *Morganella morganii,* 100% sensitive to ceftazidime, amikacin, cefepime, tazobactam-piperacillin, netilmicin, but 100 % resistant to ciprofloxacin, gentamicin, meropenem, and colistin. These results indicate that the isolation rate from tracheal aspirate samples was high, and the increasing trend of antibiotic resistance to isolated bacteria is alarming, which may lead to treatment failure.

Keywords: Tracheal aspirate sample, Bacterial pathogens, and antimicrobial resistance

Introduction

Ventilator-associated pneumonia is defined as pneumonia occurring more than 48 hours after patients have been intubated mechanical ventilation. Nosocomial infection among hospitalized patients, and those treated in the intensive care unit on ventilators, are at 2-12 times higher risk of developing nosocomial pneumonia [1.2]. The hospital-acquired infection has been associated with an increased rate of mortality and mobility, and prolonged hospital stay [3]. It is reported that mortality and morbidity rates in HAI are higher among intubated patients in the ICU (50%) than among patients in general wards (5-10%) [4]. In the ICU, most of the patients suffer from urosepsis, post-surgical infection, and lower respiratory infection [5]. The modern apparatus responsible for HAI includes are endotracheal tube, a catheter, and different surgical appliances [6]. Tracheal colonization of different bacteria may be responsible for added or superinfections and at the same time, increase the risk of mortality [8]. The etiological agents differ, depending upon the different factors like ICU type, prior use of antibiotics, and pre-existing diseases [9]. Various studies have reported that more than 30 % hospital hospital-acquired infections and more than 40% of ICU patients' infections are caused mainly by gram-negative bacteria [10,11]. The antibiotic resistance among these ICU pathogens is due to the use of broad-spectrum antibiotics [12]. The worldwide causes of resistance among these hospital-acquired organisms are misuse and overuse of antibiotics [13]. It is reported that increased use of β-lactam drugs can result in bacterial resistance towards these antimicrobial agents, and by producing β-lactamases, it develops resistance to a broad range of β-lactams antibiotics. The treatment options against the infection caused by these MDR bacteria are limited, thus, it appeared as a major challenge for clinicians [14]. Therefore, the present study was undertaken to determine the frequency and antimicrobial susceptibility of organisms isolated from tracheal aspirate specimens, which will help clinicians to choose the correct antimicrobial therapy against isolated microorganisms.

Materials and Methods

Study design

A retrospective study was conducted in the Department of Microbiology & Immunology at the Bangladesh Medical University (BMU), Dhaka, from July 2023 to June 2024 for a period of one year. Written consent was taken from the corresponding authority.

Study samples

All the Tracheal aspirate samples were collected from admitted patients of the BMU in the ICU. Patients of any age and sex with provided tracheal aspirate samples at the microbiology Laboratory during that period were included. Samples received in non-sterile containers, dry samples were excluded from the study. A comprehensive dataset regarding demographic data, previous antibiotic therapy, and laboratory results of bacterial isolation and susceptibility patterns was collected from the Laboratory specimen logbooks using the standard data collection form. The physical characteristics of the sample were noted.

Laboratory Procedure

A total of 542 tracheal aspirate samples were obtained by sterile suction through an endotracheal tube and suction catheter tip. The endotracheal aspirate was collected using a 22-inch ramsons 12-F suction catheter with a mucous extractor, which was gently introduced through the endotracheal tube. Gentle aspiration was then performed without instilling saline, and then the catheter was withdrawn from the endotracheal tube. After the catheter was withdrawn, 2 ml of sterile 0.09% normal saline was injected into it with a sterile syringe to flush the exudate into a sterile closed vacuum container using aseptic techniques, inserting a tracheal aspiration probe up to the carina. The container is sealed by physicians and sent to the microbiology laboratory for microbiological examination.

Organism Isolation and antimicrobial susceptibility

The sample was inoculated onto blood agar, Chocolate agar and MacConkey agar media and Sabouraud's dextrose agar media. The inoculated aerobic bacterial cultures were incubated overnight at 37°C for 24-48 hours. Chocolate agar was incubated in a CO_2 incubator for 24-48 hours. Organisms were identified based on colony characters, Gram staining, and biochemical reactions according to standard microbiological procedures. All the isolates were tested for antimicrobial susceptibility on Muller Hinton Agar (HI Media, India) by the Kirby Bauer disc diffusion method, according to the Clinical Laboratory Standard Institute (CLSI) guidelines

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[7]. The following antibiotics were used for gram-negative bacteria: amoxicillin (10µg), amoxicillin-clavulanic acid, ciprofloxacin (5µg), ceftriaxone (30µg), cefotaxime(30µg), ceftazidime (30µg), aztreonam(30µg), trimethoprim sulphamethoxazole (1.25/23.75µg), cefuroxime(30µg), amikacin (30µg), meropenem(10µg), piperacillin-tazobactam (100/10µg), cefepime (30µg). For gram-positive bacteria, the following antibiotics were used: amoxicillin(10µg), ciprofloxacin (5µg), cefradine (30 µg), cloxacillin (5µg), erythromycin (15µg), trimethoprim sulphamethoxazole (1.25/23.75µg), cefoxitin(30µg), vancomycin(30µg), clindamycin(2µg), fusidic acid (10µg), and linezolid (30µg). All the antibiotic disks were commercially purchased from BioMaxima, Poland. *P. aeruginosa* ATCC 27853 and S. aureus ATCC 25923 were included as quality control strains for antimicrobial susceptibility testing. Colistin susceptibility was conducted by the Broth microdilution method according to the CLSI [7].

Data analysis: Data were cleaned manually and entered and analyzed by using SPSS version 24 software. The statistical analysis used in the study was descriptive and involved categorical data analysis. Frequency and percentage were examined for categorical independent variables. Results were presented through tables. P-value < 0.05 was considered statistically significant.

Ethical statement: All participants were given informed written consent to use their data and culture susceptibility reports to be used for the research.

Results

A total of 542 tracheal aspirate samples were analyzed at the Department of Microbiology & Immunology, Bangladesh Medical University, Dhaka, during the study period, but only 371 of them had complete laboratory information for analysis. Among 542 tracheal aspirate samples cultured, bacterial growth was obtained in 371 (68.45%), and 171 (31.55%) showed no growth (Table 1). Only Gram-negative organisms were identified in this study, which was 68.45%.

Out of culture positive cases most of the bacterial isolates were found in the age group of 56-65 years 240 (44.28%) followed by 46-55 age groups 130 (23.99%), 36-35 age group 77 (14.20%), more than 65 age group (11.63%) and lowest age group 15-25 which was 6 (1.10%). (Table-2).

Table 1: Frequency of Bacterial isolates in tracheal aspirate sample (542)

Culture	Frequency	Percentage (%)
Growth	371	68.45
No growth	171	31.55
Total	542	100

Table 2: Age and Sex distribution of study population(n=542)

Age in Years	Male	Female	Percentage (%)
15-25	6	0	1.1
26-35	18	8	4.8
36-45	51	26	14.2
46-55	84	46	23.99
56-65	160	80	44.28
>65	45	18	11.63
Total	364 (67.15%)	178(32.85 %)	542 (100%)

Only gram-negative bacterial isolates were included in this study. Among them predominant bacteria were *Klebsiella spp.* 170(45.83%) followed by *Acinetobacter spp.* 142 (38.27%), Pseudomonas *spp.* 54 (14.56%), *Proteus spp.* 3(0.81%). Only one bacterium was found like *Enterobacter spp.* (0.26%) and *Morganella morganilli* (0.26%). (Table 3).

Table 3: Isolated organisms from tracheal aspirate samples (371)

Isolated organism	Number	Percentage (%)
Klebsiella spp.	170	45.83
Acinetobacter spp.	142	38.27
Pseudomonas spp.	54	14.56
Proteus spp.	3	0.81
Enterobacter spp.	1	0.26
Morganella morganii	1	0.26
Total	371	100

The antibiotic resistance pattern of the six Gram-negatives isolated from the tracheal aspirate is shown (Table 4). Among isolated gram-negative bacteria, *Klebsiella pneumoniae*. were highly resistant to ceftriaxone (95.29%), cefotaxime (91.17%0 ciprofloxacin (90.0%), cotrimoxazole (89.41**%),** cefuroxime (91.17%), amoxicillin **(**89.41%) and same resistant to ceftazidime and tazobactam piperacillin (88.23%) and moderately resistant showed against gentamicin (71.77%) and amikacin (69.41%). The highest sensitivity was exhibited for colistin, which had only 1.77% resistance among the isolates. Among *Acinetobacter spp.* showed higher sensitivity to colistin 99.29% but higher resistance 95.07% to cotrimoxazole, ciprofloxacin, cefotaxime, and 88.02% resistance to gentamicin, respectively. And lowest sensitivity showed 10.62% to tazobactam-piperacillin, 11.87% to amikacin, and 13.12% resistance to ceftazidime and meropenem, respectively. Among isolated *Pseudomonas spp.* showed highest sensitivity 98.14% to colistin, 40.74% to tazobactam, 35.18% to meropenem, and cefepime. But highest resistant to ciprofloxacin (92.60%), gentamicin (81.48%), ceftazidime (72.22%), and amikacin (70.37%), respectively. Only one *Morganella morganii* was isolated, which was sensitive to ceftazidime, cefepime, amikacin,

Table 4: Antimicrobial resistance patterns of isolated bacteria (n=371) in Tracheal aspirate

Drug tested No (%) of resistance	Microbial species isolated (No %)					
	Klebsiella spp n=170	*Acinetobacter spp* n=142	*Pseudomonas spp n=54*	*Proteus spp n=3*	*Enterobacter spp. n=1*	*Morganella morganii spp. n=1*
Amoxicillin	152	Nt	Nt	3	0	
	89.41			100	0	Nt
Cotrimoxazole	152	135		3	1	
	89.41	95.07		100	100	Nt
Ciprofloxacin	153	135	50	3	1	1
	90	95.07	92.6	100	100	100
Gentamicin	122	125	44	3	1	1
	71.77	88.02	81.48	100	100	100
Ceftazidime	150	135	39	Nt	Nt	0
	88.23	95.07	72.22			0
Cefuroxime	155	Nt	Nt	3	1	
	91.17			100	100	
Amikacin	118	125	38	3	1	0
	69.41	88.02	70.37	100	100	0
Aztreonam			Nt	Nt	Nt	0
						0
Cefepime			35	Nt	0	0
			64.82		0	0
Meropenem	120	124	35	1	1	1
	70.58	87.32	64.82	33.33	100	100
Tazobactam Piperacillin	150	124	32	2	1	0
	88.23	87.32	59.25	66.67	100	0
Colistin	3	1	1	3	0	1
	1.77	0.71	1.86	100	0	100
Ceftriaxone	162	135	Nt	2	1	1
	95.29	95.07		66.67	100	100
Cefotaxime	155	135	Nt	3	1	
	91.17	95.07		100	100	
Netilmicin	Nt	Nt		Nt	Nt	0
						0
Tigecycline		1	1	Nt	Nt	Nt
		0.71	1.86			

and aztreonam but resistant to ciprofloxacin, meropenem. Gentamicin, colistin. The most sensitive antibiotic against *Proteus spp.* was meropenem, 66.66%, and tazobactam-piperacillin, 33.33%, but 100% resistant against common drugs. But 100 % sensitive to colistin against *Enterobacter spp.* but other common drugs are 100 % resistant to it. *Proteus spp.* was 100% resistant to it because *Proteus* is intrinsically resistant to colistin.

Discussion

The most frequent bacterial infections among ICU patient are the lower respiratory tract infections [15]. The rate of nosocomial infections is increasing in the patients admitted to the ICU due to excessive invasive procedures performed including artificial ventilator support [16]. The resistance to conventional antibiotics is severely increasing in Bacteria

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in clinical and non-clinical setting [17]. This constantly emerging resistance is a serious situation implying the need of new regulations for the cautious use of antibiotics and refining the condition of hospitals to prevent further exacerbation of resistance shown by the bacteria. In our study, out of 542 samples from the tracheal aspirated ,371(68.45%) culture positive and culture negative was 171(31.55%). Similar study conducted by Begum T (2022) [18] and Ahsan at al (2016) [19] the positive samples were 64.22% and 72.3% respectively. In contrast to the study, Mallik et al. (2015) [20] and Priyanka Gohel (2022) [21] reported a higher isolation rate at 83.0% and 88.4% from the endotracheal aspirated sample in Bangladesh and India. This difference in the isolation rate might be due to difference in the number of samples, type of specimen collection procedure, specimen quality, antibiotic intake of the patients, or microbial techniques used. In our study,364(67.15%) were male and 178(32.85% were female, which was similar like the study done by Begum T(2022) [18]and Andayani (2023) [22] whose study participants were male 66.97% and 73.7% .Male are higher prevalence may be due to increase smoking habit among males [23] and reproductive hormones such as testosterone and estrogen have been linked to greater prevalence of males developing VAP [24].Testosterone trends to reduce the body's immune response to infectious agents, while estrogen increase the intensity and number of immune cells[23]. Higher estradiol levels in women allow for better protection against pathogens [23,24,25]. In our study, the majority of cases were within the 56-65 years of age group (44.28%) and the finding related to other studies by Begum T (2022) [18] and Andayani (2023) [22]. 12%and 26.3% respectively. Age has been considered because one of the risk factors for pneumonia among ventilator users [26,27]. This is because physiological functions of the respiratory system, respiratory muscle atrophy and elasticity of lung tissue decrease with an increase in age [24]

In the present study, Gram negative bacteria were the predominate organism which is similar to other studies in Bangladesh by Begum T (2022) [18] and Savanur (2019) [28], Paterson (2004) [29] Gram negative organisms have been reported as the main cause of ICU associated infections across Asian-Pacific countries [30,31]. The most commonly isolated gram negative bacteria in our study was *Klebsiella pneumonia* which is the most common isolate (45.83%), followed by *Acinetobacter spp.* (38.27%), *Pseudomonas spp.* (14.56%), *Proteus spp.* (0.81%), *Enterobacter spp*(0.26%) and *Morganella morganii* (0.26%) .Similar findings were reported in other studies by Shahunja et al (2015) [32] the most common organism was *Klebsiella spp.* 45% followed by *Acinetobacter spp*.36% and *Pseudomonas aeruginosa*14%. Similar findings also reported by Chandra (2017) [9] that gram negative bacteria were mostly isolated from tracheal aspirates with *Klebsiella pneumoniae* being the most frequent bacteria followed by *Acinetobacter spp.* and *Pseudomonas spp.* But the result differed from other studies by Dominic et al [33] showed *Pseudomonas spp.* accounted 41.14% followed by *Klebsiella spp.* 15.43% and *Acinetobacter spp.* 10.28%. This variation in the pattern of bacterial isolates may be due to the fact that the studies were done in different geographical areas. other factors are difference in patient population, exposure to antibiotic, type of ICU patient, length of ICU stay and the method used for diagnosis of ventilator-associated pneumonia (VAP) [34].

In the present study, bacterial species showed varying resistance to antibiotics in Table 3. *Klebsiella spp.* showed highest sensitivity to colistin 98.24%, amikacin 30.59%, meropenem 29.42% and gentamicin 28.24%. other drugs showed lower sensitivity same to Amoxicillin, amoxicillin plus clavulanic acid and trimethoprim sulfamethoxazole 10.59%, Ceftazidime and tazobactam piperacillin 11.77% and ciprofloxacin 10.0%. lowest sensitivity to ceftriaxone 4.71%, cefuroxime and cefotaxime 8.83%. Similar study conducted by Begum T (2022) [18] highest sensitivity to colistin 93.02%, amikacin 65.11% meropenem 37.20% and gentamicin 30.23%. But lower sensitivity to amoxicillin clavulanic acid 18.06%, ciprofloxacin 18.60%, ceftazidime and cefuroxime 4.65% and lowest sensitivity to ceftriaxone 2.32%. But study conducted by Mallik KU et al (2015) [20] reported *Klebsiella pneumonae* was sensitive to colistin 77.8% which is similar to our study but tazobactam piperacillin and meropenem 77.2%, 62.0% which dissimilar to our study. Another study done by Haque L et al (2013) [35] *Klebsiella spp.* was more than 40% to 60% sensitivity to colistin, ciprofloxacin, amikacin and meropenem.

In this present study *Acinetobacter spp*. showed higher sensitive to colistin and tigecycline 99.29%. other drugs like like meropenem, tazobactam piperacillin 12.68% and amikacin, gentamicin was 11.98%. But higher resistance to ceftriaxone, ceftazidime, cefuroxime, cefotaxime ciprofloxacin and cotrimoxazole 95.07% respectively. Similar study conducted by Begum T (2022) [18] showed higher sensitivity to colistin 100.0% and tigecycline 47.36% followed by lower sensitive to amikacin 15.78%, meropenem 10.52%. But higher resistance to ceftriaxone, ceftazidime, ciprofloxacin, cefotaxime respectively. Another study conducted by panda et al (2018) reported that *Acinetobacter spp.* showed highly sensitive to colistin followed by meropenem and imipenem but highly resistance to piperacillin tazobactam which is not accordance with our study. Another study conducted by Andayani et al (2023) [22] shows higher resistance to ceftazidime, ceftriaxone, cefotaxime, tazobactam piperacillin.100.0%, respectively. This percentage was higher compared to our study. To develop its resistance, several mechanisms such as the production of beta lactamase, increment of multidrug efflux

pumps, reduction of in membrane permeability and alteration in the target site of antibiotics have been reported [36]. In this study, *Pseudomonas spp*. showed higher sensitivity to colistin 98.15% followed by tazobactam piperacillin 40.74%, meropenem 35.18%, cefepime 35.18% but highest resistance to ciprofloxacin 59.60%, gentamicin 81.48%, amikacin 70.37% respectively. Similar study conducted by Mallik et al (2015) [20] showed *pseudomonas spp.* was highly sensitive to colistin followed by tazobactam piperacillin and meropenem which was 82.4%, 80.0% and 70.0% respectively. Another study conducted by Shaha et al (2016) [8] showed *Pseudomonas* aeruginosa sensitive to colistin and meropenem and imipenem were 100.0% and 50.0 -60.0% respectively which is near similar to our study. A study done by Karim et al (2019) [37] and Jamil et al (2016) [38] also found a similar results. The most sensitive antibiotic against *Proteus spp.* was meropenem 66.66% and tazobactam -piperacillin 33.33% but 100% resistant against common drugs. But *Proteus spp.* was 100% resistant to colistin because *Proteus* is intrinsically resistant to colistin.

In the present study, among the *Enterobacter spp.* and 100% were resistant to amoxicillin, cotrimoxazole, ciprofloxacin, cefuroxime, cefotaxime, ceftriaxone. but 100 % sensitive to colistin. similar study conducted by Andayani et al (2023) [22] showed 100% resistant to amoxicillin, cotrimoxazole, ciprofloxacin, cefuroxime, cefotaxime, ceftriaxone, but differ to meropenem, amikacin, gentamicin and piperacillin tazobactam which was highly sensitive to it. Only one *Morganella morganii* was isolated which was sensitive to ceftazidime, cefepime, amikacin, aztreonam but resistant to ciprofloxacin, meropenem, gentamicin, colistin. So, identifying the pathogens from tracheal aspirates and determining their antibiotic susceptibility is crucial for effective treatment, preventing complications, minimizing antibiotic resistance, and reducing treatment costs.

Conclusion

The present study reports the most common organism encountered in tracheal aspirate sample is *Klebsiella pneumoniae* followed by *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Proteus spp., Enterobacter spp.* and *Morganela morganii*. Most of the isolates were found resistance to commonly used drugs. Colistin, meropenem and aminoglycosides could be used as empirical therapy to cover these organisms. hence continued monitoring of susceptibility patterns need to be carried out to detect the true burden of antibiotic resistance in organisms and prevent their further emergence by judicious use of drugs.

Limitation

Due to the retrospective nature of this study, we were unable to present detailed clinical data on patient to identify predictor of all forms of tracheal aspirate sample infection and antimicrobial resistance. Limitations of this study are that the small sample size and microbiological examination for tracheal aspirate could not distinguish colonization from true infection. However long -term retrospective studies with greater samples may provide more robust information.

Conflict of interest

The author declare that the research was conducted in absence of any commercial or financial relationships that could be constructed as a potential conflict to interest.

Authors contribution

All authors contributed equally to this work.

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