ORIGINAL ARTICLE

Bacteriological profile of Tracheal aspirate and their Antimicrobial Sensitivity Pattern in a Tertiary Care Hospital in Dhaka city

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Abstract:

Background: The majority of nosocomial infections are seen in intensive care units and they course with higher rates of mortality and morbidity worldwide. Objectives: To identify the current microbial isolates and their antimicrobial susceptibility pattern from the tracheal aspirate culture in a tertiary care hospital in Dhaka city.Materials and Method: This retrospective study was conducted in the department of Microbiology at Holy Family Red Crescent Medical College Hospital, Dhaka from January 2020 to June 2021 for a period of one and a half years. Written consent was taken from the corresponding authority.A total of 109 samples were collected from tracheal aspirates of the patients who were admitted to the hospital with the critically ill patients in intensive care units.Microsoft Excel software was used for data analysis.Results: A total of 109 samples were analyzed. Of them, the predominant populations were male 73(66.97%) and the remaining were female 36(33.03%). Out of 109 samples, 70 (64.22%)was culture positive. Culture negative was 39 (35.78%). The majority of isolates were Gram-negative bacteria. Among them, predominant bacteria wereKlebsiella spp. 43(61.43%) followed by Acinetobacter spp 19(27.15%), Pseudomonas spp. 05(07.14%) & Gram-positive isolates waswereaph. Aureus 03(04.28%). Klebsiella species showed higher sensitivity totigecycline 97.67%, colistin 93.02%, amikacin 65.11% and meropenem 37.20% & gentamicin 30.23%. Other drugs showed wer sensitivity to axicillin/clavulenic acid 18.60%, and ciprofloxacin 18.60% , trimethoprim/ sulfamethoxazole 09.30%, ceftazidime & cefuroxime 04.65% and lowest sensitivity shown in ceftriaxone 02.32%. Acinetobacter speciesshowed higher sensitivityin colistin at 100% and tigecycline at 47.36%. Other drugs like amikacinand trimethoprim/ sulfamethoxazole 15.78% and the lowest sensitivity were shown in meropenem 10.52% and high resistance in gentamycin, ceftriaxone, ceftazidime, and ciprofloxacin.Pseudomonas spp. showed the highest sensitivity 60.00% to piperacillin-tazobactam, amikacin, imipenem, meropenem, and gentamycin, 40.00% to ciprofloxacin, 20.00% to ceftazidime & cefepime.All the isolate of Staph. aureus was the highest sensitive 100% to vancomycin and linezolid, 66.66% sensitive to amikacin, trimethoprim/ sulfamethoxazole, tetracycline, and gentamicin, 33.33% showed lower sensitivityto amoxicillin/ clavulanic acid, cloxacillin and ciprofloxacin.Conclusion:This study aimed to investigate thedistribution of pathogenic microorganisms isolated from tracheal aspirate and their antibiotic sensitivity profile in intensive care units. A periodic review of the bacteriological profile and antibiotic sensitivity pattern is highly essential for the clinician to treat critically ill patients in ICU. Antibiotic policy & infection control programs should be included in every hospital to reduce this drug resistance.

Key words: Tracheal aspirate, Antimicrobial sensitivity.

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Introduction:

The majority of hospital infections are seen in intensive care units(ICU). Among hospital infections, bacteremia causes serious health problems. Nosocomial infections are defined as infections that occur more than 48 hours after hospital admission and were present or incubating at the time of infection[1]. Hospital-acquired infection or nosocomial infection is a major cause of mortality and morbidity among the intubated patients in ICU. It is reported that mortality and morbidity rate by HAI is more among intubated patients in ICU(50%) than among patients in general wards(5-10%)[2].

The ICU is a unit where accurate and rapid diagnostic methods are key to initiating appropriate antimicrobial treatment and to reducing ventilator-associated pneumonia, relapse health care costs, and mortality. It has also an indirect effect on the emergence of bacterial resistance[3]. The resistant pathogens are difficult to treat and sometimes need invasive procedures such as a mechanical ventilator, and tracheostomy, and catheter application is necessary for the use of board spectrum antibiotics.

Another common hospital infection is pneumonia, where clinical and radiological findings have lower sensitivity and specificity in the diagnosis of pneumonia. Gram staining and culture of lower respiratory tract samples such as endotracheal aspirate, bronchoalveolar lavage, and protected specimen brush sample guide diagnosis and treatment[4].

In most reported studies the organisms were isolated from blood, sputum, throat swab, or nasopharyngeal swab[5,6,7]. Which either did not reflect the actual incidence or isolation of the causative organisms. Isolation of tracheal aspirates is more likely representative of the actual pathogen[8,9,10].

It is important to correctly determine the etiologic factor and to start antimicrobial treatment

earlier. It has been shown that a delay of treatment for 4-8 hours increases mortality.

Therefore, empirical antibiotic treatment is typically initiated by the clinician without waiting for laboratory results4,[11]. The study aimed to investigate the distribution of pathogenic microorganisms isolated from tracheal aspirate and their sensitivity pattern in the ICU of a tertiary care hospital in Dhaka city.

Materials and method:

This retrospective study was conducted in the department of Microbiology at Holy Family Red Crescent Medical College Hospital, Dhaka from January 2020 to June 2021 for a period of one and a half years. Written consent was taken from the corresponding authority. Microsoft Excel software was used for data analysis. A total of 109 tracheal aspirates were collected from the patients who were admitted to ICU.

Tracheal aspirations were obtained by sterile suctioning through an endotracheal tube and suction catheter tip. The endotracheal aspirate was collected by the non-bronchoscopic method. 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 the catheter was withdrawn from the endotracheal tube. After the catheter was withdrawn 2 ml of sterile 0.9% normal saline was injected into it with a sterile syringe to flash the exudates into a sterile closed vacuum container using aseptic techniques inserting a tracheal aspiration probe up to the carina. The containers were sealed by the physicians and sent to the microbiology laboratory for further processing. All samples were processed aerobically on Blood agar, and MacConkeys agar media and incubated at 370C for 24 hours.

Organisms were identified by standard procedures including colony characters, Gram staining, and biochemical reactions.[12]

Antimicrobial susceptibility testing of all the isolates was performed by Kirby Bauer's disc diffusion method by adjusting the turbidity to 0.5 McFarland standard and spread on Mueller

Hinton agar using antibiotics as per CLSI (Clinical laboratory standard institute)guidelines.[13]

Antibiotic susceptibility test: The antibiotics used for the test were amoxicillin/ clavulanic acid (30ugm), amikacin (30 µgm), ciprofloxacin (5 µgm), Ceftriaxone (30 µgm), Ceftazidime (30 µgm), Cefuroxime (30 µgm), Cloxacillin (5µgm), Gentamicin (10 µgm), Imipenem (10µgm), Meropenem (10 µgm), Trimethoprime/ sulfamethoxazole(25 µgm), Piperacillin/tazobactum (100 µgm) , colistn (10µgm), tetracycline (30µgm), vancomycin(30µgm), linezolid(30µgm), tigecycline (30µgm) cefepime(30µgm). The diameter of the zone of inhibition was measured and interpreted according to the CLSI standard.[13]

Results:

Table I: Age & sex distribution of the study population (n= 109)

Age in years	Male	Female	Total (%)
1-20	2	0	2 (01.83%)
21-40	15	5	20 (18.34%)
41-60	34	13	47 (43.12%)
61-80	19	16	35 (32.12%)
81-100	3	2	5 (04.59%)
Total	73 (66.97%)	36 (33.03%)	109 (100%)

A total of 109 samples were analyzed. Of them,predominant populations were from male 73(66.97%) and remaining were from female 36(33.03%) (Table I). The highest age group was 41-60 (43.12%) followed by 61-80 agegroup(32.12%), 21-40 age group(18.34%), 81-100 age group(04.59%) and the lowest age group was 01-20(01.83%).

Table II: Distribution of bacterial isolates from tracheal aspirate culture(n= 109)

Distribution of the isolates	Number	Percentage(%)
Growth positive culture	70	64.22
Growth negative culture	39	35.78
Total	109	100.00

Out of 109 samples, 70(64.22%) was culture positive. Culturenegative was observed in 39(35.78%). (Table II).

Table III: Organisms isolated from Tracheal aspirate (n=70)

Organisms isolated	Number	Percentage (%)
Klebsiella species	43	61.43
Acinetobacter species	19	27.15
Pseudomonas species.	05	07.14
Staphylococcus aureus	03	04.28
Total	70	100.00

Majority of isolates Gram-negative bacteria. Among them predominant bacteria was Klebsiella species43(61.43%) followed by Acinetobacter species 19(27.15%), Pseudomonas species 05(07.14%) &among the Gram positive isolates 03(04.28%) was Staph. Aureusshowed in Table III.

Table IV: Antibiotic susceptibility pattern of Gram-negative isolates

Organisms	Sensitivity (%)										
	AMC	CXM	CRO	CIP	CAZ	GN	AK	SXT	COL	TGC	MEM
Klebsiella species (n=43)	18.60	04.65	02.32	18.60	04.65	30.23	65.11	09.30	93.02	97.67	37.20
Acinetobacter species (n=19)	-	-	0	0	0	0	15.78	15.78	100.00	47.36	10.52

AMC– Amoxicillin/clavulanic acid, CXM-Cefuroxime, CTR-Ceftriaxone, CIP-Ciprofloxacin, CAZ –Ceftazidime, SXT-Trimethoprim/ Sulfamethoxazole, GN-Gentamycin, AK-Amikacin, MEM-Meropenem, COL-Colistin, GC- Tigecycline. The antibiotic susceptibility pattern of Gram-negative isolates is shown in Table 4Klebsiella species showed higher sensitivity totigecycline 97.67%, colistin 93.02%, amikacin 65.11% and meropenem 37.20% & gentamicin 30.23%. Other drugs showedlower ssensitivity toamoxicillin/clavulanic acid 18.60%, ciprofloxacin 18.60%, trimethoprim/ sulfamethoxazole 09.30%, ceftazidime & cefuroxime 04.65% and lowest sensitivity is shown in ceftriaxone 02.32%. Acinetobacter speciesshowed higher sensitivity in colistin 100% and tigecycline 47.36%.Other drugs like trimethoprim/ sulfamethoxazole 15.78% and the lowest sensitivity showed in meropenem 10.52% and high resistance in gentamycin, ceftriaxone,ceftazidime & ciprofloxacin.

Table V: Antibiotic susceptibility pattern of Pseudomonasspecies isolates (n= 05)

Organisms	Sensitivity (%)							
	CIP %	AK %	CAZ %	CFM %	GN %	IPM %	TZP %	MEM %
Pseudomonas species.	40.00	60.00	20.00	20.00	60.00	60.00	60.00	60.00

CIP-Ciprofloxacin, AK-Amikacin,CAZ – Ceftazidime, CFM- Cefepime, GN-Gentamycin, IPM - Imipenem, TZP-Piperacillin/ tazobactum, MEM-Meropenem

Pseudomonasspecies showed highest sensitivity 60.00% to piperacillin/ tazobactum, amikacin & imipenem, meropenem, and gentamycin, 40.00% to ciprofloxacin, 20.00% to ceftazidime & cefepime shown in Table V.

Table VI: Antibiotic susceptibility pattern of isolated Staph. aureus (n= 03)

Organisms	Sensitivity (%)								
	AMC %	CX %	TET %	CIP %	SXT %	GN %	AK %	VA	LZD
Staphylococcus. aureus	33.33	33.33	66.66	33.33	66.66	66.66	66.66	100.00	100.00

AMC – Amoxicillin/clavulanic acid, CX-Cloxacillin, TET- Tetracycline CIP-Ciprofloxacin, SXT-Trimethoprime/ Sulfamethoxazole, GN-Gentamycin, AK-Amikacin, VA-Vancomycin, LZD- Linezolid.

All the isolate of Staphylococcus aureus was highest sensitive 100% to vancomycin and linezolid, 66.66% sensitive to amikacin, trimethoprime/ sulfamethoxazole, tetracycline andgentamicin, 33.33% showed lower sensitivity to amoxicillin/ clavulanic acid, cloxacillin and ciprofloxacin. (Table VI)

Discussion:

Lower respiratory tract infections are the most common infections seen in the ICU.14. the rate of nosocomial infections is increasing in the patients admitted to the ICU due to excessive invasive procedures performed including artificial ventilator support.15. Bacterial sensitivity to antibiotics is decreasing day by day[16].

In our study, 109 samples of tracheal aspirates isolates were detected among them males were predominant 73(66.97%) and the remaining were females 36(33.03%). Our study also shows that the

age group 41-60 years were47(43.12%) followed by the 61-80 age group was35(32.12%), the 21-40 age group 20(18.34%), 81-100 age group 05(04.58%) and lowest age group was 01-20(01.83%) (Table 1). It could be explained by the fact that males were more predominant to infections. Another study found that culture positivity was more common in elderly male patients who were smokers and who were admitted for respiratory causes or patients who had pre-existing lung diseases. This is in coherence with the study by Ferrer et al [17].

In this study, culture positive growth was 64.22% and culture negative growth was 35.78%. The culture positivity rate in our study is similar to another study conducted by Ahsan et al, the positive samples were 72.3%.[18] However 83% culture positive isolates were also reported in a study conducted by Mallick et al.[19].

This study found that Gram-negative bacteria were the predominant organisms in comparison to Gram-positive bacteria. The common bacterial isolates found in this study were Klebsiella species 43(61.43%) followed by Acinetobacter species 19(27.15%),Pseudomonas species. 05(07.14%) & Staphylococcus aureus 03(04.28%) (Table 3). Similar findings were reported in a study done in Bangladesh by Shahunja et al the most common isolates were Klebsiella 45% followed by Acinetobacter 36% and Pseudomonas 14%[20]. Different studies have been performed to assess the bacterial profile and antibiotic susceptibility pattern in tracheal aspirate samples. This can be attributed to the fact that the majority of nosocomial infections are caused by Gram-negative bacteria which were more dangerous and difficult to treat. The causative organisms vary with the patient's demographics in the ICU, the method of diagnosis, the duration of hospital stays, and the institutional antimicrobial policies.

The antibiotic susceptibility pattern of Gram-negative isolates is shown in Table 4 Klebsiella species showed higher sensitivity to tigecycline 97.67%, colistin 93.02%, amikacin 65.11% and meropenem 37.20% & gentamicin 30.23%. Other drugs showed lower sensitivity to amoxicillin/ clavulanic acid 18.60%, ciprofloxacin 18.60%, trimethoprim/ sulfamethoxazole 09.30%, ceftazidime & cefuroxime 04.65% and lowest sensitivity shown in ceftriaxone 02.32%. Tigecycline was recommended for the treatment of complicated intraabdominal and complicated skin and soft tissue infections and community-acquired pneumonia but there are also studies on its use in high doses in clinically critical patients[21]. the fact that carbapenems are frequently preferred and prioritized antibiotics in cases where empirical treatment should first be initiated in ICU infections in our hospital, can be considered as one of the reasons for the high rates of carbapenem resistance in our hospital. Mallick Ku et al reported klebsiella was sensitive to colistin, tazobactam/ piperacillin, and meropenem at 77.8%, 72.2%, and 62%respectively.19.but the study done by Haque L et al klebsiella was more than 40% to 60% sensitivity to colistin, ciprofloxacin, amikacin, and meropenem.[22]

In our study, Acinetobacter species showed higher sensitivity in colistin 100% and tigecycline 47.36% other drugs like amikacin and trimethoprim/ sulfamethoxazole 15.78% and the lowest sensitivity showed in meropenem 10.52% and high resistance in gentamycin, ceftriaxone, ceftazidime & ciprofloxacin. The rise in Acinetobacter in another study especially in ICU set up can be attributed to the dramatic increase in the occurrence of multidrug-resistant isolates. In addition, these organisms can survive in humid and dry conditions for longer periods resulting in nosocomial outbreaks[23]. A study by Rani et al reported Acinetobacter with 80% to 90% sensitivity to colistin[24] Pseudomonas species showed the highest sensitivity 60.00% to piperacillin-tazobactam, amikacin & imipenem, meropenem, and gentamycin, 40.00% to ciprofloxacin, 20.00% to ceftazidime &cefepime shown in Table 5. Mallick Ku et al revealed that Pseudomonas had sensitivity to colistin, tazobactam/ piperacillin, and meropenem, and it was 82.4%, 80%, and 70% respectively[19]. but a study was done by Saha, et al[25]. showed pseudomonas sensitive

to colistin, and carbapenem were 100% and 50%-60% respectively. A study done by Karim et al and Jamil et al also found a similar result[26, 27].

In present study shows the isolation of Staph. aureus was the highest sensitive 100% to vancomycin and linezolid, 66.66% sensitive to amikacin, trimethoprim/ sulfamethoxazole, tetracycline, and gentamicin, 33..33% showed lower sensitivity to amoxicillin/ clavulanic acid, cloxacillin and ciprofloxacin. (Table 6). In the SENTRY antimicrobial survey program (1997-2008) staphylococcus aureus was found to be 28% of nosocomial and ventilator-associated pneumonia agents in a study by Kollef et al[28], evaluating the bacterial growth of deep tracheal aspirates cultures in patients with pneumonia. In our study staphylococcus aureus was found to be (04.28%) of isolated microorganisms. Different hospital deals with different infections, so the isolation rate of the bacteria may be variable from hospital to hospital. Mallick ku et al[19] observed that staphylococcus was sensitive to linezolid(87.8%) and vancomycin(82.2%) and less sensitive to commonly used antibiotics such as gentamycin, erythromycin, tetracycline, and cephradine which is similar to study done by Gitau et al[29].

Conclusion: In our study, the commonest organism which was isolated from tracheal aspirate culture were Klebsiella species followed by Acinetobacter species, Pseudomonas species, and Staphylococcus aureus, which were sensitive to colistin, tigecycline, meropenem and also imipenem, piperacillin tazobactam. Bacterial sensitivity to major antibiotics is decreasing day by day and complicating the empirical selection of antibiotics in the ICU. Our data shows an alarming pattern of poor antibiotic sensitivity of the majority of ICU isolates to most of board spectrum antibiotics. To prevent the emergence of multidrug-resistant bacteria in the ICU we must take some strategies immediately. Judicious use of older and newer antimicrobial agents according to antibiogram and help clinicians in choosing appropriate empirical antibiotics to maximize the patient's chances of receiving early and effective treatment. A periodic review of the bacteriological profile and antibiotic sensitivity pattern is highly essential. Antibiotic policy & infection control programs should be included in every hospital to reduce this drug resistance.

Limitation:

Limitations of this study are that the small sample size and microbiological examination for tracheal aspirate culture could not distinguish colonization from true infection. Clinical correlations were not done, so we could not point to colonization. However long-term prospective studies with greater samples may provide more robust information.

Acknowledgment:

We acknowledge the staff and doctors of the department of Microbiology and Immunology, HFRCMCH, Dhaka for their constant support in collecting samples, laboratory work, and management of patients.

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