Original Article

Identification of Bacterial Isolates from Endotracheal Aspirate of Patients in Intensive Care Unit and Their Antimicrobial Susceptibility Pattern

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Abstract

***Background**: Nosocomial infections have been discussed as a critical issue among intubated patients leading to significant morbidity and mortality. Tracheal colonization of different bacteria may be responsible for added or superinfections and may increase the risk of mortality. Irrational use of antibiotics also increases the emergence of drug-resistant bacteria. **Objectives**: We aimed to investigate the bacterial isolates in the endotracheal aspirates of ICU patients and to see the pattern of antibiotic susceptibility. **Materials and Methods**: This cross-sectional study was based on 40 specimens of endotracheal aspirates which were collected from ICU patients of Dhaka Medical College Hospital. All the specimens were processed and cultured on MacConkey and blood agar media. The isolated organisms were identified by different biochemical tests. **Results**: Among the 40 specimens, 38 (95%) yielded growths of different bacteria. Of them, Acinetobacter baumanni were 13 (34%), Pseudomonas aeruginosa were 6 (16%), Klebsiella pneumoniae were 3 (8%), Klebsiella oxytoca were 3 (8%) and Staphylococcus aureus were 3 (8%). All the isolated bacteria were sensitive to colistin and most of the Acinetobacter baumanni were resistant to different antibiotics. Among the 32 isolated gram-negative bacteria, 10 (31.25%) were ESBL producers. **Conclusion**: Most of the bacteria showed antibiotic resistance to different common antibiotics, which is very alarming for the ICU patients.*

***Key words**: Endotracheal aspirate; ICU; Culture; Antibiotic susceptibility*

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Introduction

Hospital-acquired infection (HAI) is a serious and burning problem worldwide and is responsible for high rate of morbidities and mortalities.[1] It has been shown that in developed countries 5−15% patients in general wards and 50% patients in intensive care units (ICU) suffered from HAI.[2] In developing countries this burden is somewhat underestimated, which may be due to lack of knowledge of proper surveillance, proper resources and proper guidance.[2] In ICU most of the patients suffer from urosepsis, post-surgical infection and lower respiratory infection.[3] The modern apparatus responsible for HAI are endotracheal tube, catheter and different surgical appliances.[4] Tracheal colonization of different bacteria may be responsible for added or superinfections and at the same time, increases the risk of mortality.[5] Again due to irrational use of antibiotics, there is increasing emergence of

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drug-resistant bacteria.[6] These gram-negative drug resistant bacteria are prevalent all over the world.[7] The aim of our study was to detect different pathogens from endotracheal aspirate in ICU patients and to see their antimicrobial susceptibility pattern.

Materials and Methods

This cross-sectional study was carried out on 40 specimens of endotracheal aspirates collected from ICU patients of Dhaka Medical College Hospital. This study was done in the department of Microbiology, Dhaka Medical College (DMC), Dhaka from July 2013 to June 2014. Patients with clinically suspected respiratory tract infection hospitalized in ICU of Dhaka Medical College Hospital (DMCH) irrespective of age, sex and antibiotic used were included in this study. Informed written consent was taken from all patients or their legal guardians before specimen collection.

Specimen collection

Endotracheal tube aspirates were collected from clinically suspected respiratory tract infected patients in ICU of DMCH. With all aseptic precautions, endotracheal aspirates were collected by using a 50 cm and 14 Fr suction catheter. The suction catheter was gently introduced through the endotracheal tube for a distance of approximately 25−26 cm. The endotracheal aspirate was obtained by suction, without instilling saline and the catheter was withdrawn from the endotracheal tube. After the catheter was withdrawn, 2 mL of 0.9% sterile normal saline was injected into the suction catheter with a sterile syringe to flash the exudates. The aspirates were collected into a sterile falcon tube and were transported immediately to the laboratory for further processing.[8]

Specimen processing

Endotracheal aspirates were mechanically liquefied and homogenized by vortexing for one minute.[9] After vortexing specimens were centrifuged at 2000 g for 10 minutes. Two mL supernatant was discarded using a sterile pipet and the deposit was further mixed by vortexing. The processed specimen was used for culture.

Gram staining of aspirate and microscopic examination

Smears were prepared from the aspirate, gram staining was done and then examined under microscope for the presence of gram-positive or gram-negative organisms.

Isolation of organisms

Specimens were inoculated by using a sterile wire loop on the blood agar medium and MacConkey agar medium (three sectors consecutively). After inoculation, all the media were incubated overnight at 37°C aerobically. The microbiological growths of endotracheal aspirates were classified as rare, light, moderate or heavy, based on the number of colonies in each of two sectors shown in the table (Table I).[10] Moderate to heavy growth was considered as significant.[11] For confirmation of *Staphylocoocus spp.* subculture was done on mannitol salt agar medium from blood agar medium and incubated at 37ºC for 24 hours and examined after 24 hours.

Identification of organisms

All the isolated organisms were identified by their colony morphology, staining characters and further confirmed by relevant biochemical tests.

Table I: Semi-quantitative reporting of microbial growth in culture performed in petri-dishes

Report	Number of colonies in each sector		
	1st	2nd	3rd
Rare	<10	0	0
Light	≥10	<5	0
Moderate	≥10	≥5	<5
Heavy	≥10	≥5	≥5

Antibiotic susceptibility test

Using Kirby-Bauer modified disc-diffusion technique, antibiotic susceptibility test was performed as described by Clinical and Laboratory Standards Institute (CLSI).[12] Antibiotic discs of oxacillin (1 µg⁄disc), vancomycin (30 µg⁄disc), amoxiclav (amoxicillin and clavulanic acid, 20⁄10 µg per disc), cefotaxime (10 µg⁄disc), cefoxitin (30 µg⁄disc), ceftriaxone (30 µg⁄disc), linezolid (30 µg⁄disc), cefepime (30 µg⁄disc), gentamicin (10 µg⁄disc), imipenem (10 µg⁄disc), ciprofloxacin (5 µg⁄disc), amikacin (30 µg⁄disc), combination of piperacillin-tazobactam (100⁄10 µg/per disc), combination of cefoperazone-sulbactam (105 µg⁄disc), colistin (30 µg⁄disc), aztreonam (30 µg⁄disc) were used (Oxoid Ltd. UK). Mueller-Hinton agar medium was used for antibiotic susceptibility test. Resistant and sensitive bacteria were defined according to CLSI guidelines.

Data analysis

After compiling data were analyzed using 'Microsoft Office Excel 2007' program and χ^2 test was used to compare the results.

Results

Among the 40 endotracheal aspirates, 2 (5%) yielded no growth in culture. Of the 38 isolated organisms, 13 (34%) were *Acinetobacter baumanni*, 6 (16%) were *Pseudomonas aeruginosa*, 6 (16%) were *Klebsiella pneumoniae*, 3 (8%) were *Klebsiella oxytoca*, 3 (8%) were *Staphylococcus aureus* and 2 (5%) were *Moraxella catarrhalis* (Table II).

According to antimicrobial resistance pattern to different antibiotics, none of the isolates were resistant to colistin and 12 (92%) *Acinetobacter baumanni* were resistant to amoxiclav, ceftazidime, ciprofloxacin and cefoxitin. Among the 3 isolated *Staphylococcus aureus*, 2 (66.67%) were resistant to ciprofloxacin and all were sensitive to linezolid, oxacillin, cefoxitin and vancomycin (Table III). No MRSA and VRSA were isolated.

Table II: Distribution of bacteria isolated from endotracheal aspirate samples (n=38)

Bacteria	Number	Percentage
Acinetobacter baumannii	13	34.21
Pseudomonas aeruginosa	6	15.79
Klebsiella spp.	9	23.68
Citrobacter koseri	1	2.63
Enterobacter aerogenes	1	2.63
Staphylococcus aureus	3	7.89
Staphylococcus epidermidis	1	2.63
Moraxella catarrhalis	2	5.26
Proteus spp.	2	5.26
Total	38	100.00

Screening of ESBL producers by double disc synergy assay

The test was performed on Muller-Hinton agar medium. Disc containing 30 µg of ceftazidime and a disc containing amoxicillin plus clavulanic acid (20 µg + 10 µg) were placed 20−25 mm apart (center to center). The Muller-Hinton agar plate was incubated at 37ºC for 24 hours. A clear extension of the edge of inhibition zone of cephalosporin disc towards amoxiclav disc was interpreted as ESBL production.[13]

Among the 32 isolated gram-negative bacteria, 10 (31.25%) were ESBL producers. Among them, 5 (50%) were *Klebsiella pneumoniae* and there were no ESBL producing *Acinetobacter baumannii* (Table IV).

Table III: Antibiotic resistance pattern of major isolated bacteria to different antibiotics

Antibiotics	*Acinetobacter* (n=13)	*Pseudomonas aeruginosa* (n=6)	*Klebsiella Spp.* (n=9)	*Proteus spp.* (n=2)	*Moraxella catarrhalis* (n=2)	*Staph. aureus* (n=3)
Imipenem	11 (85.00)	1 (16.66)	2 (22.22)	0 (0.00)	0 (0.00)	-
Amikacin	12 (92.00)	3 (50.00)	3 (33.33)	2 (100.00)	1 (100.00)	-
Ciprofloxacin	12 (92.00)	4 (66.67)	8 (88.89)	2 (100.00)	1 (100.00)	2 (66.67)
Ceftriaxone	-	3 (50.00)	8 (88.89)	1 (50.00)	1 (100.00)	1 (33.33)
Ceftazidime	12 (92.00	4 (66.67)	9 (100.00)	1 (50.00)	1 (100.00)	-
Gentamicin	-	6 (100.00)	7 (77.78)	2 (100.00)	1 (50.00)	1 (33.33)
Colistin	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	-
Piperacillin	-	2 (33.33)	3 (33.33)	1 (50.00)	0 (0.00)	-
Amoxiclav	12 (92.00)	6 (100.00)	7 (77.78)	2 (100.00)	1 (100.00)	-
Aztreonam	6 (46.00)	2 (33.33)	7 (77.78)	2 (100.00)	0 (0.00)	-
Linezolid	-	-	-	-	-	0 (0.00)
Cefoxitin	12 (92.00)	4 (66.67)	7 (77.78)	2 (100.00)	1 (100.00)	0 (0.00)
Oxacillin	-	-	-	-	-	0 (0.00)
Vancomycin	-	-	-	-	-	0 (0.00)
Cefepime	10 (77.00)	-	-	-	-	-
Sulbactam-Cefoperazone	7 (54.00)	-	-	-	-	-
Piperacillin-Tazobactam	11 (85.00)	-	-	-	-	-

Table IV: Distribution of ESBL producing gram-negative bacteria identified by DDS test

Gram-negative bacteria	Number	ESBL producers
Acinetobacter baumannii	13	0
Pseudomonas aeruginosa	6	1
Klebsiella pneumoniae	6	5
Klebsiella oxytoca	3	1
Enterobacter aerogenes	1	1
Citrobacter koseri	1	0
Proteus vulgaris	1	1
Proteus mirabilis	1	1
Total	32	10

Discussion

Infections are the most important and the leading cause of mortality and morbidity in ICU.[14] Endotracheal tubes are susceptible to infection and therefore it is important to be aware of the relevant factors and responsible organisms to take prompt action.[5] The findings of this study would be helpful in selection of appropriate antibiotics. In this study, all the positive colonies of significant growth obtained from endotracheal aspirates were considered.

In this study, 40 endotracheal aspirates were studied and organisms were isolated from 38 specimens. Among the isolated organisms, 13 (34%) were *Acinetobacter baumannii*, 6 (16%) were

Pseudomonas aeruginosa, 6 (16%) were *Klebsiella pneumoniae*, 3 (8%) were *Klebsiella oxytoca*, 3 (8%) were *Staphylococcus aureus*. Out of 38 significant growths in semiquantitative culture positive cases, gram-negative bacteria were isolated in 32 (84.21%) cases. Amini et al[15] reported that gram-negative bacilli accounted for 83% among all isolates. The most commonly identified organism was *Acinetobacter spp*. followed by *Pseudomonas aeruginosa* in that study. In another study, gram-negative bacilli accounted for 86% among all isolates and the most commonly identified organism was *Acinetobacter spp*. followed by *Klebsiella pneumoniae*.[16] *Klebsiella pneumoniae* (34%) was the most common isolate, followed by *Pseudomonas aeruginosa* (20%) and *Acinetobacter species* (18%) in another study.[9] In a study done by Dominic et al[17] in Kasturba Medical College Hospital, Mangalore, it was reported that the majority of bacteria in their study were gram-negative bacilli (81.14%), among them *Pseudomonas spp*. accounted for 41.14%, *Klebsiella spp*. 15.43%, *Acinetobacter spp*. 10.28%. In this study, 7.5% *Staphylococcus aureus* was found but incidence of *Staphylococcus aureus* was 15.2% in another study which is significantly higher than findings in our study.[18] 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 differences 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).[15]

In this study, most (85%) of the *Acinetobacter baumannii* were resistant to imipenem. High percentages (29%) of *Acinetobacter baumannii* strains resistant to imipenem were reported in another study.[19] A study done by Shaheda[20] in BSMMU found that 62% of the *Acinetobacter* were imipenem sensitive. Since last 10 years, resistance to imipenem has been increasingly reported worldwide in non-fermenting gram-negative bacilli (NFGNB) including *Acinetobacter spp*..[21] In this study, most (92%) of the *Acinetobacter baumannii* were resistant to ceftazidime and amoxiclav and amikacin. *Acinetobacter baumannii* was found highly resistant (89%) to ceftazidime in another study that was almost in agreement with the present study.[22] Infection by metallo-β-lactamase (MBL) producing organism including $bla_{\text{NDM-1}}$ producers are increasing in the last few years in Bangladesh, which are resistant to most of the commonly used third generation cephalosporin including imipenem.[23,24]

In the present study, among the 13 *Acinetobacter baumannii*, 12 (92%) were multidrug-resistant. Two previous studies reported 85.4% and 68.1% multidrug-resistant *Acinetobacter spp*. were isolated from ICU patients.[25,26] These results are very much similar to the present study.

In the present study, all the six isolated *Pseudomonas aeruginosa* (100%) were resistant to amoxiclav and gentamicin. Imipenem was found most sensitive against *Pseudomonas aeruginosa*. Isolated *Pseudomonas aeruginosa* were imipenem sensitive in 83.44% and piperacillin sensitive in 67.67% cases. In contrast, *Pseudomonas* was most sensitive to amikacin (52.78%) and ceftazidime (47.22%) in a study done by Taj et al[27] in Pakistan.

Among the nine isolated *Klebsiella spp*., 8 (88.89%) were resistant to ceftriaxone and ciprofloxacin, 7 (77.78%) were resistant to gentamicin. All (100%) of the isolated *Klebsiella spp*. were sensitive to colistin and 77.78% to imipenem. Dominic et al[17] reported that 66.67% *Klebsiella spp*. were sensitive to amikacin, which is similar to the present study. Among the three isolated *Staphylococcus aureus*, 2 (66.67%) were resistant to ciprofloxacin. Taj et al[27] reported 63.7% *Staphylococcus aureus* were resistant to ciprofloxacin, which is close to the present study. In this study, all (100%) isolated *Staphylococcus aureus* were sensitive to linezolid. Another study also found that all the *Staphylococcus* was sensitive to linezolid.[27]

In the present study, among the 32 isolated gram-negative bacteria 10 (31.25%) were ESBL producers. Among them *Klebsiella pneumoniae* were 50%. Another study carried out in Bangladesh in 2012 reported that among 169 gram-negative bacteria isolated from wound swab and urine, 42 (25%) were ESBL producers, which is slightly lower than the present study.[23] In that study, most of the isolates of *Klebsiella pneumoniae* (83.33%), *Klebsiella oxytoca* (33.33%), *Enterobacter aerogenes* and *Proteus spp*. produced ESBL, which is in accordance with the present study. In the present study, out of 13 *Acinetobacter baumannii* isolates, none were found positive for ESBL production. Higgins et al[28] reported that 9.09% ESBL producer was *Acinetobacter*

baumannii in their study.

In conclusion, this study presents the most common microorganisms colonized from endotracheal tube of hospitalized patients and their pattern of antibiotic resistance. This study shows that *Acinetobacter* are the most common bacteria and most of them are resistant to common antibiotics, which is alarming for developing countries like Bangladesh. The antibiotic colistin was found sensitive for all the organisms in the present study.

References

1. Pele AV, Hooper DC. Hospital acquired infections due to gram negative bacteria. N Engl J Med 2010; 362: 1804−1843.
2. Bagheri NS, Allegranzi B, Syed SB, Elli B, Pitter D. Health-care associated infection in Africa: a systematic review. Bull World Health Organ 2011; 89: 757−765.
3. Adir CG, Gorman SP, Byers LM, Jones DS, Feron B, Crowe M. Eradication of endotracheal biofilm by nebulized gentamicin. Intensive Care Med 2012; 28: 426−431.
4. Hoque L, Mostofa KSM, Ahmed Z. Isolation, identification and antimicrobial sensitivity patterns of bacterial isolates from tracheal aspirate of ICU patients of central Dhaka, Bangladesh. Int J Res App Natural and Social Sci 2013; 1: 11−16.
5. Saha AK, Nandi S, Dhar P. Prevalence of bacterial isolates in endotracheal tube according to culture and sensitivity in patients of ICU of a tertiary medical college and hospital, Kolkata, West Bengal. Int J Contem Med Res 2016; 3(6): 1775−1781.
6. Khosravi AD, Parhizgari N, Montazeri EA, Mozaffari A, Abbasi F. The prevalence of bacteria isolated from endotracheal tube of patients in Golestan Hospital, Ahvaz, Iran and determination of their antibiotic susceptibility patterns. Jundishapur Microbiol 2013; 5: 67−71.
7. Rosenthal VD, Maki DG, Salomao R, Moreno CA, Mehta Y. Device associated nosocomial infections in 55 intensive care units of 8 developing countries. Ann Intern Med 2006; 345: 82−91.
8. Dey A, Bairy I. Incidence of multidrug resistant organisms causing ventilator-associated pneumonia in a tertiary care hospital: a nine month prospective study. Ann Thorac Med 2007; 2(2): 52−57.
9. Jakribettu RP, Boloor R. Characterisation of aerobic bacteria isolated from endotracheal aspirate in adult patients suspected ventilator associated pneumonia in a tertiary care center in Mangalore. Saudi J Anaesth 2012; 6(2): 115−119.
10. Fujitani S, Mark H, Cohen-Melamed RRT, Raymond P, Tuttle RRT, Edgar Delgado RR et al. Comparison of semi-quantitative endotracheal aspirates to quantitative non-bronchoscopic bronchoalveolar lavage in diagnosing ventilator-associated pneumonia. Respiratory Care 2010; 54(11): 1453−1461.
11. Craven DE, Chroneou A, Zias N, Hjalmarson KI. Ventilator-associated tracheobronchitis. The impact of targeted antibiotic therapy on patient outcomes. Chest 2012; 135: 521−528.
12. CLSI (Clinical and Laboratory Standard Institute). Performance standards for antimicrobial disc susceptibility testing; twenty-first informational supplement. CLSI document M100-S21. Wayne, PA: CLSI; 2011.
13. Collee JG, Marr W. Specimen collection, culture containers and media. In: Collee JG, Fraser AG, Marmion BP, Simmons A (eds). Mike & McCartney Practical Medical Microbiology. 14th edn. USA: Churchill Livingstone, 1996: 95−111.
14. Abdullahi A, Shoar S, Shoar N. Micro-organisms colonization and their antibiotic resistance pattern in oro-tracheal tube. Iranian J Microbiol 2013; 5(2): 102−107.
15. Amini M, Javanmard A, Davati A, Azimi G. Bacterial colonization in tracheal tube of ICU patients. Iranian J Path 2012; 4: 123−127.
16. Shrestha RK, Dahal RK, Mishra SK, Parajuli K, Rijal BP, Sherchand JB et al. Ventilator associated pneumonia in tertiary care hospital, Maharajgunj, Kathmandu, Nepal. J Int Med 2013; 35(3): 21−28.
17. Dominic RMS, Prashanth HV, Shenoy S, Baliga S. A clinical-microbiological study of ventilator-associated pneumonia in a tertiary care hospital. Int J Bio Med Res 2012; 3(2): 1651−1654.
18. Rahbar M, Hajia M. Detection and quantification of the etiologic agents of ventilator-associated pneumonia in endotracheal aspirates from patients in Iran. Infect Control Hosp Epidermiol 2006; 27: 884−885.
19. Joshi SG, Litake GM, Niphadkar KB, Ghole VS. Multidrug resistant *Acinetobacter baumannii* isolates from teaching hospital. J Infect Chemother 2011; 9(2): 187−190.
20. Shaheda A. Phenotypic detection of metallo-beta-lactamase among clinical isolates of *Pseudomonas*

spp. and *Acinetobacter spp*. [M Phill thesis]. Dhaka: Bangabandhu Sheikh Mujib Medical Unuversity; 2010.

21. Safari M, Saidijam M, Bahador A, Jafari R, Alikhani MY. High prevalence of multidrug resistance and metallo-beta-lactamase (MβL) producing *Acinetobacter baumannii* isolated from patients in ICU wards, Hamadan, Iran. J Res Health Sci 2013; 13(2): 162−167.
22. Nahar A. Association of virulence factors − gelatinase production and biofilm formation with antimicrobial resistance among the *Acinetobacter* isolates [M Phil Thesis]. Dhaka: Bangabandhu Sheikh Mujib Medical Unuversity; 2011.
23. Farzana R, Shamsuzzaman SM, Mamun KZ. Isolation and moleculer characterization of New Delhi metallo-beta-lactamase-1 producing superbug in Bangladesh. J Infect Dev Ctries 2013; 7(3): 161−168.
24. Shamsuzzaman SM. NDM-1 producing new superbug bacteria: a threat to control infection. Bangladesh J Med Microbiol 2011; 5(1): 1−2.
25. Khanal S, Joshi DR, Bhatta DR, Devkota U, Pokhrel BM. β-Lactamase-producing multidrug-resistant bacterial pathogens from tracheal aspirates of intensive care unit patients at National Institute of Neurological and Allied Sciences, Nepal. ISRN Microbiol 2013; 2013: 847569. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3775398/. Accessed July 2017.
26. Sivaranjani V, Umadevi S, Srirangaraj S, Kali A, Seetha KS. Multi-drug resistant Acinetobacter species from various clinical samples in a tertiary care hospital from South India. Aus Med J 2013; 6(12): 697−700.
27. Taj Y, Abdullah FE, Kazmi SU. Current pattern of antibiotic resistance in *Staphylococcus aureus* clinical isolates and the emergence of vancomycin resistance. J Coll Physicians Surg Pak 2010; 20: 728−732.
28. Higgins PG, Poirel L, Lehmann M, Nordmann P, Seifert H. OXA-143 a novel carbapenem hydrolyzing class D beta-lactamase in *Acinetobacter baumannii*. Antimicrobial Agents and Chemotherapy 2009; 53(12): 5035−5038.