Original Article

Microbial isolates and their antimicrobial resistance pattern from endotracheal aspirates: a retrospective observational study over one year at a tertiary care hospital of Bangladesh

Saha MR[a], Rahman T[a], Hasan MR[b], Islam KMS[c]

ABSTRACT

Background: *Emergence of multidrug resistant (MDR) organisms causing ventilator-associated pneumonia (VAP) is an increasing threat with substantial mortality in critically ill patients. Aetiology and treatment of VAP in mechanically ventilated patients can be evaluated by analysis of culture reports periodically for better understanding of microbial isolates and their antimicrobial susceptibility pattern. The present study aimed to analyze the culture and sensitivity pattern of endotracheal aspirates in intensive care unit (ICU) patients.*

Methods: *A hospital-based retrospective study was done in the Department of Microbiology, BIRDEM General Hospital, Dhaka, Bangladesh from January to December, 2019. Data were obtained from the laboratory record book conserved in the Department of Microbiology. Isolates of endotracheal aspirates from 715 patients were identified and their antibiotic susceptibility were (done by standard microbiological methods) recorded.*

Results: *Out of total 715 endotracheal aspirates, growth was obtained from 482 (67.4%) samples. Among the 482 culture positive samples, 714 isolates were identified of which 250 (51.9%) yielded single organism and 232 (48.1%) were double organisms. Among total isolates, 642 (89.9%) were bacteria and 72 (10.1%) were fungi. Gram negative bacilli were mostly isolated organisms (85.6%) followed by fungi (10.1%) and gram positive cocci (4.3%). Acinetobacter sp. (40.6%) was the most prevailing of all isolates followed by Klebsiella sp.(29.4%), Pseudomonas sp. (11.2%), Candida sp. (8.7%), Staph. aureus (3.8%), Esch. coli (2.8%) and Aspergillus sp. (1.3%). Almost all (90-100%) of the Acinetobacter sp., Klebsiella sp., and Esch. coli showed resistance to 3rd generation cephelasporin, aztreonam, amoxicillin-clavulunate and ciprofloxacin and lowest resistance was noted to tigecycline (5.2%, 13% and 0% respectively). Imipenem was found resistant to 97.6%, 77.4% and 30% in Acinetobactersp, Klebsiella sp. and Esch.coli respectively. Resistant pattern of Pseudomonas sp. to ceftazidime (72.5%), aztreonam (81.2%), imipenem (75%), aminoglycosides (70-76%), ciprofloxacin (72.5%), piperacillin-tazobactum (41.2%) and colistin (11.2%) were also noted. Methicillin-resistant Staph. aureus (MRSA)were 77.8% and 75% Enterococcus sp. showed high level resistance to gentamicin (HLGRE). No vancomycin resistant Staph. aureus(VRSA) or Enterococcus sp. (VRE) was detected in this study.*

Conclusion: *Gram negative isolates were large-scale than any other microorganism. Of them, Acinetobacter sp. was most quotidian followed by Klebsiella sp. Acinetobacter sp. and Klebsiella sp. were highly resistant to different antibiotics including imipenem and piperacillin-tazobactum. High rate of colistin resistant Pseudomonas sp. were also observed. Combined clinical, microbiological and infection control strategies can guide appropriate patient management and antimicrobial stewardship program to combat antibiotic resistance in critical care set up in Bangladesh.*

Key words: *Antibiotic resistance, methicillin-resistant Staph. aureus, tertiary care hospital, ventilator-associated pneumonia.*

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Author information

a. Mili Rani Saha, Tanjila Rahman, Assistant Professor, Department of Microbiology, BIRDEM General Hospital, Dhaka, Bangladesh.

b. Md. Rokibul Hasan, Research Assistant, Department of Microbiology, BIRDEM General Hospital, Dhaka, Bangladesh.

c. KM Shahidul Islam, Retired Professor (Microbiology), BIRDEM General Hospital, Dhaka, Bangladesh.

Address of correspondence: Mili Rani Saha, Assistant Professor, Department of Microbiology, BIRDEM General Hospital, Dhaka, Bangladesh. Email: milisaha77@yahoo.com

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INTRODUCTION

Mechanical ventilation is a life-saving procedure for many patients in intensive care unit (ICU). Patients who are intubated and mechanically ventilated are at high risk of acquiring respiratory infections including ventilator associated pneumonia (VAP).[1] VAP constitutes 80% of nosocomial pneumonia and is considered as the second most common hospital acquired infection with higher mortality and morbidity.[2] The tube inserted in trachea reduces the clearance of bacteria, increases the leakage of secretion around the cuff of the tube and disables the cilliary tract by damaging it.[3] As a consequence of decreased salivary secretion, colonization of oropharynx with different bacteria may be responsible for super infections.[4] The etiological agents may vary according to the population of patients in ICU, duration of hospital stay, pre-existing and prior antimicrobial therapy.[5] Multi-drug resistant (MDR) bacteria are rapidly emerging across the world and pose a big challenge to health care system. Understanding such context, appropriate antimicrobial stewardship that includes optimal selection, dose and duration of treatment, as well as control of antimicrobial use will prevent or slow the emergence and dissemination of resistant microorganisms and also reduce adverse events and costs.[6] For instance, routine endotracheal aspirate cultures of critically ill patients in ICU may guide the selection of appropriate empirical therapy based on the predominant pathogens identified in microbial cultures in VAP.[7] It is difficult to manage these infections effectively unless physicians are armed with adequate and good quality data about the antibiotic susceptibility pattern of organisms causing respiratory infections among mechanically ventilated patients in ICU. As such data are scanty, this type of study update the knowledge of local epidemiology and susceptibility profile and guide the clinicians regarding empirical choice of antibiotics along with adequate clinical diagnosis and bacterial confirmation.[8] This study was undertaken to determine the pathogens and their antimicrobial resistance pattern over one year period in endotracheal aspirates in ICU of a tertiary care hospital in Dhaka city.

METHODS

This retrospective study was conducted in the Department of Microbiology, BIRDEM General Hospital, Dhaka, Bangladesh. A total 715 culture and antimicrobial sensitivity results of endotracheal aspirates from ICU patients during January to December, 2019 were included in this study. Culture was done by standard method[9] and antimicrobial sensitivity test of isolated bacteria by Kirby Bauer disc diffusion techniques.[10] Extended spectrum beta-lactamase (ESBL) was detected by Double Disc Synergy Test[11]and MRSA was detected by cefoxitin disc.[10] Data were collected from laboratory record book and analyzed by WHONET-5 software. As antimicrobial susceptibility test of fungus was not performed routinely, it was left out for analysis.

RESULTS

A total of 715 endotracheal aspirates from critically ill patients on mechanical ventilation were analyzed. Among these samples, 369(51.6%) were from male patients and 346(48.4%) were from female patients. Out of 715 samples, culture was positive in 482(67.4%) samples showing growth of bacteria and fungi either singly or in combination. Among the 482 culture positive samples, 250(51.9%) yielded single organism and 232(48.1%) double organisms (Table I).

Table I Frequency of isolates in culture positive endotracheal aspirates (N=714)

Culture yielding character	Culture positive samples No. (%)	Number of isolates
Single organism	250 (51.9)	250
Double organism	232 (48.1)	464
Total	482 (100)	714

The culture positive samples revealed 714 organisms of which 611 (85.6%) were gram negative, 31 (4.3%) were gram positive bacteria and 72 (10.1%) were fungi. Among total isolates *Acinetobacter sp.* (290, 40.6%,) was the most prevailing isolate followed by *Klebsiella sp.* (210, 29.4%), *Pseudomonas sp.* (80, 11.2%), *Candida sp.*(62, 8.7%), *Staph. aureus* (27,3.8%), *Esch. coli* (20, 2.8%), *Aspergillus sp.* (09, 1.3%), *Enterobacter sp.* (06, 0.8%), *Flavo-bacterium sp.* (05, 0.7%), *Enterococcus sp.* (04, 0.6%) and *Rhizopus sp*. (01, 0.1%). *Acinetobacter sp.* showed 90-100% resistance to 3rd generation cephalosporins, monobactum, amoxicillin-clavulunate, aminoglycosides and lowest resistance was noticed against tigecycline (5.2%). Most of *Klebsiella sp*. (95-97%) were resistant to 3rd generation cephalosporins, monobactum, amoxicillin-clavulunate, ciprofloxacin, 73-76% to aminoglycosides but 13% to tigecycline. No isolates of *Acinetobacter sp.* and *Klebsiella sp.* were resistant to colistin (Table II). Resistance to ceftazidime, monobactum, carbapenem, aminoglycosides in *Pseudomonas* sp. were 72.5%, 81.2%, 75% and 70-76% respectively. Resistance was also observed to piperacillin-tazobactum (41%) and colistin (11.2%) in *Pseudomonas sp.* (Table II). Among *Esch. coli,* all were resistant to 3rd generation cephalosporins, monobactum, amoxicillin-clavulunate and ciprofloxacin. All isolates were sensitive to tigecycline and colistin (Table II).

Table II Antimicrobial resistance pattern of isolated major gram negative bacteria (N=606)

Antibiotics	Resistance (%)				
	Acinetobacter sp. (n=290)	*Klebsiella sp.* (n=210)	*Pseudomonas sp.* (n=80)	*Esch. coli* (n=20)	*Enterobacter sp.* (n=06)
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
3rdgen Cephalosporin					
Ceftriaxone	290(100)	203(96.6)	ND	20(100)	4(66.7)
Ceftazidime	290(100)	203(96.6)	58(72.5)	20(100)	4(66.7)
Cefotaxime	290(100)	203(96.6)	ND	20(100)	4(66.7)
Cefuroxime	290(100)	203(96.6)	ND	20(100)	4(66.7)
Monobactum					
Aztreonam	290(100)	203(96.6)	65(81.2)	20(100)	4(66.7)
Beta-lactamase inhibitor combinations					
Amoxicillin-clavulunate	290(100)	203(96.6)	ND	20(100)	4(66.7)
Piperacillin–tazobactum	282(97.2)	168(79.8)	33(41.2)	8(40)	2(33.3)
Carbapenem					
Imipenem	283(97.6)	163(77.4)	60(75)	6(30)	1(16.7)
Aminoglycosides					
Amikacin	279(96.2)	156(75.0)	56(70.0)	3(15.8)	2(33.3)
Gentamicin	277(95.5)	160(76.1)	61(76.2)	5(25.0)	2(33.3)
Netilmicin	262(90.3)	155(73.6)	59(73.8)	4(20.0)	2(33.3)
Fluroquinolones					
Ciprofloxacin	288(99.3)	201(95.7)	58(72.5)	20(100)	4(66.7)
Folate pathway inhibitors					
Cotrimoxazole	239(82.4)	150(71.2)	ND	10(50)	0(0)
Glycycline					
Tigecycline	15(5.2)	27(13.0)	ND	0(0)	0(0)
Lipopeptides					
Colistin	0(0)	0(0)	9(11.2)	0(0)	0(0)

ND= Not done

The antibiotic resistance pattern of *Staph. aureus* to oxacillin and cefoxitin were noted in 77.8%. All the strains of *Enterococcus sp*. were found resistance to ciprofloxacin and 50% to penicillin and ampicillin. Vancomycin was found sensitive to all gram positive isolates (Table III). Out of 236 *Enterobacteriaceae* isolates, 38% *Klebsiella sp*. (80/210), 35% *Esch.coli* (7/20) and 33.3% *Enterobacter sp*. (2/6) were ESBL producers.

Table III Antimicrobial resistance pattern of isolated gram positive bacteria (N=31)

Antibiotics	Resistance (%)	
	Staph. aureus (n= 27) No. (%)	*Enterococcus* sp. (n= 04) No. (%)
Cell wall inhibitor		
Penicillin	ND	2(50)
Ampicillin	ND	2(50)
Oxacillin	21(77.8)	ND
Cefoxitin	21(77.8)	ND
Cefalexin	21(77.8)	ND
Augmentin	21(77.8)	ND
Vancomycin	0(0)	0(0)
Nucleic acid synthesis inhibitor		
Rifampicin	05(18.5)	ND
Ciprofloxacin	23(85.2)	4(100)
Cotrimoxazole	6(22.2)	4(100)
Protein synthesis inhibitor		
Amikacin	3(11.1)	ND
Gentamicin	4(14.8)	3(75)
Netilmicin	2(7.4)	ND
Erythromycin	21(77.8)	ND
Clindamycin	20(74.1)	ND
Fusidic acid	4(14.8)	ND

ND= Not done

DISCUSSION

The study revealed high rate growth of microbes and preponderance of MDR gram negative bacilli in mechanically-ventilated patients.

Out of 715 samples, positive culture was observed in 482(67.41%) samples. Similarly high rate of culture positivity was also reported by several recent reports, among ICU admitted patients of Bangladesh[12] and India.[13]The inserted mechanical tube, co-morbidity of the patient and use of immunosuppressive drugs are mainly responsible for the high rate of culture positivity in endotracheal aspirates.[5,7]

Among the culture positive cases, 51.9% were single organisms and 48.1% were double organisms, yielding a total of 714 isolates. The high rate of single organisms over double organisms was also reported in other studies.[14]

The etiological agents were gram negative bacilli (85.6%), gram positive cocci (4.3%) and fungus (10.1%) in this study. Several previous studies reported that predominant isolation rate was gram negative bacilli[15] in endotracheal aspirates. The cause of predominant isolation rate of the gram negative organism among hospitalized patient might be due to selective pressure of broad spectrum antibiotics causing persistent of drug resistance genes/ plasmids, virulence factors like flagella, capsule, outer membrane in this class compared to gram positive bacteria.[16] Another possible explanation of the predominance of gram negative bacteria is that asymptomatic colonization of patients, the contaminated environment or both can serve as reservoirs for these pathogens, which are then transmitted by the hands of health care workers.[17]

The most commonly identified organism was *Acinetobacter sp.* (40.6%) followed by *Klebsiella sp.* (29.4%)*, Pseudomonas sp*. (11.2%) and *Candida sp.* (8.7%) in the current study. Similar study was carried out by several investigators reported that most frequently isolated organisms were *Acinetobacter sp.* (37.8%) followed by *Pseudomonas sp.*(24.3%), *Klebsiella sp.* (13.5%).[18] But Jakribettu et al., noted that *Klebsiella sp.* (34%) was the most common isolate, followed by *Pseudomonas sp.* (20%) and *Acinetobacter sp.* (18%).[19] The changing pattern of causative agent in endotracheal aspirates might be due to predominant organism residing in the hospital environment.

The present study detected,4.3% gram positive bacteria in which 3.8% *Staph. aureus* and 0.6% *Enterococcus sp.* The observation is consistent with a study where 5.4% were *Staph. aureus*.[18] A study done by Quartin et al was reported that gram positive pathogens were the predominant isolates (42.7 %).[20] This disparity 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 antibiotics, type of ICU admitted patient, length of ICU stay and the method used for diagnosis of VAP.[15]

The isolated *Acinetobacter sp.* of this study showed higher resistance (82.4%-100%) to all antibiotics

(Table II) except tigecycline (5.2%) and colistin (0.3%). Similar findings have been reported by other investigators.[21] Al-Sweih reported that 13.6% *Acinetobacter sp.* were resistant to tigecycline where rate was higher than the present study.[22]

The isolated *Klebsiella sp.* of this study showed higher resistance (71.2%-96.6%) to all antibiotics (Table no II) except tigecycline (13.0%) and colistin (0%). *Esch. coli* showed higher resistance to all antibiotics but all were sensitive to tigecycline and colistin. Similar findings were also compatible with Khatun's study. [23]

Isolated *Pseudomonas sp*. in the present study, showed high resistance (72.5%-81.2%) to almost all anti-pseudomonal panel of antibiotics. The present study showed 75% of *Pseudomonas sp.* were carbapenam resistant. Similar observations were also made by Dey et al (50%).[5] Moreover, in the present study, *Pseudomonas sp.* was 41.2% resistant to piperacillin–tazobactum and 11.2% to colistin. Piperacillin–tazobactum was also ineffective against most of the organisms reported by Sarkar et al which is similar to this study.[24]

The emergence of MDR bacteria has forced us to think about polymyxins, especially colistin.[25] But the frequent use of this reserve antibiotic, colistin resistance strains have developed.[26] A study in Bangladesh reported in 2019 that, *Klebsiella sp.* was found highest resistant to colistin with 16.6%, *Pseudomonas sp*. with 11.76% and *Acinetobacter sp.* with 9.8%.[27] These results are very much similar to the present study. Therefore, colistin use should be restricted to control the rate of colistin resistance bacteria.

Staph. aureus showed 77.8% resistance to cefoxitin, oxacillin, and 85.2% resistance to ciprofloxacin. *Enterococcus sp.* were 100% resistant to ciprofloxacin and cotrimoxazole. This study also reported that 75% *Enterococcus sp.* high level resistant to gentamicin (HLGRE). This finding is similar to the reports of Taj et al.[28] The gram positive isolates of the current study were 100% sensitive to vancomycin which is in concord to the findings of Taj et al.[28] Out of 27 isolates of *Staph. aureus,* 21(77.8%) were MRSA. As the total number of *Staph. aureus* were 27, this observation cannot be considered as high percentage. 18.2% MRSA was reported by Veena Krishnamurthy et al.[29] This finding is inconsistent with the present study.

This study presents a high rate of ESBL producers which were common among Enterobacteriaceae members like *Klebsiella sp.* (38%), *Esch. coli* (35%) and *Enterobacter sp*.(33.33%). Similar results have been reported in Bangladesh.[23]

Candida species was the fourth frequently isolated organism but high figure isolation in this study. It might be due to long-term antibiotic use or severe immunosuppressive states in critically ill patients. Identification of *Candida species* and sensitivity test were not analyzed as routine practice of this test was limited in our laboratory.

Conclusion

In conclusion, this study presents that the most common microorganisms are *Acinetobacter sp.* and *Klebsiella sp.* in endotracheal aspirates. This study also delineate aetiological agents of VAP, their antimicrobial resistance pattern, high rate of imipenem resistant gram negative bacteria, ESBL producers and MRSA. So, the data collected from this study could present a current overview in microbes in endotracheal aspirates of mechanically ventilated patients in an ICU of a tertiary care hospital in Bangladesh. Periodic monitoring and antimicrobial resistance surveillance are necessary for proper patient management, selection of antibiotic and also to combat the emergence of resistant organisms in the critical care setup of Bangladesh.

Authors' contribution: MRS prepared the study design, collected data, writing the manuscript, TR helped in draft, MRH compiled data and KMSI supervised and edited the manuscript. All authors read and approve the final version for submission.

Conflicts of interest: Nothing to declare.

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