

Original Research Article

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Antibiogram of Bacterial Isolates from Endotracheal Aspirates in a Tertiary Care Hospital in North India

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ABSTRACT

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Respiratory tract infections are very common in critically ill patients in hospital settings. Endotracheal aspiration is a simple and easy method to perform especially in ICU patients for obtaining samples from lower respiratory tract. Therefore, this study was carried out to determine the microbiological spectrum and antibiotic susceptibility profile of Endotracheal aspirates among patients admitted in our institute. The present study was conducted on 348 ETT aspirate samples received in the Department of Microbiology, GMC, Jammu for a period of 1 year i.e. April 2017- 2018. Bacteria were identified using standard bacteriological and biochemical methods. 285 (81.89%) samples were culture positive. The most commonly isolated bacteria were *Acinetobacter*, followed by *Klebsiella*, *Pseudomonas* and *Staphylococcus aureus*. Meropenem, Amikacin and Tobramycin showed good sensitivity against GNB. Colistin, Polymyxin-B, Cefepime-tazobactam were highly effective against *Acinetobacter* and *Pseudomonas* isolates. Multi-drug resistant microorganisms are on a rise in our hospital. Strict implementation of antibiotic policy and creating awareness through education programmes can help in combating rapid spread of drug resistance.

Introduction

Respiratory infections in critically ill patients are associated with high morbidity and mortality. Investigation of the lower respiratory tract infections in hospitalized patients particularly in ICUs is difficult due to limited access to the site of infection. Endotracheal aspiration is a relatively noninvasive method that can be easily performed for obtaining early, accurate and representative sample of lower respiratory tract pathogens (Mouli *et al.*, 2016). Once respiratory infection is diagnosed, treatment

strategy is early, swift administration of antibiotics as per culture results.

The etiological agents may vary according to duration of stay in hospital, the population of patients admitted, pre-existing illness and prior antimicrobial therapy. Internationally several studies done on ET aspirates reported predominantly gram negative organisms including *Klebsiella spp*, *Pseudomonas spp*, *Enterobacter spp*, *Acinetobacter spp*. and gram positive bacteria like *Staphylococcus aureus*. In India studies have been carried out to evaluate the organisms isolated from the

ET aspirates and demonstrated the significant isolation of gram negative organisms including *Klebsiella spp*, *Acinetobacter spp*, *Pseudomonas spp*. (A. Swati *et al.*, 2018)

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 of resistance among microorganisms in the hospital settings. (S. Priyanka *et al.*, 2015) With this objective in mind this study was carried out to determine the microbiological spectrum and antibiotic susceptibility profile of Endotracheal aspirates among patients admitted in our institute. This will provide a valuable guidance for antibiotic therapy.

Materials and Methods

Study design

This was a retrospective study conducted in the Department of Microbiology, Government Medical College, Jammu. All endotracheal aspirate specimens received in the Microbiology department for culture and sensitivity testing over a period of 1 year i.e. from April 2017- April 2018 were included in the study.

Sample collection

Endotracheal aspirate

Sampling was done by introducing a catheter aseptically through the endotracheal tube and secretions aspirated into a sterile syringe.

Endotracheal tube tip

The endo-tracheal tube tip was cut aseptically into a sterile container and sent to the Microbiology laboratory.

Sample processing

Sample processing was done using standard aseptic precautions.

Endotracheal aspirate

1 µl of the specimen was inoculated on Blood agar and MacConkey agar.

Endotracheal tube tip

ET tip was rubbed on Blood agar and MacConkey agar plates for inoculation of sample.

Bacterial isolation and identification

A Gram stain of the endotracheal secretions/endotracheal tube tip fluid was done to assess the number of pus cells and the presence of bacteria. The inoculated plates were incubated overnight at 37°C. All plates were examined for visible growth. The colonies were identified as per standard microbiological procedures. The bacteria were identified by their colony morphology, Gram staining, biochemical and sugar fermentation tests. Antibiotic sensitivity testing of the microorganisms was done by modified Kirby Bauer Disc Diffusion method on Muller Hinton agar and the results were interpreted as was recommended by the CLSI (Clinical Laboratory Standards Institute) guidelines.

Results and Discussion

ET tube tip provides the most representative sample of the pathogenic bacteria without any chance of contamination. Out of the 348 Endotracheal aspirates received in the Microbiology laboratory for analysis, 285 (81.89%) were culture positive. 316 isolates were obtained from 285 culture positive samples i.e. 254 (89.12%) yielded single

bacterial isolate and 31 (10.87%) yielded two bacterial isolates.

Among the isolates, Gram negative bacilli accounted 268 (84.81%), Gram positive cocci 46 (14.55%) and 2 (0.63%) isolates of *Candida* species were obtained (Table 1).

Acinetobacter sp. was the predominant Gram negative bacilli which constituted 96 (35.82%) followed by *Klebsiella sp.* 76 (28.35%), *Pseudomonas sp.* 41 (15.29%), *Citrobacter sp.* 24 (8.95%), *Escherichia coli* 16 (5.97%), *Proteus sp.* 10 (3.73%), *Enterobacter sp.* 4(1.49%) and *Serratia* 1(0.37%). Similar results were noted by (Modi *et al.*, 2011) and (Mouli *et al.*, 2016). *Acinetobacter sp.* is a persistent colonizer due to its ability of forming biofilm which is an important virulence factor for device associated infections (Ejaz *et al.*, 2016)

Among the Gram positive bacteria *Staphylococcus aureus* 40 (86.95%) was the leading isolate. Less frequently encountered organisms were *Streptococcus pneumoniae* and *Enterococcus sp* 3 (6.52%) isolates each.

The culture positive specimens belonged to 188 (65.96%) male and 97 (34.03%) female hospitalized patients.

During the study it was noted that most of the isolates were obtained from intubated patients in MICU 129 (40.82%) and EICU 65 (20.56%) (Table 2). This is a matter of concern as patients admitted in these units are seriously ill and are more prone to get infection. Our findings are in agreement with (Khosravi *et al.*, 2013).

Antimicrobial resistance is becoming a critical issue in hospital settings, both for the management and prevention of serious infections in patients (Ejaz *et al.*, 2016). It leads to increase in cost of patient care and also a rise in morbidity and mortality rate.

Studying the antibiogram of the bacterial isolates, both gram negative and gram positive bacteria showed multi-drug resistance (Table 3 and 4). Similar results were seen in many studies (Vedavathy *et al.*, 2016). The major reasons for multidrug resistance are ventilators, prolonged hospital stay and irrational usage of antibiotics. (Santharam *et al.*, 2018).

The non- fermenters *Acinetobacter sp.* and *Pseudomonas sp.* showed high sensitivity to Cefoperazone-sulbactam (88.54%, 95.12%), Colistin (87.5%, 87.54%), Polymyxin-B (84.37%, 92.68%), Cefepime-tazobactam (92.70%, 90.24%) respectively. This is in concordance with Mst *et al.*, (2018). Hence, its use should be restricted to severe nosocomial infections, in order to avoid rapid emergence of resistant strains.

Studying the antibiotic sensitivity profile of the Enterobacteriaceae most isolates showed high resistance pattern. The sensitivity pattern of *Escherichia coli* and *Klebsiella sp.* were as follows: Amoxicillin-clavulanic acid (6.25%, 1.31%), Piperacillin-tazobactam (31.25%, 50%), Ceftriaxone (56.25%, 55.26%), Meropenem (87.5%, 81.57%), Amikacin (75%, 68.42%), Gentamicin (56.25%, 55.26%), Tobramycin (75%,72.36%) , Ciprofloxacin (6.25%, 7.89%), Co-trimoxazole (12.5%,6.57%) respectively. Similar result was seen in study conducted by Syal *et al.*, (2018). Aminoglycosides and Quinolones are a good option for treatment but due to their unnecessary injurious use, resistance against them is also on a rise.

The antibiotic profile of *Citrobacter sp.* showed poor sensitivity to Cefepime (62.5%), Cefuroxime (29.16%), Ceftriaxone (58.33%). High rate of resistance shows that Cephalosporins in monotherapy are no longer an effective treatment option.

Table.1 Isolate frequency in endotracheal aspirate samples

Total Samples	Total Isolates	Single type of Growth	Two types of Growth	Bacteria		Fungi
				GNB	GPC	
348	316	254	31	268	46	2

Table.2 Ward-wise distribution of various isolates

Wards	E.coli	Kle	Citro	Enb	Pseu	Acinet	Prot	Ser	Stap	Strep	Ent	BY	Total
EICU	8	17	4	3	10	18	2	-		1	2	-	65
MICU	4	36	10	1	6	51	4	1	12	2	-	2	129
SICU	1	7	2	-	15	11	-	-	7	-	-	-	43
PICU	2	12	4	-	5	13	3	-	6	-	1	-	46
Surgery ward	1	4	4	-	5	3	1	-	7	-	-	-	25

E.coli=*Escherichia coli*, Kle=*Klebsiella*, Citro=*Citrobacter*, Enb=*Enterobacter*, Pseu=*Pseudomonas*, Acinet=*Acinetobacter*, Prot=*Proteus*, Ser=*Serratia*, Stap=*Staphylococcus*, Strep=*Streptococcus*, Ent=*Enterococcus*, BY=Budding yeast

Table.3 Antibiotic sensitivity profile of gram negative isolates (N= 268)

	E.coli (N=16)	Kle (N=76)	Citro (N=24)	Enb (N=4)	Pseu (N=41)	Acinet (N=96)	Prot (N=10)	Ser (N=1)
AMP	0 (0%)	3(7.89%)	2(8.33%)	0(0%)	5(12.19)	10(10.41%)	2(20%)	0(0%)
AMC	1 (6.25%)	1(1.31%)	0(0%)	0(0%)	0(0%)	4(4.16%)	1(10%)	0(0%)
PIT	5 (31.25%)	38 (50%)	10(54.16%)	2(50%)	31(75.60%)	65(67.70%)	4(40%)	1(100%)
CPM	8 (50%)	45(59.21%)	15(62.5%)	2(50%)	33(80.48%)	70(72.91%)	5(50%)	0(0%)
CXM	0 (0%)	6 (7.89%)	7(29.16%)	1(25%)	10(24.39%)	20(20.83%)	0(0%)	0(0%)
CTR	9(56.25%)	42(55.26%)	14(58.33%)	2(50%)	21(51.21%)	51(53.12%)	2(20%)	0(0%)
AO	12(75%)	51(67.10%)	16(66.66%)	1(25%)	30(73.17%)	60(62.5%)	2(20%)	1(100%)
MR	14(87.5%)	62(81.57%)	21(87.5%)	3(75%)	35(85.36%)	68(70.83%)	8(80%)	1(100%)
AK	12(75%)	52(68.42%)	18(75%)	2(50%)	32(78.04%)	65(67.70%)	6(60%)	1(100%)
GEN	9(56.25%)	42(55.26%)	15(62.5%)	3(75%)	28(68.29%)	58(60.41%)	5(50%)	1(100%)
TOB	12 (75%)	55(72.36%)	20(83.33%)	4(100%)	35(85.36%)	72(75%)	6(60%)	1(100%)
CIP	1 (6.25%)	6(7.89%)	5(20.83%)	1(25%)	21(51.21%)	48(50%)	1(10%)	0(0%)
COT	2 (12.5%)	5(6.57%)	3(12.5%)	1(25%)	12(29.26%)	20(20.83%)	1(10%)	0(0%)
CEF-SUL	-	-	-	-	39(95.12%)	85(88.54%)	-	-
CL	-	-	-	-	36(87.80%)	84(87.5%)	-	-
PB	-	-	-	-	38(92.68%)	81(84.37%)	-	-
CPM-TZ	-	-	-	-	37(90.24%)	89(92.70%)	-	-

Table.4 Antibiotic sensitivity profile of gram positive isolates (N=46)

	Stap. (N=40)	Strep. (N=3)	Enterococcus (N=3)
PG	7(17.5%)	1(33.3%)	1(33.3%)
VA	36(90%)	3(100%)	2(66.6%)
CD	30(75%)	3(100%)	3(100%)
E	11(27.5%)	2(66.6%)	1(33.3%)
LZ	36(90%)	3(100%)	3(100%)
C	37(92.5%)	2(66.6%)	3(100%)
GEN	18(45%)	-	-
CIP	20(50%)	-	-
COT	23(57.5%)	-	-
TET	36(90%)	-	-
CX	25(62.5%)	-	-
CEF	10(25%)	-	-
CTR	-	2(66.6%)	2(66.6%)
CPM	-	3(100%)	2(66.6%)

Pencillin G=PG, VA= Vancomcin, CD= Clindamycin, E=Erythromycin, LZ= Linezolid, C= Chloramphenicol, GEN=Gentamycin, CIP=Ciprofloxacin, COT= Co-trimoxazole, TET= Tetracycline, CX= Cefoxitin, CEF=Ceftazidime, CTR=Ceftriaxone, CPM=Cefipime

The *Proteus sp.* isolates showed high resistance against Ampicillin (20%), Amoxicillin-clavulanic acid (10%), Piperacillin-tazobactam (40%), Ciprofloxacin (10%) and Co-trimoxazole (10%). Good sensitivity was seen against Meropenem (80%).

Antibiotic sensitivity pattern of Gram positive isolates revealed good sensitivity of *Staphylococcus aureus*, *Streptococcus sp.* and *Enterococcus sp.* against Vancomycin, Linezolid and Chloramphenicol. 15 (37.5%) isolates of *Staphylococcus aureus* were methicillin resistant. Similar results were seen in studies conducted by Chandra *et al.*, (2017).

Our study concluded that multi drug resistant micro-organisms are on a rise in our hospital setting especially in ICU patients. Amongst Endotracheal tube aspirates the commonest organisms isolated were *Acinetobacter*, followed by *Klebsiella* and *Pseudomonas*.

There was an alarming increase of resistance against Penicillins, Cephalosporins and Quinolones. Imipenems still show good sensitivity but resistance is on a rise. Antibiotics such as Colistin and Polymyxin-B were very effective against *Acinetobacter* and *Pseudomonas* infections and should be used as reserve drugs. In order to fight the high antimicrobial resistance there is a need for development of strict antibiotic policy in the hospital so that injurious and irrational use of antibiotics is prevented. The infection control committee plays an important role in implementation of hand hygiene practices and creating awareness through education programmes.

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