BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF TRACHEAL SECRETIONS ISOLATES AMONG ICU PATIENTS AT TERTIARY CARE HOSPITAL

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ABS TRACT

Tracheobronchial secretions are produced by mucous glands and goblet cells of the tracheobronchial tree. These secretions are not only involved in the protection of the respiratory system but are also responsible for the exchange of heat and water during breathing.

Respiratory infections are associated with high morbidity and mortality, especially in ICU patients. Such patients are commonly maintained using invasive devices which itself tend to be a major reservoir for hospital-acquired infections. Among Respiratory infections, Lower Respiratory Tract Infections (LRTI) are the most common infectious diseases affecting humans causing significant morbidity and mortality for all age groups. It is responsible for 4.4% of all hospital admissions. It also accounts for 3%–5% of deaths in adults. LRTI are often misdiagnosed, mistreated, and underestimated due to its nonspecific presentation in community or hospital setting. Etiological agents of an LRTI vary geographically and timely.These problem is much greater in developing countries.

Critically ill patients of ICUs are at greater risk for acquiring hospital-associated infections with multidrug-resistant microorganisms. This is because of their prolonged hospital stay, immunocompromised profile, serious illness, use of invasive devices, catheters, and prolonged use of antibiotics. The frequent and unselective usage of broad-spectrum antibiotics without reporting of culture and sensitivity leads to development of these multidrug resistant superbugs in the world of microbi- ology and this creates problem for the treatment of ICUs patients.

KEYWORDS
Respiratory infections, Lower Respiratory Tract Infections (LRTI).

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INTRODUCTION

Tracheobronchial secretions are produced by mucous glands and goblet cells of the tracheobronchial tree [1]. These secretions are not only involved in the protection of the respiratory system but are also responsible for the exchange of heat and water during breathing [2].

Respiratory infections are associated with high morbidity and mortality, especially in ICU patients [3]. Such patients are commonly maintained using invasive devices which itself tend to be a major reservoir for hospital-acquired infections [4,5].

Among Respiratory infections, Lower respiratory tract infections (LRTI) are the most common infectious diseases affecting humans causing significant morbidity and mortality for all age groups. It is responsible for 4.4% of all hospital admissions. It also accounts for 3%–5% of deaths in adults[6].

LRTI are often misdiagnosed, mistreated, and underestimated due to its nonspecific presentation in community or hospital setting. Etiological agents of an LRTI vary geographically and timely.These problem is much greater in developing countries[7,8].

Critically ill patients of ICUs are at greater risk for acquiring hospital-associated infections with multidrug-resistant microorganisms. This is because of their prolonged hospital stay, immunocompromised profile, serious illness, use of invasive devices, catheters, and prolonged use of antibiotics[9]. The frequent and unselective usage of broad-spectrum antibiotics without reporting of culture and sensitivity leads to development of these multidrug resistant superbugs in the world of microbi- ology and this creates problem for the treatment of ICUs patients[10,11].

Ventilator-Associated Pneumonia (VAP) is defined as pneumonia that occurs 48–72 hours or there after following endotracheal intubation, characterized by the presence of a new or progressive infiltrate, signs of systemic infection (fever, altered white blood cell count), changes in sputum characteristics, and detection of a causative agent[12].

The longer the hospital stay is as in the case of head trauma, the more are the chances of a change of bacterial flora causing infection, eventually complicating therapy because of alteration of susceptibility pattern[13,14].

Ventilator-Associated Pneumonia (VAP) is one of the commonest and important nosocomial infections, which is acquired by ICUs admitted patients, who are intubated for mechanical ventilation. The mechanical- ventilation is one of the lifesaving practices for ICUs admitted patients but it has a greater risk of developing respiratory infections. The morbidity of these patients is increasing due to the invasion of MDR strains of microorganisms. Among these superbugs, who are associated with pneumonia, are Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella spp., and Acinetobacter spp. Apart from this, ICUs patients developed multi- bacterial infections during their prolonged stay in hospitals. These changing floras also complicate the therapy by developing MDR and their sensitivities pattern[15].

Family Enterobacteriaceae comprising of Gram-negative bacteria, their common prevalent bacteria can develop resistance against different β- lactam agents, which attributes their resistance to broad-spectrum cephalosporin. The antimicrobials of this group were primarily administrated or the treatment of ICUs patients in hospitals previously. Along with them, the bacteria also developed antimicrobial resistance to other groups of antibiotics like Trimethoprim Sulphamethoxazole, Fluoroquinolones, and Aminoglycosides[16]. Similarly, MRSA strains are also one of the most important microorganisms regarding nosocomial infections in ICUs. MRSA developed due to excessive usage of antibiotics in ICUs settings. About 70% of Staphylococcus aureus isolated from ICUs are MRSA[17].

The etiology of these MDR superbugs may vary according to the different ICUs settings along with the patient's illness and their antibacterial treatment. Therefore, it is mandatory to have knowledge about the bacterial pattern of the hospital ICUs settings and their local antimicrobial susceptibility pattern, which provides guidelines to the clinicians for prompt and empirical treatment with appropriate antibiotics[18].

Objective

The aim of this study was to identify the common bacterial pathogens in tracheal secretions and to study the patterns of their sensitivity to various antibiotics.

MATERIALS AND METHODS

A retrospective study was conducted in tertiary care hospital in Ahmedabad over a period of 4 months from November 2019 to February 2020.The analysis of the reports was done at the Microbiology Department.

Sample Collection

The respiratory tract samples (sputum, Bronchoealveolar Lavage [BAL], endotracheal aspirate, gastric lavage, etc.) from ICU patients of all the age and sex groups were included in the study.

Patient Enrollment

Samples from patients admitted in ICU for More than 48 hrs on Mechanical Ventilation, Clinical History of fever ≥ 38°C,whose WBCs count ≥ 10,000/mm^3 or ≤ 3000/mm, having purulent tracheal secretions and with diffused or patchy infiltration in chest radiograph were included in our study.

Specimen selected in the study

Gram's staining of these samples was done to rule out that whether the bacteria were a colonizer or pathogen using Q score. It also provided an initial clues about the type of bacteria, whether the material was purulent or not, (≥ 25 neutrophils and ≤ 10 squamous cells per Low power field).

All patients whose quantitative culture had revealed a colony count of <105 CFU/ml of tracheal aspirate and who did not show clinical evidence of pneumonia were excluded from the study.

Total 632 tracheal secretions specimen enrolled in the study. The received samples of endotracheal secretions were inoculated on blood agar and Mac Conkeys agar and incubated for 24 hours in an incubator at 37oC.The cultures were read next day for any positive or negative growth. The culture read as semi quantitatively when growth was moderate or heavy and quantitatively when more than 105 colony- forming units CFU/mL of bacteria were isolated on culture. The bacteria were preliminary identified on basis of their colonial morphology, presence or absence of hemolysis on blood agar, fermenter or non-fermenter. Then Gram's staining was done to confirm whether Gram-positive or Gram-negative cocci or bacilli.

Thereafter, Identification with the VITEK-2 compact system was performed using a Gram Negative (GN) card and Gram Positive (GP) card according to manufacturer's instructions.

Antibiotics susceptibility testing with the VITEK-2 compact system was performed using an AST N281(for Acinetobacter and Pseudomonas aeruginosa) and N280 (for other gram negative organisms) susceptibility card for Gram negative organisms and GP628 susceptibility card for Gram positive organisms[19].
AST card 280 contains antibiotics Amikacin, Ampicillin, Amoxicillin/clavulanic acid, Cefepime, Cefoperazone/Sulbactam, Ceftriaxone, Cefuroxime , Ciprofloxacin, Colistin, Ertapenem, Gentamicin, Imipenem, Meropenem, Nalidixic acid, Nitrofurantoin, Piperacillin/Tazobactam, Tigecyclin, Trimethoprim/Sulfamethoxazole.
AST card 281 contains antibiotics Amikacin, Cefepime, Cefoperazone/Sulbactam, Ceftazidime , Ciprofloxacin, Colistin, Doripenem, Gentamicin, Imipenem, Meropenem, Minocycline, Piperacillin/Tazobactam, Ticarcillin/clauvulanic acid, Trimethoprim/Sulfamethoxazole.
AST card 628 contains antibiotics Ciprofloxacin, Clindamycin, Daptomycin, Erythromycin, Gentamicin, Levofloxacin, Linezolid, Nitrofurantoin, Oxacillin, Rifampicin, Teicoplanin, Tetracycline,Tigecycline, Trimethoprim/Sulfamethoxazole, Vancomycin.

RESULTS

In total, 632 tracheal secretions were received during the study period, 559 cultures yielded significant pathogens and no organisms were isolated in 73 cultures. Among 559 cultures,172 samples showed polymicrobial infection.
Gram negative bacteria contributed to major number of isolates (97%) including Klebsiella pneumoniae(30%) being most common followed by Acinetobacter baumanni complex (28%),Pseudomonas spp.(21%), E.coli (6%) and other gram negative bacteria includes Proteus mirabilis, Providencia rettegeri, Providencia stuartii, Serratia marcescens, Enterobacter cloacae complex, Stenotrophomonas maltophilia.
Gram positive bacteria (2%) includes S.aureus , S.hemolyticus, Streptococcus pneumoniae, Enterococcus faecium. Among Candida spp.(1%) C. tropicalis, C. famata, C.albicans and C.lustianae Shown in chart 1.

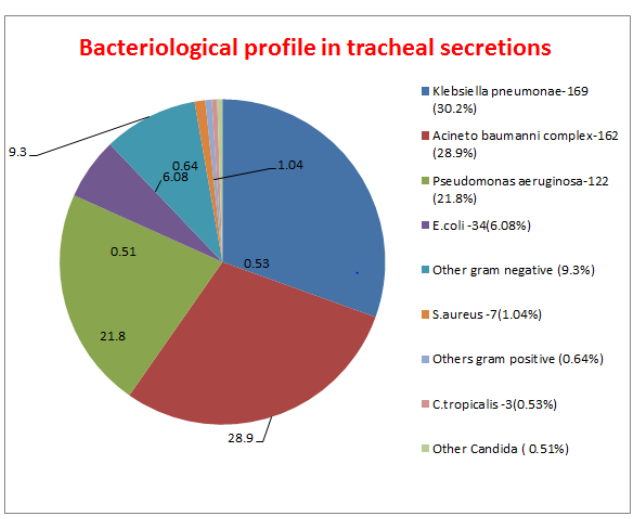

Chart 1 : Bacteriological Profile In Tracheal Secretions

In our study, Staphylococcus aureus is 100% sensitive to Vancomycin , Linezolid and Tigecyclin followed by 85.7 % sensitivity to Daptomycin shown in chart 2.

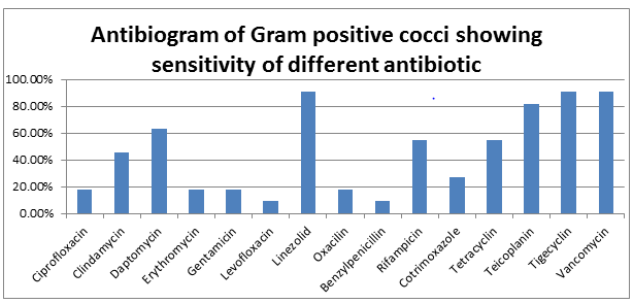

Chart 2 : Antibiogram Of Gram Positive Cocci Showing Sensitivity Of Different Antibiotic

In our study, Candida spp. showed 100% susceptibility to amphotericin B, Caspofungin, Flucytosin, Fluconazole, Micafungin, Voriconazole.
Our results showed that Gram negative bacilli were most susceptible to colistin.
E.coli were sensitive to amikacin (82.3%) with MIC <=2 , imipenem(67.6%)with MIC <=0.25, meropenem(67.6%) with MIC <=0.25 and tigecyclin(94.1%) with MIC <=0.5 (table 1).

Pseudomonas were sensitive to gentamicin(55.7%) with MIC <=1 , cefepime(53.2%) with MIC OF 8 , amikacin(51.6%)with MIC <=2 . (See Table 2)

In our study phenotypic detection by VITEK-2 AES system; In Gram positive cocci , Staphylococci aureus shows most commonly resistance to penicillin, Aminoglycoside, Streptogramins and Oxacillin. In gram negative Bacilli; non lactose fermenter Acinetobacter spp. shows commonly resistance to gentamicin and Carbapenems(139)and in Pseudomonas spp. shows common resistance to Aminoglycosides and Carbapenemase(34). (Table 1)
In lactose fermenter organisms E.coli and Klebsiella pneumoniae most common phenotype is ESBL producing. (Table 1)

Antibiotics	Klebsiella pneumoniae 169(30.2%)				E. coli 34(6.08%)				
	No.(%)	MIC			No.(%)	MIC			
Amikacin	54(31.95%)	≤ 2			28(82.30%)	≤ 2(14)	4(6)	8(7)	16(1)
Amoxicillin/Clavulanic acid	20(11.80%)	4(5)	8(15)		7(20.50%)	4(5)	8(2)		
Ciprofloxacin	10(5.90%)	≤ 0.25			4(11.70%)	≤0.25			
Ceftriaxone	3(1.77%)	≤ 1							
Colistin	155(99.40%)	≤ 0.5			33(97.00%)	≤0.5			
Cefuroxime	3(1.00%)	≤ 1(1)	2(1)	4(1)					
Doripenem	1(0.59%)	≤ 0.12			2(5.80%)	≤0.12			
Ertapenem	29(17.15%)	≤ 0.5			21(61.70%)	≤0.5			
Cefepime	20(11.83%)	≤ 1(3)	2(17)		4(11.70%)	2			
Gentamicin	52(30.76%)	≤ 1			22(64.70%)	≤ 1(18)	2(1)	4(3)	
Imipenem	37(21.89%)	≤ 0.25			23(67.60%)	≤ 0.25(20)	0.5(2)	1(1)	
Meropenem	35(20.71%)	≤ 0.25			23(67.60%)	≤ 0.25			
Minocyclin	6(3.55%)	4			3(8.80%)	≤ 1			
Cefoperazone/sulbactam	28(16.50%)	≤ 8(22)	16(6)		10(29.40%)	≤ 8/16			
Cotrimoxazole	42(24.85%)	≤ 20			17(50.00%)	≤ 20			
Tigecyclin	115(69.04%)	≤ 0.5(15)	1(35)		32(94.10%)	≤ 0.5			
Piperacilin/tazobactam	23(13.60%)	≤ 4(18)	8(3)	16(2)	6(17.60%)	≤ 4			

Table 1 : Antibiotic Sensitivity Pattern Of Klebsiella Spp. & E.Coli

Antibiotics	Pseudomonas spp. 122(21%)					Acinetobacter spp. 162(28%)				
	No.(%)	MIC				No.(%)	MIC			
Amikacin	63(51.60%)	≤ 2 (56)	4(4)	8(2)	16(1)	33(20.30%)	≤ 2(5)	4(5)	8(5)	16(12)
Ceftazidime	58(47.50%)	≤ 1(4)	2(4)	4(45)	8(5)	2(1.20%)	4			
Ciprofloxacin	58(47.50%)	≤ 0.25(50)		0.5(8)		5(3.00%)	≤ 0.25			
Colistin	108(88.50%)	≤ 0.5				154(95.00%)	≤ 0.5			
Doripenem	49(40.10%)	≤ 0.12(16)	0.25(9)	0.5(12)	1(12)	3(1.85%)	≤ 0.12			
Cefepime	65(53.20%)	≤ 1(9)	2(38)	4(8)	8(10)	5(3.08%)	2(1)	4(1)	8(3)	
Gentamicin	68(55.70%)	≤ 1(53)		2(8)	4(7)	11(6.70%)	≤ 1			
Imipenem	49(40.10%)	≤ 0.25(4)	0.5(4)	1(26)	2(15)	3(1.85%)	≤ 0.25			
Levofloxacin	43(35.20%)	≤ 0.25(4)	0.5(15)	1(23)	≤ 0.12(1)	6(3.60%)	≤ 0.12(3)	1(1)	2(2)	
Meropenem	49(40.10%)	≤ 0.25(25)	0.5(7)	1(12)	2(25)	3(1.85%)	≤ 0.25			
Minocyclin	INTRINSIC RESISTANCE					63(38.80%)	≤ 1(39)	2(9)	4(15)	
Cefoperazone /sulbactam	57(46.70%)	≤ 8(44)		16(13)		10(6.17%)	≤ 8(4)	16(6)		
Cotrimoxazole	INTRINSIC RESISTANCE					15(9.25%)	≤ 20			
Ticarcillin/Clavulanic acid	17(13.90%)	16				3(1.85%)	≤ 8			
Tigecycline	INTRINSIC RESISTANCE					137(84.5%)	≤ 0.5(24)	1(45)	2(68)	
Piperacilin/tazobactam	51(41.80%)	≤ 4(14)		8(29)	16(8)	3(1.85%)	≤ 4(2)	8(1)		

Table 2 : Antibiotic Sensitivity Pattern Of Pseudomonas Spp. & Acinetobacter Spp.

Klebsiella pneumoniae	Total 169 (30.2%)
CARBAPENEMASE (+ OR - ESBL) ONLY	23(13.6%)
EXTENDED SPECTRUM BETA-LACTAMASE ONLY	25(14.8%)
NOTA	4(2.4%)
RES TO GEN TOB NET AMI +ESBL	3(1.8%)
CARBAPENEMASE +IMPERMEABILITY CARBAPENEMASE	22(13.0%)
RES TO GEN TOB NET AMI + CARBAPENEMASE +IMPERAMEABILITY CARBA	92(54.4%)
Acinetobacter spp.	Total 164 (28.9%)
RESISTANT (GEN TOB AMI) + CARBAPENEMASE	139(84.7%)
RESISTANT (GEN TOB AMI) ONLY	1(0.6%)
NONE OF THE 2	7(4.3%)
CARBAPENEMASE ONLY	17(10.3%)
P.aeruginosa	Total 122 (21.8%)
RESISTANT (GEN NET AMI TOB) ONLY	6(4.9%)
ACQUIRED PENICILLINASE ONLY	32(26.3%)
CARBAPENEMASE ONLY	6(4.9%)
HIGH LEVEL CEPHALOSPORINASE ONLY	3(2.5%)
NOTA	31(25.4%)
RES TO GEN NET AMI TOB + ACQ PENICILLNASE	2(1.6%)
CARBAPENEMASE +HIGH LEVEL CEPHALOSPORINASE	1(0.8%)
RESISTANT (GEN NET AMI TOB) + HIGH LEVEL CEPHALOSPORINASE	1(0.8%)
RESISTANT (GEN NET AMI TOB) + CARBAPENEMASE	34(27.9%)
RESISTANT (GEN NET AMI TOB) + CARBAPENEMASE +HIGH LEVEL CEPHALOSPORINASE	6(4.9%)
E. coli	Total 34(6.08%)
ONLY CARBAPENEMASE	4(11.7%)
ONLY ESBL	15(44.1%)
NOTA	2(5.9%)
RES GEN TOB NET AMI+ CARBAPENEMASE + IMPERMEABILITY CARBA	3(8.8%)
RES TO GEN TOB NET AMI + ESBL	7(20.5%)
CARBAPENEMASE + IMPERMEABILITY CARBA	3(8.8%)
Staph.aureus	Total 7 (1.04%)
PENICILLINASE + RES TO KAN TOB + RES TO STREPTOGRAMINS	2 (28.6%)
RES TO KAN TOB GEN + RES TO RIFAMYCINS + OXACILLIN RES	2(28.6%)
RES TO KAN TOB GEN + OXACILLIN RES	1(14.3%)
RES TO KAN TOB GEN + RES TO RIFAMYCINS + OXACILLIN RES	1(14.3%)
ONLY OXACILLIN	1(14.3%)

Table 3 : Relevant Phenotype Study By Vitek-2 AES System

DISCUSSION

The resistance to conventional antibiotics is severely increasing in bacteria in clinical and non- clinical settings [20]. The rate of nosocomial infection is also increasing in the patients admitted in the ICU due to excessive invasive procedures performed including artificial ventilator support [21]. This constantly emerging resistance is a serious situation implying the need for new regulations for the cautious use of antibiotics and refining the conditions of hospitals to prevent further exacerbation of resistance shown by the bacteria.

The percentage of samples showing positive growth in our study was 88.4%. In a study by Chandra et al., the positive samples were 72.3% [18]. In a study conducted by Malik et al., the positive cultures came out to be 83% [22] Organisms not isolated in samples may be due to inability to culture fastidious organisms or anaerobic organisms.

In our study, gram-negative bacilli were more common causative agents (97%) as compared to gram-positive cocci, which were (2%) of the total positive cultures. Similar findings are observed in study Chandra et al., in which the gram-negative bacilli were 85% and Gupta et al., in which 86% of the samples were gram-negative bacilli [18,23]. A study by Chandra et al.showed that more of the isolates from the patients were gram-negative enteric aerobic bacteria, with Klebsiella being the most common species followed by Acinetobacter and Pseudomonas. The gram negative predominace might partly be due to the unequal distribution of patients with community acquired and hospital acquired infections and also due to the spread of antibiotics resistance in hospital settings.This can be attributed to the fact that the majority of the nosocomial infections are caused by gram-negative bacteria which are difficult to treat. This calls for strict measures against the spread of gram-negative bacilli, especially in the ICU setting.

In our study, Klebsiella pneumoniae (30.2%) was the most common isolate followed by Acinetobacter spp. (28%). In a study by Malik et al., the commonest bacterium isolated from tracheal secretions was Klebsiella pneumoniae (35.4%) [22]. Also, in a study by Chandra et al., Klebsiella (32.35%) was the most common isolate [18] Because of their ability to spread rapidly in the hospital environment, Klebsiella pneumonia tend to cause nosocomial outbreaks.

In our study, amongst gram negative bacteria, Klebsiella isolates and Acinetobacter spp. were resistant to group of antibiotic namely cephalosporins and aminoglycosides. Most common phenotype in Klebsiella was resistant to Aminoglycosides, Carbapenemase producing and impermeability carbapenem while in Acinetobacter spp. phenotype was resistant to Aminoglycosides, Carbapenemase producing. This might be due to continuous exposure of these bacteria to a variety of β-lactams that leads to the production of beta-lactamases. Moreover, plasmid and chromosomal gene mediated beta-lactamase enzymes are major reasons for antibiotic resistance.

Klebsiella most sensitive to Colistin followed by tigecycline with most common MIC of 1, and than Amikacin with MIC of <=2. Acinetobacter spp. most sensitive to Colistin followed by tigecycline with most common MIC of 1, followed by Minocycline with MIC of <=1.

On the other hand, E.coli was the least resistant organism being sensitive to colistin, imipenem, meropenem, tigecyclin. Phenotype most common is ESBL.

In Pseudomonas spp. most common pattern is resistant to aminoglycosides and carbapnemase. Among these Colistin is the most sensitive drug for Pseudomonas followed by Gentamicin with MIC of <=1.

Amongst Gram positive bacteria S. aureus was shown to 100 % Susceptibility to higher antibiotics viz. teicoplanin, linezolid , vancomycin, tigecyclin. While resistance was observed towards cotrimoxazole ,oxacillin, benzylpenicillin and fluoroquinolons.

It is necessary to have policies regarding restrictive use of antibiotics such as aminoglycosides and carbapenems. Regular monitoring of such resistant isolates would be important for infection control in critical units.

CONCLUSION

In our study, gram-negative bacilli were more common causative agents (97%) as compared to gram-positive cocci, which were (2%) of the total positive cultures. Among Gram negative bacteria Klebsiella pneumoniae (30.2%) was the most common isolate followed by Acinetobacter spp. (28%). Klebsiella isolates and Acinetobacter spp. were resistant to antibiotics namely cephalosporins and aminoglycosides. Most common phenotype in Klebsiella was resistant to Aminoglycosides, Carbapenemase producing and impermeability carbapenem while in Acinetobacter spp. phenotype was resistant to Aminoglycosides, Carbapenemase producing. Proper identification of the pathogens and their antibiotic susceptibility pattern with MIC can help our health professionals to choose the right antibiotic therapy and improve the outcome.

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