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ORIGINAL ARTICLE

Microbiological Profile and Antimicrobial Susceptibility Pattern of Microorganisms Isolated from Endotracheal Tube Tips and Tracheal Aspirates Specimens: A Hospital Based Study

Alia Batool,[1] Sana Ashiq,[2] Durre Shahwar Lone,[1] Ayaz Lone,[1] Kanwal Ashiq,[3] Sabiha Riaz[1]

1. Department of Pathology, Fatima Memorial Hospital, FMH College of Medicine and Dentistry Shadman, Lahore-Pakistan.
2. Center for Applied Molecular Biology, University of the Punjab, Lahore-Pakistan.
3. Faculty of Pharmaceutical Sciences, Superior College, Raiwind Road, Lahore-Pakistan.

Correspondence to: Ms. Sana Ashiq, Email: sanaashiq72@gmail.com, ORCiD: 0000-0003-0418-4022

ABSTRACT

Objective: To determine the microbiological profile and antimicrobial susceptibility pattern of microorganisms isolated from specimens of tracheal aspirate and endotracheal tube (ETT) tip.
Methods: A descriptive cross-sectional study was conducted in a tertiary care hospital, Lahore, Pakistan during July 2016 to July 2017. The laboratory records of ETT tips and tracheal aspirate specimens was retrospectively reviewed. Standard microbiological procedures were followed for the isolation and the identification of microorganisms. Clinical and Laboratory Standards Institute (CLSI) 2016, guidelines were used for the antimicrobial susceptibility testing.
Results: A total of 126 organisms were isolated from 121 specimens [Tracheal Aspirate 103 (85.1%) and ETT Tip 18 (14.9%)]. Most of the organisms were gram-negative 98 (77.78%), 17 (13.49%) were gram-positive and 11 (8.73%) were yeast. Of 98 gram-negative organisms, Acinetobacter spp was found in majority 61 (62.24%) followed by Klebsiella spp in 17 (17.34%), and Pseudomonas spp in 12 (12.24%). Of 17 gram-positive samples, Streptococcus spp was found in majority 6 (35.29%), followed by Methicillin-Resistant Staphylococcus aureus (MRSA) in 5 (29.41%), Staphylococcus epidermidis in 2 (11.76%), and Streptococcus Group D in 1 (5.88%). Candida was the only specie found in yeast. Acinetobacter was found highly resistant to all antibiotics except doxycycline, colistin and polymixin-b. Pseudomonas was sensitive to all the antibiotics except to ceftazidime (100% resistant). Klebsiella and MRSA showed high resistance to all the tested antibiotics.
Conclusion: Gram-negative was the most common isolated bacteria from ETT tips and tracheal aspiration. Moreover, the specific isolates pattern of antimicrobial susceptibility showed a high resistance to widely used antibiotics.
Keywords: Bacterial infection, Tracheal Aspirate, Antimicrobial Susceptibility, Endotracheal Tube (ETT) tip

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INTRODUCTION

Among hospitalized patients nosocomial infections emerged as a major threat of mortality and morbidity.[1] According to the World Health Organization (WHO) highest percentage of nosocomial infections occurs in intensive care units (ICUs) which is 5 to 7 fold higher as compared to others.[2]
The major risk factors among critically ill patients are defective immune system and excessive use of invasive devices.[3] In developing countries, elevated rate of device associated infections such as ventilator associated pneumonia (VAP) observed.[4] Approximately 24-50% mortality occurs due to VAP and depending upon the specific setting and host pathogen relationship it can rise up to 76%.[5] The length of hospital stay, exposure to ICU, use of invasive procedures along with inappropriate or prolong exposure of broad spectrum antimicrobial agents are the certain risk factors which can help to multi-drug resistant (MDR) pathogens to cause infections among the hospitalized patients.[6]
The use of endotracheal tube (ETT) and tracheal incubation can also inhibits innate immunity elements to work properly.[7,8] The biofilm presence on ETT and pathogenic organism aspiration from upper respiratory tract plays a significant role in the pathogenesis of VAP.[9] The risk for developing pneumonia increases by the use of ETT which promotes the accumulation of tracheobronchial secretions by impairing the mucocilliary clearance and disturbing the cough reflex. Injury and colonization of tracheal mucosa by the endogenous and exogenous bacteria is also facilitated by the insertion of ETT.[10] Moreover, the progression of bacteria in airway tract also depends on ETT which act as a source of bridge between the oropharynx and

trachea.[11] The presence of biofilm in the lumen of ETT also imposes a great threat for developing antibiotic resistance as it helps in bacterial proliferation by creating a microenvironment which inhibits antibiotic access to the bacteria.[12] The etiologic agents differs, depends upon the different factors like ICU type, prior use of antimicrobial therapies, and pre-existing disease.[13] Various studies have reported that more than 30% hospital acquired infections and more than 40% of ICU patients infections are caused by mainly gram-negative bacteria.[14–17]

Another major threat is antibiotic resistance among these ICU pathogens due to the use of broad spectrum antibiotics.[18] The worldwide causes of resistance among these hospital acquired organisms are misuse and overuse of antibiotics.[19] It is reported that increase use of β-lactam drugs can results in bacterial resistance towards these antimicrobial agents and by producing β-lactamases it develops resistance to a broad range of β-lactams antibiotics. The treatment options against the infection caused by these MDR bacteria are limited thus it appeared as a major challenge for clinicians.[6] To the best of our knowledge, in Pakistan there is insufficient data regarding tracheal aspirate and ETT tip specimen pathogens and their antimicrobial susceptibility pattern. Therefore, the present study was undertaken to determine frequency and antimicrobial susceptibility of organisms isolated from ETT and tracheal aspirate specimens which will help clinicians to choose correct antimicrobial therapy against these MDR bacteria and control serious infections.

METHODS

This descriptive cross-sectional study was carried out by retrospective analysis of lab records of ETT tips and tracheal aspirates of patients admitted in the tertiary care hospital, Lahore, Pakistan during July 2016 to July 2017. Approval from the Ethics Committee of The FMH College of Medicine and Dentistry Lahore, Pakistan was obtained prior conduction of the study.

The consecutive ETT tips and tracheal aspirate specimens were recruited from different sources of hospital (ICUs, out born nursery, new born nursery). On the basis of cultural characteristics, morphology and biochemical profile the isolates were identified. Moreover, appropriate labelling of specimen in a sterile container was also noted prior to the selection of the sample. Whereas those specimens other than tracheal aspirate and ETT tips were excluded. All specimens were cultured on Blood and MacConkey agar and incubated aerobically overnight at 37°C. By using Gram staining technique, identification of organism from culture media was made into Gram-positive or Gram-negative. Then for identification basic biochemical tests including catalase, coagulase and oxidase test were performed. Based on the sensitivity to Novobiocin (5μg disc), coagulase and DNAase tests the Staphylococci were divided into two major groups either as *Staphylococcus aureus* and *Staphylococcus epidermidis*. Bacteria species identification was further confirmed by using Analytical Profile Index (API-20 NE Biomeurix France). The antimicrobial susceptibility testing was done according to Clinical and Laboratory Standards Institute (CLSI) 2016 guidelines by Kirby-Bauer Technique using Mueller -Hinton agar (Oxoid UK). As per CLSI guidelines zone diameter interpreted and was measured in millimeters (mm). The antimicrobial agents used in study were Aminoglycosides [Amikacin (30μg), Gentamicin (10μg)], Carbapenems [Meropenem (10μg), Imipenem (10μg)], Tetracyclines [Doxycycline (30μg)], β-lactamase inhibitor combinations [Piperacillin-Tazobactam (100/10μg)], Flouroquinolones [Ciprofloxacin (5μg)] Cephalosporin's [Ceftazidime (30μg), Ceftriaxone (30μg)], Penicillinase labile Penicillins [Penicillin (10units)], Penicillinase labile Penicillins [Oxacillin (30μg) cefoxitin (surrogate test for oxacillin)] and Glycopeptides [Vancomycin (30μg)]. Since Lipopeptides [Polymixin B (300units) and Colistin (10μg)] disk diffusion method was not established for *Acinetobacter* spp in CLSI 2016, hence zone diameter interpretation was adopted from Zafar *et al* method.[20]

Statistical analysis was done by using Statistical Package for the Social Sciences [SPSS 22.0].

RESULTS

Out of total 121 specimens, 103 (85.1%) were from tracheal aspirate while 18 (14.9%) were from ETT Tip (Table 1). From these 121 specimens, 126 organisms were isolated. Most of the organisms were gram-negative 98 (77.78%), 17 (13.49%) were gram-positive and 11 (8.73%) were yeast. Of 98 gram-negative organisms, Acinetobacter spp was found in majority 61 (62.24%) followed by Klebsiella spp in 17 (17.34%), and Pseudomonas spp in 12 (12.24%). (Figure 1) Of 17 gram-positive samples, Streptococcus spp was found in majority 6 (35.29%), followed by Methicillin-Resistant Staphylococcus aureus (MRSA) in 5 (29.41%), Staphylococcus epidermidis in 2 (11.76%), and Streptococcus Group D in 1 (5.88%). (Figure 2) Candida was the only specie found in yeast.

Acinetobacter was found highly resistant to all antibiotics except doxycycline, colistin and polymixin-b. Pseudomonas was sensitive to all the antibiotics except to ceftazidime (100% resistant). Klebsiella and MRSA showed high resistance to all the tested antibiotics. Except both MRSA and Staphylococcus aureus was 100% sensitive to vancomycin. The most of the isolates antibiotic susceptibility pattern exhibit high level of antibiotic resistance to the tested antibiotics as described in Table 2.

DISCUSSION

The most frequent bacterial infections among ICU patient are the lower respiratory tract infections. The three categories of bacteria which include carbapenem resistant *Acinetobacter* spp, ESBL producing *Echerichia coli* and *Klebsiella* spp and MDR pseudomonas are recently placed on the top list of bacterial pathogens by Infectious Disease Society of America.[21] One of the

Table 1: Frequency of isolated pathogens from ETT tip and tracheal aspirates

Total Specimens	Specimen Types		Total Organisms	Two Types of Growth	Organisms		
	Tracheal Aspirate	ETT Tips			Gram -ve	Gram +ve	Yeast
121	103 (85.1%)	18 (14.9%)	126	5 Specimens	17 (13.5%)	98 (77.8%)	11 (8.7%)

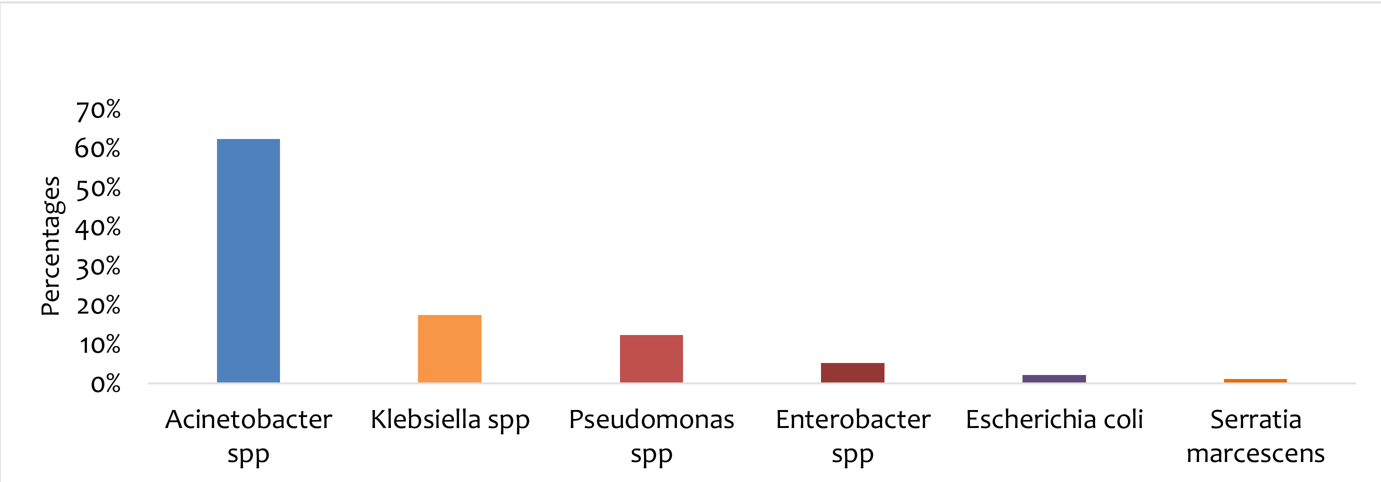

Figure 1: Frequency of gram-negative isolated organisms from tracheal aspirate and ETT tip specimens (n=98)

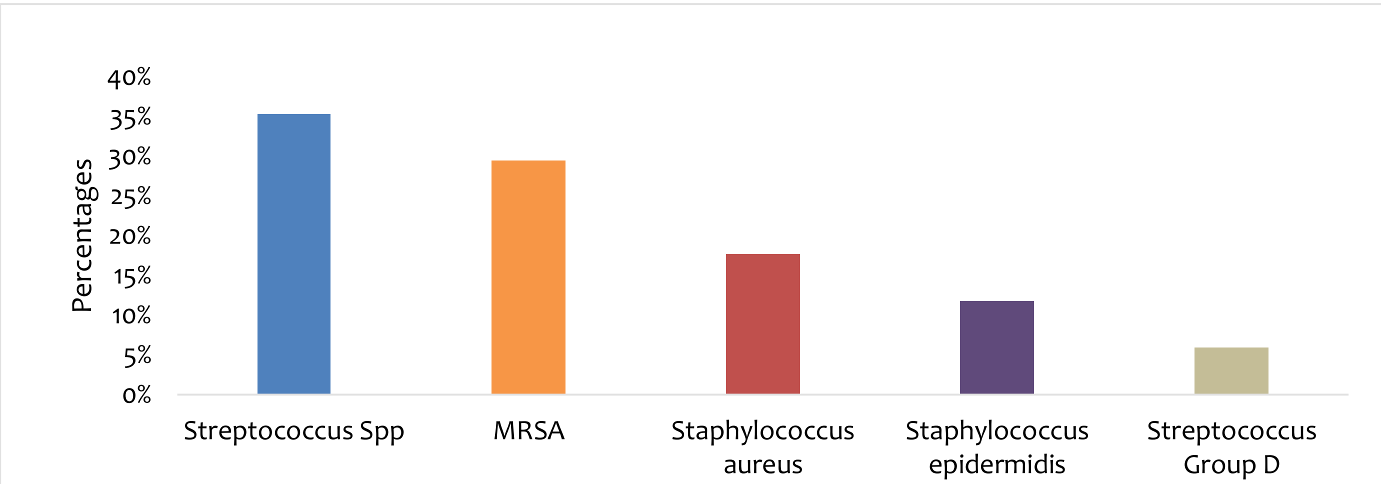

Figure 2: Frequency of gram-positive isolated organisms from tracheal aspirate and ETT tip specimens (n=17)

Table 2: Susceptibility Pattern of various causative bacteria to antibiotics (%)

Antibiotics	Acinetobacter spp			Klebsiella spp			Pseudomonas spp			Staphylococcus aureus			MRSA		
Susceptibility Pattern	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Amikacin	8.2	24.6	67.2	41.2	0	58.8	100	0	0	-	-	-	-	-	-
Gentamicin	-	-	-	-	-	-	-	-	-	0	0	100	0	0	100
Meropenem	8.2	3.3	88.5	47.1	5.9	47.1	100	0	0	-	-	-	-	-	-
Imipenem	-	-	-	64.7	0	35.3	100	0	0	-	-	-	-	-	-
Polymixin-B	98.4	-	1.6	-	-	-	-	-	-	-	-	-	-	-	-
Colistin	65.6	-	34.4	-	-	-	-	-	-	-	-	-	-	-	-
Piperacillin-Tazobactam	4.9	3.3	91.8	47.1	11.8	41.2	100	0	0	-	-	-	-	-	-
Doxycycline	80.3	14.8	4.9	-	-	-	-	-	-	-	-	-	-	-	-
Ciprofloxacin	-	-	-	29.4	0	70.6	100	0	0	33.3	0	66.7	0	0	100
Ceftriaxone	-	-	-	11.8	0	88.2	-	-	-	66.7	33.3	0	0	0	100
Ceftazidime	-	-	-	-	-	-	0	0	100	-	-	-	-	-	-
Aztreonam	-	-	-	-	-	-	91.7	0	8.3	-	-	-	-	-	-
Penicillin	-	-	-	-	-	-	-	-	-	66.7	-	33.3	0	-	100
Oxacillin	-	-	-	-	-	-	-	-	-	100	-	0	0	-	100
Vancomycin	-	-	-	-	-	-	-	-	-	100	0	0	100	0	0

major issues is the emergence of bacterial resistance appearing as a major problem for the management and prevention of serious infections among patients.[22] Therefore identification of local microbial flora and their antimicrobial susceptibility pattern with the infection control practices are necessary for the better clinical outcomes.[23,24]

Similar to the current study findings, previous studies also reported that Gram-negative bacteria were mostly isolated from tracheal aspirates and ETT tips with *Klebsiella* species being the most frequent followed by *Acinetobacter*, *Pseudomonas* and *Staphylococcus aureus.*[13]

The present study reported Acinetobacter spp be the most predominant isolate followed by Klebsiella spp, Psuedomonas spp and Staphylococcus aureus. The results are in accordance to many published studies as in Pakistan and Iran reported Acinetobacter being the most common isolate.[16,25]

According to the current study findings, Acinetobacter spp isolates were resistant to most commonly used antibiotics while polymixin-b, colistin and doxycycline proved effective. The results are in accordance to many published studies. Panda et al also observed Acinetobacter as highly sensitive to polymixin-b and colistin with intermediate resistant to meropenem and imipenem while highly resistant to piperacillin-tazobactam.[26] In Pakistan Kidwai et al also observed very similar findings reported 0% resistance to polymixins while 91% resistance to amikacin and 69.4% resistance to piperacillin-tazobactam.[16] Similar results obtained by study conducted in India showed 100% susceptibility to polymixin-b, 85.71% to colistin, 71.42% to meropenem while 28.57% to amikacin.[13] Goel et al also showed doxycycline effective for Acinetobacter and the resistance rate was 2.6% while we also reported 4.9% resistance to doxycycline.[21]

The current study found Klebsiella spp highly resistance to tested antibiotics while Pseudomonas spp exhibit 100% resistance to ceftazidime, 8.3% to aztreonam while 0% resistance against amikacin, meropenem, imipenem, ciprofloxacin and piperacillin-tazobactam. Ranjan et al revealed similar results as resistance to amikacin 53.3%, ciprofloxacin 66.6%, meropenem 20%, piperacillin-tazobactam 13.3% and ceftazidime 93.3%.[27] Juayang et al reported in their study of review on antimicrobial resistance of pathogens isolated from tracheal and endotracheal aspirates that highest resistance of Pseudomonas against ceftazidime 65.8%, ciprofloxacin 40.6%, aztreonam 33.3%, meropenem and imipenem

25%, piperacillin-tazobactam 26.3%, and amikacin 5.1%.[28] While another study conducted in Iran reported high resistance of Pseudomonas against commonly used antibiotics except colistin with 0% resistance.[25] In India research report showed that Klebsiella susceptibility to amikacin 27.7%, ceftriaxone 9.09%, meropenem 90.90%, polymixin-b and colistin 100% while for Pseudomonas susceptibility pattern was amikacin 60%, ceftazidime 20%, meropenem 80%, colistin and polymixin-b 100%.[13]

In our study among Gram-positive Staphylococcus aureus showed 100% resistance to gentamicin, 66.7% while for MRSA resistance pattern as 100% resistance to all used antibiotics except for vancomycin, for which both Gram-positive bacteria showed 0% resistance. The results are in accordance to many published studies which report 0% resistance to vancomycin.[25,27] A study report from India showed methicillin-sensitive Staphylococcus aureus (MSSA) resistance to penicillin 50% while 0% resistance for oxacillin, ciprofloxacin, gentamicin and vancomycin in comparison to MSSA the MRSA showed 100% resistance to penicillin, oxacillin while 0% resistance for vancomycin, ciprofloxacin and gentamicin.[29]

Thus, patients under the procedure of ETT are more prone to acquire the respiratory tract infections due to colonization of bacteria. The inapt consumption of broad-spectrum antibiotics leads to the emergence of multi-drug resistant bacteria. There is an urgent need for the prevention of antibiotic resistance among these MDR bacteria and in future appropriate empirical antibiotic therapy may proves helpful.

CONCLUSION

It is concluded from this study that most commonly isolated pathogens were belonged to the Gram-negative bacteria while among Gram-positive bacteria Staphylococcus aureus and MRSA were most frequent. Antibiotic susceptibility testing has revealed that isolated pathogens were resistant to most commonly used antibiotics in our setup.

ETHICAL APPROVAL: The study protocol was approved by the Ethics Committee of The FMH College of Medicine and Dentistry Lahore, Pakistan.

AUTHORS' CONTRIBUTION: AB conceived the idea, conducted the analyses, provided the data, written and revised. SA Critical feedback and discussion write up. DSL, IL, KA and SR conceived the idea, written and revised.

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