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Bacteriological Profile and Antibiotic Sensitivity Pattern of Endotracheal Tube Tip Culture

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ABSTRACT:

Introduction: Endotracheal tubes act as reservoirs for pathogens by providing a surface for adherence and forming biofilms. Most ventilator-associated pneumonia is due to the dissemination of these biofilms into the mechanically ventilated lung. This study will provide an idea about different bacteria that remained within the endotracheal tube and provide a reference for the selection of empirical antibiotic therapy. **Methods:** A descriptive cross-sectional study was conducted in the Microbiology laboratory of Lumbini Medical College. A total of 114 endotracheal tubes, received from different ICU departments of Lumbini Medical College and Teaching Hospital, during one year from May 2022 to April 2023, were collected. The collected specimens were processed in the Microbiology lab for isolation of colonized bacteria and antibiotic sensitivty test was performed for all isolates by the Kirby-Bauer disc diffusion method. **Results:** Among the total 114 endotracheal tubes, 97 (85.05%) showed growth and 17 (14.91%) showed no growth. Seventeen specimens showed growth of two different bacteria, whereas 80 showed growth of only one. Among these isolates, 89 were Gram-negative bacilli and only 14 were Gram-positive cocci. Pseudomonas spp. 24(21.05%) was the most frequently isolated bacterium, followed by Klebsiella pneumoniae 23(20.17%) and Acinetobacter spp. 22(19.2%). Most Gram-negative bacilli were sensitive to Piperacillin Tazobactam, Imipenem, and Meropenem. Among 12 isolated Staphylococcus aureus, six were MRSA positive. MRSA showed sensitivity towards vancomycin and linezolid. **Conclusions:** Pseudomonas spp, Klebsiella pneumoniae and Acinetobacter spp were the most common isolates with high resistance to most antibiotics.

Keywords: Antibiotic sensitivity test; Bacteriological profile; Endotracheal tubes; Ventilator-associated pneumonia.

INTRODUCTION:

Hospital-acquired infection (HAI) is one of the most common problems in hospitalized patients especially in Intensive care units (ICUs) due to the high prevalence of invasive procedure.[1] In developed countries, healthcare-associated infections are reported to occur in 5-15% of hospitalized patients in general wards and upto 50% in intensive care units.[2] Ventilator-associated pneumonia (VAP) is one of the most common healthcare-associated infections among mechanically ventilated patients in intensive care units, with reported incidence rates ranging from approximately 5% to 40% depending on patient population, diagnostic criteria, and healthcare setting.[3]

Endotracheal tubes, the most commonly used device in clinical management of hospitalized patients who require life-support measures, appear to be a risk factor for VAP.[4] Several studies have demonstrated that the endotracheal tube culture is an additive tool, along with the routine tests, in the detection of plausible pneumonia pathogens.[2,4,5] Several bacterial pathogens, Acinetobacter spp, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Staphylococcus aureus have been commonly implicated in VAP, and these organisms were frequently isolated from endotracheal tube tip culture.[2,4]

This study aimed to detect common pathogens within the endotracheal tube and to determine their sensitivity to various antibiotics, which serve as guidelines to physicians for empirical treatment, and this will also help to formulate an antibiotic policy for the ICU.

METHODS:

All the endotracheal tube tips sent for culture from various ICU departments in sterile airtight containers were included. Endotracheal tube tips received

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in open containers were excluded. A total of 114 endotracheal tubes fulfilling the inclusion criteria within the study period were collected using a consecutive sampling method and included in the final analysis.Culture and Identification of Isolates:The tips of the endotracheal tubes within the sterile containers were inoculated on Mac-Conkey agar, Blood agar, and Chocolate agar, and incubated at 37°C for 48 hours. After incubation, the isolates obtained were identified based on standard microbiological criteria such as colony characteristics, Gram stain, and biochemical properties. Budding yeast cells isolated in culture were further identified as Candida ablicans by performing the germ tube test.[6]

Antibiotic susceptibility test: Antibiotic-susceptibility test was performed for all bacterial isolates by a modified Kirby – Bauer disk diffusion method according to the guidelines of Clinical and Laboratory Standards Institute on Mueller-Hinton agar. Staphylococcus aureus resistant to cefoxitin (30 mcg) was considered as methicillin-resistant Staphylococcus aureus (MRSA).[7] Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 25923 were used as reference strains for quality control.The data were entered and analysed using Statistical Package for Social Sciences (SPSS) software (version 18.0). Categorical data were summarized as frequencies and percentages. Associations between categorical variables were assessed using the Chi-Square (χ^2) test, where appropriate. Statistical significance was defined as a p-value < 0.05 (95% confidence interval).

RESULTS:

Out of 114 samples, 97 (85.08%) showed growth of aerobic organisms, whereas 17 (14.91%) showed no growth. Seventeen (17.52%) [reviewer 1.1]samples showed growth of two different bacteria, so the total isolates were 114. Among 114 isolates, 89 (78.07%) were gram-negative bacteria, 14 (12.28%) were gram-positive bacteria, and 11 (9.64%) were Candida albicans.

A total of 114 samples, 65 (57.01%) were from males and 49 (42.98%) were from females. A Chi-square test between sex and growth was not significant ($\chi2 = 0.027$, $p = 0.870$).

The age-wise distribution showed 54 (47.36%) samples were from the less than 20 years age group, 13 (11.40%) from the 21-40 years age group, 9 (7.89%) from the 41-60 years age group and 38 (33.33%) from the more than 60 years age group.

As shown in Fig. 1, out of all gram-negative organisms isolated, Pseudomonas spp (n=24, 21.05%) was the most predominant organism and Citrobacter spp (n=1, 0.87%) was the least isolated organism.As depicted in Table 1, most of the Pseudomonas spp were highly resistant to piperacillin (79.16%) and ceftazidime (75%) but were comparatively less resistant to piperacillin-tazobactam (25%), imipenem (37.5%), and meropenem (41.6%).

Enteric Gram-negative bacteria (Escherichia coli,Citrobacter spp) were totally resistant to ampicillin (100%) and were sensitive to imipenem and meropenem.All 12 (100%) isolated Staphylococcus aureus were resistant to Ampicillin and 6 (50%) were sensitive to both cefoxitin and cloxacillin. Linezolid was the most sensitive antibiotic, showing 100% sensitivity (Table 2)

DISCUSSION:

Lower respiratory tract infection is the most frequent bacterial infection among ICU patients.[8] Many bacteria, either of endogenous or exogenous origin, remain colonized in mechanically ventilated and tracheostomized patients, which might develop ventilator-associated tracheobronchitis or ventilator-associated pneumonia.[9] So bacteria isolated and identified by endotracheal tube cultures can be a determinant of the responsible microbial agents.

Our study showed 85.08% growth from endotracheal tube cultures, which was nearly identical to the studies carried out by Rathod et al. and Samal et al.[5,10] However, Swati et al. and Shrestha et al. showed somewhat lower growth rates than our study.[2,11] The lower growth rate may be due to previous antibiotic therapy.

Our study showed that most of the samples belonged to the 0-20 years age group compared to the>60 years age group. Kaur et al. showed majority of patients belonged to the age group > 60 years.[12] The higher prevalence of infection in these age groups may be attributed to their reduced immune competence. Additionally, neonates frequently suffer from congenital conditions requiring intensive care and ventilatory support, which may predispose them to the development of ventilator-associated pneumonia (VAP).

Out of 114 isolates, 89 (78.07%) were Gram-negative bacteria which was significantly higher than Gram-positive cocci, 14 (12.2%). This is concordant with studies conducted by Ahmad et al. and Kaur et al.[12,13] This indicates that the majority of nosocomial infections were caused by gram-negative bacteria.

The most common isolate in our study was Pseudomonas spp (n=24, 21.09%), followed by Klebsiella pneumoniae (n=23, 20.17%) and Acinetobacter spp (n=22, 19.29%). Most of the other studies showed Acinetobacter spp as the most isolated bacteria, followed by either Klebsiella pneumoniae or Pseudomonas spp.[2,5,14] These three bacteria, Pseudomonas spp, Acinetobacter spp, and Klebsiella pneumoniae, have overtaken the pathogenic rate in ventilated patients. These three bacteria are potent biofilm producers and can show multidrug resistance patterns leading to high mortality rates.[5]

In our study, four enteric gram-negative bacteria, Klebsiella pneumoniae, Escherichia coli, Enterobacter spp., and Citrobacter spp were sensitive to imipenem and meropenem but less sensitive to ceftazidime and cephalexin. This is in accordance with a study done by Rathod et al.[10] Among aminoglycosides they were most sensitive to amikacin in comparison with gentamicin. Kaur et al. also found high effectiveness of amikacin over gentamicin.[12] Ampicillin was a highly resistant antibiotic show-

Table 1: Sensitivity pattern of Gram-negative bacteria (n=89).

Antibiotics	Pseudomonas spp(n = 24)	Klebsiella pneumoniae(n = 23)	Escherichia coli(n = 6)	Acinetobacter spp(n = 22)	Enterobacter spp(n = 13)	Citrobacter spp(n = 1)
Ampicillin	Nt*	Nt*	0%	0%	Nt*	0%
Ciprofloxacin	58.34%	30.43%	16.67%	13.64%	38.47%	100%
Gentamicin	45.90%	39.14%	66.67%	13.64%	23.08%	100%
Cotrimoxazole	41.67%	26.09%	16.67%	9.1%	46.16%	100%
Amikacin	62.5%	47.83%	83.34%	18.19%	38.47%	100%
Ceftazidime	25%	26.09%	16.67%	0%	15.39%	100%
Cephalexin	Nt*	13.05%	0%	0%	0%	0%
Piperacillin	20.84%	Nt*	Nt*	13.64%	Nt*	Nt*
Piperacillin tazobactam	75%	Nt*	Nt*	63.64%	Nt*	Nt*
Imipenem	62.50%	69.57%	83.33%	50%	69.24%	100%
Meropenem	58.40%	78.27%	83.33%	50.1%	76.93%	100%
Carbenicillin	41.67%	Nt*	Nt*	Nt*	Nt*	Nt*

**Nt = Not tested*

Table 2: Sensitivity pattern of Gram-positive bacteria (n=14).

Antibiotics	Staphylococcus aureus (n= 12)	Enterococcus spp (n = 2)
Ampicillin	0%	50%
Ciprofloxacin	66.67%	50%
Gentamicin	50%	0%
Cotrimoxazole	58.34%	Nt*
Amikacin	83.34%	50%
Cloxacillin	50%	Nt*
Cefoxitin	50%	Nt*
Cephalexin	41.67%	0%
Vancomycin	83.34%	50%
Linezolid	100%	Nt*

**Nt = Not tested*

ing 0% sensitivity. Rathod et al. and Juayang et al. also recorded the least sensitivity of ampicillin.[10,15] Pseudomonas spp showed 75% sensitivity to piperacillin-tazobactam and 62.5% to amikacin and imipenem. Batool et al. found 100 % sensitivity to imipenem and meropenem, and piperacillin-tazobactam.[8]

Our study represented Acinetobacter spp as the most resistant bacteria showing high resistance to the most commonly used antibiotics. All were resistant to ampicillin, ceftazidime and only 50% sensitivity toward imipenem and meropenem which are antibiotics of choice against multidrug-resistant bacteria in the ICU limiting the antibiotics for treatment. Other studies also found high resistance to ceftazidime along with carbapenems.[11,13] This is an alarming situation as multidrug-resistant bacteria are emerging in tracheal secretion, making treatment of the patient more difficult and expensive.Among the 12 isolated Staphylococcus aureus, 6 (50%) were found to be Methicillin-resistant Staphylococcus aureus. Most of the Staphylococcus aureus were sensitive to amikacin and vancomycin, with a sensitivity rate of 83.34% and showed 100% sensitivity towards linezolid. Rashid et al. also showed 100% sensitivity towards linezolid.[16] Ampicillin was found to be a very resistant antibiotic, showing 0% sensitivity. Most of the other studies also revealed the same.[5,8]

In this study, a MIC-based antibiotic sensitivity test method was not used due to resource limitations, and the study was done for a short period with a small sample.

CONCLUSION:

Our study showed predominantly Gram-negative bacilli in endotracheal aspirates, with Pseudomonas spp being the most common bacteria isolated.

Ampicillin and ceftazidime were the least sensitive antibiotics, and imipenem and meropenem were sensitive antibiotics for all gram-negative bacilli except Acinetobacter spp, which showed only 50% sensitivity showed only 50% sensitivity.

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