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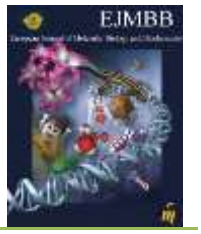

ANTIMICROBIAL SENSITIVITY AND MICROBIAL PROFILES IN VENTILATOR-ASSOCIATED PNEUMONIA: A CRITICAL ANALYSIS FOR TAILORED THERAPEUTIC STRATEGIES

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ABSTRACT

Ventilator-associated pneumonia (VAP) poses a significant threat to critically ill patients, leading to increased morbidity and mortality. The emergence of multidrug-resistant bacteria, including Acinetobacter spp., Pseudomonas spp., and drug-resistant Staphylococcus aureus, further complicates treatment. Understanding local antimicrobial resistance patterns becomes crucial for initiating timely and effective empirical antimicrobial therapy. This study investigates the antimicrobial sensitivity of organisms isolated from endotracheal aspirates in a hospital's intensive care unit, where a majority of patients are mechanically ventilated. Gram stain and semiquantitative cultures were conducted on 70 endotracheal aspirates, revealing a positive culture rate. Acinetobacter spp. and Pseudomonas spp. were the most common isolates, with Klebsiella and Staphylococcus aureus also identified. Notably, a significant portion of Staphylococcus aureus demonstrated resistance to cefoxitin, indicating methicillin-resistant Staphylococcus aureus (MRSA). Imipenem was effective against most Gram-negative isolates, while extended-spectrum beta-lactamase (ESBL) resistance was observed. Strikingly, most of the isolates exhibited biofilm production, with associated higher mortality rates. This underscores the importance of tailoring empirical treatment based on the hospital's bacteriological profile, local antibiogram patterns, and patient susceptibilities, emphasizing the need for institution-specific antibiotic policies.

INTRODUCTION

Mechanical ventilation is a crucial life-saving intervention in intensive care, but it poses an increased risk of respiratory infections and heightened morbidity and mortality for critically ill patients [1,2]. Ventilator-associated pneumonia (VAP) is a complex condition resulting from the interaction of invading bacteria, the endotracheal tube (ET tube), and host immunity [3]. Characterized by new or progressive infiltrates, systemic infection signs, and changes in sputum characteristics, VAP arises from factors like oropharyngeal bacterial colonization, aspiration into the lower respiratory tract, and compromised host defenses [4,5].

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The causes of VAP vary based on factors such as onset, ICU type, preexisting illness, patient age, sex, and prior antibiotic therapy [6]. Despite bronchoalveolar lavage and protected specimen brush offering high diagnostic sensitivity and specificity, their invasiveness limits their practicality. The Infectious Disease Society recommends noninvasive sampling, such as ET aspirate with semiquantitative culture, for diagnosing VAP [7]. This approach is relatively noninvasive, easy to perform, and cost-effective. ET tubes act as reservoirs for infecting microorganisms, especially in the distal third of the tube, playing a significant role in pulmonary infections [8]. The polymeric matrix encasing these organisms allows for potent biofilm production, contributing to high mortality rates associated with multidrug resistance patterns [9]. This study aims to determine the local bacteriological

profile of ET aspirates, elucidate their resistance patterns, and assess their virulence by investigating the presence of biofilms in these aspirates.

METHODOLOGY

Endotracheal aspirates were collected from mechanically ventilated patients in various ICUs of our hospital, where ventilation was required for over 48 hours due to different reasons. A 22-inch Ramson's 12F suction catheter was employed for non-bronchoscopic collection, gently aspirating without saline instillation. The catheter tip with secretions was transferred to BHI broth post-suctioning. Gram staining and semi-quantitative cultures were conducted, with cultured samples expected to have 1-10 squamous cells. Semi-quantitative culture on Blood agar (BA) and MacConkey agar (MA) involved 4 quadrant streaks with a calibrated loop for 24 hours. Plates showing moderate to heavy growth, indicative of colony counts >105 CFU/mL, were subjected to standard biochemical identification and KirbyBauer Disk Diffusion testing on MHA for antimicrobial susceptibility [10].

For Gram-negative isolates, antimicrobial testing included gentamicin, cefotaxime, ceftazidime, cefepime, levofloxacin, tobramycin, piperacillin-tazobactam, ceftazidime-clavulanic acid, and imipenem. Gram-positive isolates were tested against gentamicin, azithromycin, cotrimoxazole, moxifloxacin, linezolid, and cefoxitin. Zone diameters were interpreted following CLSI guidelines. Extended spectrum beta-lactamases (ESBLs) were detected using cefotaxime (30 mg) and cefotaxime clavulanic acid (30/10 mg) on Muller Hinton agar, where clavulanate significantly increased zone diameters, indicating ESBL production.

AmpC beta-lactamase detection involved swabbing MHA with Escherichia coli ATCC 25922 as the indicator organism, placing a cefoxitin disc (30 mg), and inoculating a sterile filter paper disc with test organism colonies. An indentation or flattening of the cefoxitin zone indicated AmpC production. Methicillin resistance in Staphylococcus spp. was assessed by incubating MHA with a 30 mg cefoxitin disc for 16–18 hours, where a zone of inhibition of 21 mm in Staphylococcus aureus was considered resistant (mecA positive). Biofilm production was tested on Congo Red Agar, with black colonies formed after 24 hours of incubation at 37°C.

RESULT

The study spanned a considerable timeframe, encompassing the collection of 70 endotracheal aspirates from various intensive care units nationwide, with representation from the neonatal intensive care unit. The prevalence of ventilator-associated pneumonia (VAP) was markedly higher in men than in women, particularly prevalent in the 0-14 age group. The results revealed a higher likelihood of VAP in patients with severe pneumonia, while hollow viscous perforation was more prevalent in patients without severe pneumonia and those with cerebrovascular accidents. Identified predisposing factors included general anesthesia and type 2 diabetes mellitus. Culture results indicated a significant count of 105 CFU/mL in 60 samples (85.7%). Gram-negative bacilli dominated with 55 isolates, primarily Acinetobacter spp., Pseudomonas species, Klebsiella spp. and Citrobacter species (8%).

The prevalent gram-positive isolate was Staphylococcus aureus. Most Gram-negative isolates demonstrated susceptibility to carbapenems (Imipenem) and Piperacillin-tazobactam combinations, though some strains exhibited multidrug resistance. Staphylococcus aureus isolates were sensitive to Linezolid but resistant to Cefoxitin, categorizing all samples as methicillin-resistant Staphylococcus aureus (MRSA). The double disc diffusion method identified 25% of Klebsiella species with extended-spectrum beta-lactamase (ESBL), while 33% and 27% of Pseudomonas species exhibited Amp C resistance. Acinetobacter spp. displayed similar resistance patterns.

Biofilm production, detected through inoculating Congo red agar, was observed. However, the biofilm production was prominent in Staphylococcus aureus (100%), Acinetobacter species (81.8%), and Pseudomonas species (75%), reinforcing the correlation between biofilm production and mortality. Patients with biofilm-producing organisms in ET tube aspirates exhibited a higher mortality rate.

Table 1: Case distribution

Ward	Number
NICU	20
PICU	15
MICU	17
SICU	13
O&G ICU	5

Table 2: Demographic Characteristics

Age in years	Male	Female
0-14	15	20

15-30	0	5
31-45	5	0
46-60	5	2
>60	13	5

Table 3: Disease comparison profile

Diseases	Patients in number
HIE with ARDS 15	7
Severe pneumonia 25	13
RPOC (septic shock) 10	5
Hollow viscus perforation 20	10
Pneumothorax 5	2
VLBW 10	5
Febrile encephalopathy 10	5
Acyanotic heart disease with MR 10	5
Malaria with encephalitis 10	5
CVA (restroke) 15	8
Meningitis 5	2
Pancreatitis 5	3

Table 4: Risk factor associated with patients

Risk factors	Patients in number
Type 2 Diabetes Mellitus 15	7
PLHA 10	5
Old CVA 10	5
Post‐.General Anesthesia 30	15
VLBW 10	5
Old pulmonary tuberculosis 5	3

Table 5: Mortality rate over bio-film production

Isolated bacteria	Bio-film	Mortality
Staphylococcus aureus	7	3
Acinetobacter spp	23	5
Pseudomonas	7	5
Klebsiella spp	0	0
Citrobacter	0	0

DISCUSSION

Ventilator-associated pneumonia (VAP) poses significant mortality and morbidity risks for mechanically ventilated patients. This study aimed to explore noninvasive techniques for early VAP detection, addressing the high fatality rate of 20% and a prevalence ranging from 10% to 65%. While previous studies often focused on elderly males, this study, conducted in a tertiary care center, found a majority of patients to be in the 0–14 age range [11-16], with a significant representation from the Neonatal ICU. General anesthesia emerged as a primary risk factor for aspiration, attributed to patient sedation delaying ambulation. Acinetobacter spp. and Pseudomonas spp. were the predominant isolates, consistent with other studies [17]. Gram-negative bacteria exhibited susceptibility to carbapenems, aligning with existing literature [18]. The study highlighted the epidemiological challenges posed by ESBL-producing organisms, AmpC resistance, and MRSA. Biofilm production in endotracheal tubes was observed in 62.5% of isolates, with a notable correlation between biofilm presence and increased mortality.

CONCLUSION

Endotracheal aspirates commonly featured Acinetobacter spp. and Pseudomonas, with susceptibility to carbapenems observed in bacteria like *Klebsiella spp*. The presence of biofilm producers among isolates correlated with increased mortality. ETT biofilms housing multidrug-resistant microorganisms were linked to treatment failures and relapses. Early treatment stages could benefit from an empirical anti-biogram specific to the hospital. Implementing rotational antibiotic therapy, emphasizing proper hand-washing and employing decontamination techniques, coupled with educational programs, may prove effective in addressing multidrug-resistant pathogens and reducing the incidence of ventilator-associated pneumonia.

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