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Original Article

BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF GRAM-NEGATIVE BACILLI ISOLATED FROM ENDOTRACHEAL TUBE SECRETIONS AND TRACHEAL ASPIRATES FROM PATIENTS ON MECHANICAL VENTILATION: A HOSPITAL-BASED CROSS-SECTIONAL STUDY

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ABSTRACT

Objective: Nosocomial infections have become a serious threat to mortality and morbidity among hospitalized patients. Major risk factor among critically ill patients is excessive use of invasive devices like Endotracheal tube (ET) and tracheal intubation. The development of biofilm and their subsequent dislodgement aids in lung colonization and may cause Ventilator-associated pneumonia (VAP) [1].

Methods: Present study was conducted on ET tube tips and Tracheal aspirates received in microbiological laboratory from various ICU's (Medical, surgical and pediatric) in Andhra Medical College, Visakhapatnam over a period of one year from September 2022 to August 2023. A total of 116 samples were processed according to standard protocols and Antibiotic Susceptible Testing (AST) was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per CLSI guidelines.

Results: In total, 116 samples were processed. ET tube tips were 76/116 and tracheal aspirates were 40/116, with males (69/116) outnumbered females (47/116). Out of a total of 116 samples, 72 (62%) were culture-positive and 44 (38%) were sterile. Acinetobacter spp. was the most common pathogen among 72 culture positives (31/72, 43%), followed by Klebsiella spp. (20/72, 28%), Pseudomonas spp. (13/72, 18%) and Escherichia coli (8/72, 11%). A total of 11 (11/72, 15%) GNB were found as multi-drug resistant. The multi-drug resistant GNB isolated were Acinetobacter (6/72), Klebsiella (3/72) and Pseudomonas (2/72). Meropenem is the most susceptible antibiotic, followed by Piperacillin-Tazobactam.

Conclusion: It was concluded from this study that most commonly isolated pathogen was Acinetobacter spp. Most of these isolates were sensitive to Carbapenem and BLBLI combination drugs. Microbial persistence and impaired response to the treatment were more frequent, when multi-drug resistant organisms were present. Hence a local combined antibiotic approach, based on bacteriological profile and AST is essential to initiate empirical therapy which will minimize the bacterial colonization and prevent the incidences of VAP.

Keywords: Nosocomial infections, Ventilator associated pneumonia, Endo tracheal aspirates, Gram negative bacteria

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INTRODUCTION

Nosocomial infections have become a serious threat to mortality and morbidity among hospitalized patients. Major risk factors among critically ill patients are defective immune system and excessive use of invasive devices. The use of Endo tracheal [ET] tube and Tracheal intubation causes an increased risk of pneumonia which promotes the accumulation of trachea-bronchial secretions by impairing mucociliary clearance and disturbing cough reflex. The development of biofilm around the ET tubes by bacteria and their subsequent dislodgement after ET secretions aids in lung colonization and may cause Ventilator associated pneumonia (VAP) [1]. Three factors are critical in the pathogenesis of VAP: colonization of oropharynx with pathogenic organisms, aspiration of these organisms from oropharynx into lower respiratory tract, and compromise of the normal host defense mechanism. The etiologic agent of VAP may vary according to the onset of VAP, type of ICU, preexisting illness, age and sex of the patients, and prior antimicrobial therapy [2]. The mortality among these patients is on the rise due to the advent and constant increase in the number of MDR pathogens such as MDR Gram-negative rods including extended-spectrum beta-lactamase (ESBL) producers, carbapenem, and colistin-resistant organisms, and methicillin-resistant *Staphylococcus aureus* (MRSA). Concerning pathogens associated with lRTI, Gram-negative rods such as *Acinetobacter spp.*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* are predominant, whereas Gram-positive bacteria such as *S. aureus* including *MRSA* are comparatively less common [3]. Broncho alveolar lavage and protected specimen brush have been reported to have high sensitivity and specificity for the diagnosis of VAP, but are invasive and difficult to perform. The procedure of ET aspirate collection is easily performed at the bedside, relatively simple, minimally invasive and inexpensive, has a proven acceptable accuracy and requires minimal investment for the training of health professionals [2]. Therefore, the present study was aimed to determine the bacterial profile and their Antibiotic susceptibility pattern (AST) of Endo tracheal tube and Tracheal secretions of ventilated patients which will help clinicians to choose correct antimicrobial therapy against these MDR bacteria and control serious infections.

MATERIALS AND METHODS

The present study was conducted on ET secretions and Tracheal aspirates collected from the patients of various ICU's (Medical, surgical and pediatric) in Andhra Medical College, Visakhapatnam over a period of one year from September 2022 to August 2023. A total of 116 samples were collected aseptically in a labelled sterile container and transported immediately to the laboratory. All the samples were processed according to standard protocols. All specimens were cultured on Blood agar and MacConkey agar, and then incubated aerobically at 37 °C overnight. The isolates were identified based on their cultural characteristics, morphology in Gram staining and motility by hanging drop preparation. Among the isolated organisms, Gram negative bacilli were further processed. All standard biochemical reactions were performed to identify the isolated Gram negative bacilli. The antimicrobial susceptibility testing was performed on the entire Gram negative bacilli according to Clinical and laboratory Standards Institute (CLSI) 2022 and 2023 guidelines by Kirby-Bauer Technique [4, 5]. The antimicrobial agents used in this study were Amikacin (30µg), Aztreonam (30µg),

Ceftazidime (30µg) and Cefepime (30µg), Doxycycline (30µg), levofloxacin (5µg), Meropenem (10µg), Piperacillin-Tazobactam (100/10µg). As per CLSI 2022 guidelines zone diameters were measured in millimeters (mm) and interpreted as sensitive (S), intermediate (I) and resistant (R).

RESULTS

In total, 116 samples were processed. ET tube tips were 76/116 and tracheal aspirates were 40/116, with males (69/116) outnumbered females (47/116). Most commonly effected age group was 41-50 y (33/116) (Fig. 1). Majority of samples received from medical ICU's (81/116, 70%) (Fig. 2). Out of a total of 116 samples, 72 (62%) were culture positive and 44 (38%) were sterile. Acinetobacter spp. was the most common pathogen among 72 culture positives (31/72, 43%), followed by Klebsiella spp. (20/72, 28%), Pseudomonas spp. (13/72, 18%) and Escherichia coli (8/72, 11%) (Fig. 3). A total of 11 (11/72, 15%) GNB were found as multi drug resistant. The multi drug resistant GNB isolated were Acinetobacter (6/72), Klebsiella (3/72) and Pseudomonas (2/72) (Fig. 4). Meropenem is the most susceptible antibiotic, followed by Piperacillin-Tazobactam (table 1).

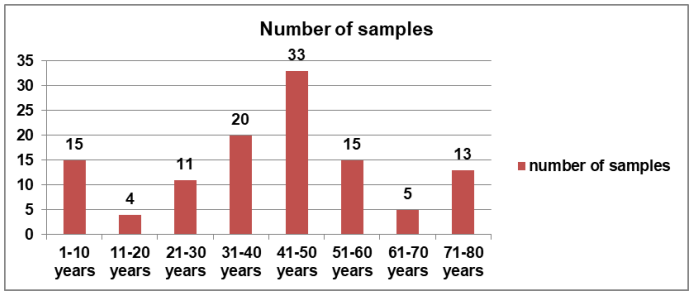

Fig. 1: Age wise distribution of patients samples (N=116)

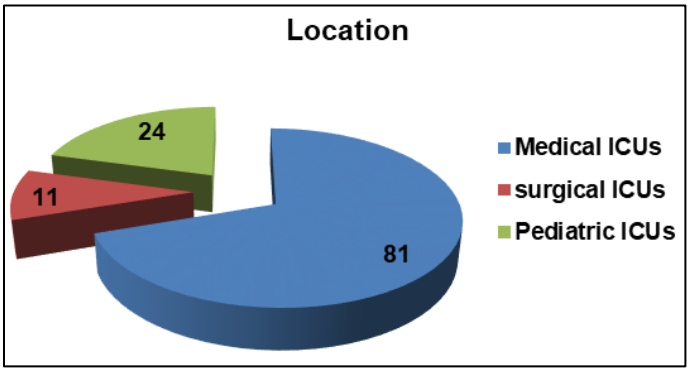

Fig. 2: location wise distribution of samples (N=116)

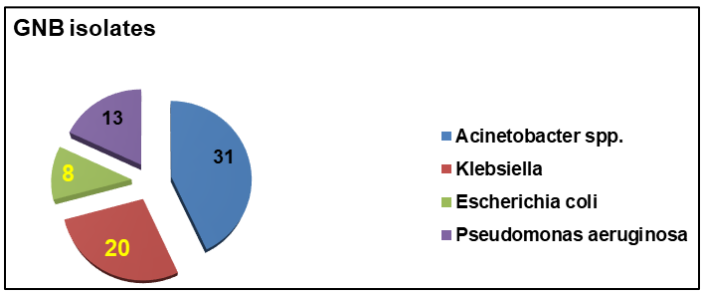

Fig. 3: Distribution of GNB isolates among culture positive samples (n=72)

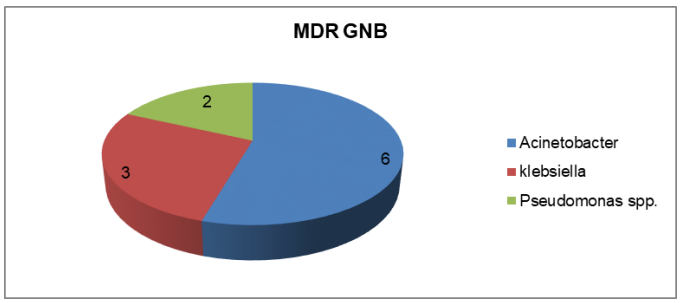

Fig. 4: Distribution of multidrug resistant GNB isolates (n=72)

Table 1: Percentage susceptibility of GNB isolates (n=72)

Antibiotics/Organism	Acinetobacter spp. [31]	Klebsiella spp. [20]	Escherichia coli [8]	Pseudomonas aeruginosa [13]
Amikacin	35% (11/31)	40% (8/20)	38% (3/8)	31% (4/13)
Azetreonam	-	-	-	69% (9/13)
Ceftazidime	16% (5/31)	15% (3/20)	25% (2/8)	15% (2/13)
Cefeipime	35% (11/31)	45% (9/20)	50% (4/8)	69% (9/13)
Doxycycline	71% (22/31)	-	-	-
Levofloxacin	26% (8/31)	30% (6/20)	38% (3/8)	38% (5/13)
Meropenem	81% (25/31)	85% (17/20)	100% (8/8)	85% (11/13)
Piperacillin Tazobactam	61% (19/31)	70% (14/20)	75% (6/8)	85% (11/13)

DISCUSSION

Lower respiratory tract infections are the most common healthcare-associated infections among intensive care unit patients. The Infectious Disease Society of America recently listed carbapenem-resistant Acinetobacter spp., ESBL-producing Escherichia coli and Klebsiella spp., and MDR pseudomonas as the top bacterial pathogens [1].

Present study has showed that the Gram-negative bacteria were primarily isolated from tracheal aspirates and ET secretions, with Acinetobacter species being the most common, followed by Klebsiella spp., Pseudomonas, and Eschericia coli. The findings are consistent with many previously published studies, including those by Batool *et al.* [1], Samal *et al.* [2] and Swathi *et al.* [6] who also reported Acinetobacter as the most common isolate. In the current study, only 15% of Acinetobacter were sensitive to 3rd gen. cephalosporins, whereas 85% were sensitive to carbapenems, which is consistent with Samal *et al.* [2] and Deepthi Chandra *et al.* [7], who found 82% and 72%, respectively, to carbapenems. In contrast, other studies, such as Sannathimmappa *et al.* [3], Ahmed *et al.* [8], S. K. Mishra *et al.* [9] and Kar *et al.* [10] show extremely high carbapenem resistance. In the present study, other GNB like Klebsiella spp., Escherichia coli and Pseudomonas aeruginosa also shows highest susceptibility Meropenem and Piperacillin-Tazobactam which is coinciding with Samal *et al.* [2] and Deepthi Chandra *et al.* [7].

Thus, patients undergoing the ETT procedure are more likely to develop respiratory tract infections as a result of bacterial colonization. The inappropriate use of broad-spectrum antibiotics results in the emergence of multidrug resistant bacteria. There is an urgent need to prevent antibiotic resistance among these MDR bacteria, and in the future, appropriate empirical antibiotic therapy may prove useful [1].

CONCLUSION

It is concluded from this study that most commonly isolated pathogen was Acinetobacter spp. Most of these isolates were sensitive to Carbapenems, Beta-lactam and Beta-lactam inhibitors combination. Microbial persistence and impaired response to the treatment were more frequent, when multi-drug resistant organisms were present. Hence a local combined antibiotic approach, based on bacteriological profile and AST is essential to initiate empirical therapy which will minimise the bacterial colonization and prevent the incidences of VAP.

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AUTHORS CONTRIBUTIONS

First author of the study K Snehitha contributed conceptual design, literature search, collection of patient samples and data collectiion. The second author S Swapna contributed sample processing, data analysis, statistical analysis and wrote the first draft of the manuscript. The third author P Kamala guided the work and corrected the manuscript.

CONFLICTS OF INTERESTS

The study declared 'no conflicts of interest

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