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Original Article

STUDY OF PREVALENCE OF MULTI-DRUG RESISTANT ACINETOBACTER SPECIES IN VENTILATOR-ASSOCIATED PNEUMONIAE CASES IN A TERTIARY CARE HOSPITAL, VISAKHAPATNAM

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ABSTRACT

Objective: Acinetobacter sps are important oppurtunistic pathogens with their significance in colonising and persistent biofilm production capabilities. The aim of the present study was to assess the prevalence of multidrug-resistant Acinetobacter sps in ventilator-associated pneumoniae cases in a tertiary care hospital Visakhapatnam.

Methods: A prospective study was conducted on 328 endotracheal aspirate samples received in Clinical Laboratory, Department of Microbiology for a period of 1 y, from June 2023 to June 2024, in mechanical ventilated cases, admitted in intensive care units, in a tertiary care hospital, Visakhapatnam.

Results: Out of 328 samples received, 42 (12.8%) were Acinetobacter isolates with resistance to Aminoglycosides (66.7%), Cephalosporins (57.1%), Quinolones (52.4%), Carbapenems (42.9%). Biofilm production was identified in 31 isolates (73.8%) by Congo Red Agar method in this study.

Conclusion: The potential ability of Acinetobacter sps to form biofilms and antibiotic selective pressure leading to emergence of resistant clones is directly proportional to the injudicious use of antibiotics. There is a strict need of antibiotic stewardship measures in intensive care units to restrict development of pan-resistant strains.

Keywords: Acinetobacter sps, Ventilator-associated pneumoniae, Biofilm production, Multidrug resistance, Carbapenems

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INTRODUCTION

Acinetobacter are common, free-living, ubiquitous organisms in the clinical environment. They have been recognised increasingly as notorious nosocomial pathogens involved in outbreaks of hospital infection, particularly in high-dependency or intensive care units, where they rapidly develop resistance to even the most potent antimicrobials [1]. Usually, Acintobacter sps are non-pathogenic but have components like endotoxin siderophores, that are capable to enhance virulence in debilitated individuals with life support. Endotoxin is a lipopolysaccharide moiety which is considered responsible for febrile response in sepsis cases [2]. Development of multidrug resistance in Acinetobacter can also be attributed to its ability of biofilm production, which often requires aggressive treatment measures to reduce morbidity and mortality in ICU settings [3].

MATERIALS AND METHODS

A total of 328 endotracheal aspirate samples received in Clinical Laboratory, Department of Microbiology, Andhra Medical College, Visakhapatnam, were processed during 1 y study period from June 2023 to June 2024. Samples collected aseptically in mechanical ventilated pneumoniae cases admitted in ICUs were included in the study. These samples were processed immediately by subculturing on nutrient agar, 5% sheep blood agar, mac conkey agar and biochemical reactions were performed for species identification. Biofilm producingh ability of Acinetobacter species was tested by Congo Red Agar method. Antimicrobial resistance pattern was recorded by *in vitro* conventional Kirby bauer disk diffusion method according to standard laboratory protocols.

Table 1: Biochemical reactions for Acinetobacter species, n=42

Reaction	Acinetobacter baumannii (n=38)	Acinetobacter lwoffii (n=04)
Catalase	+	+
Citrate	+	-
Indole	-	-
Methyl Red	-	-
Nitrate reduction	-	-
Urease	V	-
Voges Proskauer	-	-
Glucose	+	-
Mannitol	-	-
Mannose	+	-
Sucrose	-	-
Xylose	+	-
Oxidase	-	-
Citrate	+	-
TSI	K/K, no H_2S	K/K, no H_2S

RESULTS

Out of total 328 samples, 42 were identified as Acinetobacter species (12.8%). Based on biochemical reactions, predominant isolate in this study was identified as Acinetobacter baumannii (38/42, 90.5%), followed by Acinetobacter lwoffii (4/42, 9.5%) table 1. Biofilm production was evident in 31 isolates (73.8%) by congo red agar method, producing dry crystalline black-colored colonies.

Multidrug resistance to carbapenems, quinolones, cephalosporins, aminoglycosides was recorded in 18 isolates (42.8%). Resistance patterns of different group of antimicrobials was studied and recorded as per standard CLSI guidelines table 2. Least resistance was recorded in tigecycline (9.5%) and colistin (11.9%). Beta-lactamase inhibitors like tazobactam and avibactum have shown promising efficacy in multidrug-resistant strains in this study.

Table 2: Antimicrobial class resistance pattern, n=42

Antimicrobial class	Total resistant	Percentage %
Aminoglycosides	28	66.7
Cephalosporins	24	57.1
Quinolones	22	52.4
Carbapenems	18	42.9

DISCUSSION

Ventilator-associated pneumoniae is a term used to describe penumoniae that develops in a patient who has been on mechanical ventilatory support for more than 48 h. Exponential increase of multidrug-resistant microorganisms in ICU settings has grown as an epidemic which directly effects the patient's outcome. Acinetobacter has evolved as heterotrophic aerobic isolate in hospital settings [4]. They were considered as commensal, but some strains possess virulent determinants like endotoxin, fimbriae, polysaccharide capsule, siderophores, iron-repressible outer membrane receptor proteins, O antigens [5].

Identification and speciation of Acinetobacter isolates was done by subculturing samples on nutrient agar, 5% sheep blood agar, mac conkey agar and incubated at 37 °C for 18 to 24 h. Growth on the media was selected for performing routine Grams stain to identify the morphology as Gram-negative coccobacilli. Biochemical reactions were done from nutrient agar colonies and results read after 24 to 48 h [6]. Acinetobacter baumannii (90.5%) is the predominant isolate in this study. Antimicrobial resistance patterns of different classes of antibiotics was done by *in vitro* conventional kirby bauer disk diffusion method in a 90 mm Mueller Hinton agar petri plate, with inoculum matching 0.5 Mc Farland turbidity. Interpretation of result was doing by standard CLSI and EUCAST guidelines [7].

Biofilm production plays a crucial role in pathogenesis, making treatment options more difficult. Medical devices act as an excellent reservoir in mechanical ventilated scenarios. Biofilm-encased cells are shielded by an extracellularly produced polymeric matrix, making them more resistant to antibiotics and innate components of the host [8, 9]. Congo red agar method was used in this study, biofilm producing colonies were dry, crystalline and black-colored.

Multidrug resistance may have been attributed by different genotypic and phenotypic characteristics. Aminoglycoside resistance in Acinetobacter is due to decreased membrane permeability by loss of porins, acquisition of extended spectrum beta-lactamase and multidrug efflux system [10]. Cephalosporin resistance may be conferred to inactivation by hydrolysis, imbalance between influx and efflux and protection of antibiotic target [11]. Mutations at residues Ser 80 and Glu 84, when combined with mutations at Ser 83 of Gyr A or alterations in target enzymes like DNA gyrase and topoisomerase IV may render quinolone resistance in Acinetobacter baumannii [12]. CRAB, which stands for carbapenem resisatant Acinetobacter baumannii, results from porin or penicillin-binding protein modifications. Several porins, including the 33-kDa Car O Protein, that constitute a pore channel for influx of carbapenems, might be the involved mechanism [13].

Higher resistance in this study was seen with aminoglysosides (66.7%) and least resistance in tigecycline (9.5%). In multidrug resistant cases, combination therapy with antibiotic and beta-lactamase inhibitors had shown effective efficacy. Piperacillin+Tazobactam was the resort of choice in pan-resistant strains of Acinetobacter [14] in this study (table 3).

Table 3: Drug resistance pattern, n=42s

Antimicrobial agent	Total resistant	Percentage %
Imipenem	18	42.9
Meropenem	11	26.2
Ciprofloxacin	22	52.4
Levofloxacin	08	19
Colistin	05	11.9
Cefotaxime	24	57.1
Ceftriaxone	16	38.1
Amikacin	28	66.7
Gentamicin	26	62
Ceftazidime+Avibactum	12	28.6
Ceftazidime	20	47.6
Piperacillin+Tazobactam	08	19
Polymyxin B	06	14.3
Tigecycline	04	9.5

CONCLUSION

The potential ability of Acinetobacter species to form biofilms and antibiotic selective pressure leading to emergence of multidrug-resistant clones is directly proportional to the injudicious use of antibiotics. Strict antibiotic stewardship measures in ICU, along with combination therapy with beta-lactamase inhibitors may support to enhance the treatment response and minimal evolution of drug resistance.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICTS OF INTERESTS

There are no conflicts of interest

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