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A Study of prevalence and Antimicrobial Resistance of *Acinetobacter Species* in a Tertiary Care Hospital, Chidambaram, India

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ABSTRACT

Acinetobacter species has emerged as one of the most important hospital pathogens which are becoming increasingly multi drug-resistant. They cause outbreaks in intensive care units and health care units. A cross-sectional study was conducted to assess the prevalence and antibiotic susceptibility pattern of *Acinetobacter* spp isolated from various clinical samples collected from 720 patients admitted in all wards and intensive care units of the hospital for one year (September 2021 to August 2022). Out of 720 samples, 390 (54%) showed significant growth. These 390 positive cultures were further processed for identification and out of these positive cultures, 93 (23.8%) *Acinetobacter* spp were isolated. The majority of *Acinetobacter* spp 46 (49%) were isolated from the Intensive Care Unit (ICU) followed by Surgery ward 18 (19%), Medicine ward 15 (16%) and Orthopaedics ward 5 (5%). Among the 93 isolates of *Acinetobacter*, 100% sensitivity was to Colistin and 96% to Polymyxin B followed by Meropenem (81%), Levofloxacin (78%), Cefoperazone-Sulbactam (73%), Ampicillin-Sulbactam (68%), Ceftazidime (48%), Norfloxacin (40%), Piperacillin-Tazobactam (39%), Ciprofloxacin (35%), Gentamicin (32%), Amikacin (32%), Cefepime (29%), Cefotaxime (16%). High levels of resistance were seen for Cefotaxime (84%) and Cefepime (71%). *Acinetobacter* isolated in this study showed multidrug-resistant patterns mostly in ICU patients. To avoid multidrug resistance, antibiotics should be used judiciously. The importance of hand washing and the use of disinfectants to prevent the transmission of infection in health care setup has to be emphasized.

Keywords: *Acinetobacter* spp, Prevalence, Antibiotic resistance, Intensive care units, Multidrug resistance.

INTRODUCTION

The genus *Acinetobacter* is Gram-negative, strictly aerobic non-fermenting, non-fastidious, non-motile, catalase-positive and oxidase-negative coccobacillus bacteria. They prefer moist environments and can easily be obtained from soil, water, food and sewage [1,2]. They are usually considered to be opportunistic pathogens, and of recently been reported to cause several outbreaks of nosocomial infections in hospitalized patients like septicaemia, pneumonia, wound sepsis, endocarditis, meningitis and urinary tract infections (UTI) [3,4]. Such infections are often extremely difficult for the clinician to treat because of the widespread resistance of these bacteria to the major group of antibiotics. More than two third of *Acinetobacter* infections are due to *Acinetobacter* baumannii which causes healthcare-associated infections [5-8]. It also can form biofilms, which may play a role in the process of colonization. Biofilms help the bacteria resist disinfection while also allowing the participating cells to trading resistance genes, further facilitating the persistence of the pathogen [9]. *Acinetobacter*-associated infections represent a tough challenge to control in severely ill patients especially those in the Intensive Care Unit (ICU). *Acinetobacter* species can acquire resistance to almost all presently existing antimicrobial agents [10]. Despite the increasing significance and frequency of multidrug-resistant *Acinetobacter* infections, many clinicians and microbiologists still lack an appreciation of the importance of these organisms because of their confused taxonomic status [11]. Because of their increasing importance in nosocomial infections and multidrug-resistant patterns, further study is warranted.

In the present study, an attempt was made to find out the prevalence of *Acinetobacter* isolates obtained from various clinical samples collected from patients admitted in various ICUs and wards by phenotypic identification scheme and also determine their antimicrobial susceptibility at the Department of Microbiology (RMMCH), Government Cuddalore Medical College and Hospital.

METHODS

The present study was conducted in the Department of Microbiology, Faculty of Medicine, Government Cuddalore Medical College and Hospital, Formerly Rajah Muthiah Medical College and Hospital (RMMCH), Annamalai University, Chidambaram, Tamilnadu, India. During a period of one year (September 2021 to August 2022). The study included all the patients who had been admitted to various wards and ICUs whose various clinical samples were sent to the microbiology laboratory for routine culture and antibiotic susceptibility tests. Blood samples are inoculated into BHI broth and other samples were inoculated onto MacConkey Agar and Blood Agar plates.

All isolates obtained were further processed and identified by standard routine microbiological processes. Genus *Acinetobacter* was identified by Gram staining as Gram-negative coccobacilli, colony morphology, non-motile, oxidase negative, catalase positive, TSI reaction (AK/AK) and citrate utilization test positive. Identification of *Acinetobacter* species was done based on glucose oxidation (OF test), haemolysis on blood agar, growth at 37°C and 44°C, citrate utilization, Arginine decarboxylation, Glucose utilization[12,13].

Antibiotic susceptibility testing was performed by standard Kirby Bauer disc diffusion method for the following antimicrobial agents' Gentamicin (GEN), Amikacin (AK), Ciprofloxacin (CIP), Levofloxacin (LE), Ceftazidime (CAZ), Cefotaxime (CTX), Cefepime (CPM), Ampicillin-Sulbactam(A/S), Cefoperazone-Sulbactam (CFS), Piperacillin-Tazobactam (PIT), Meropenem (MRP), Polymyxin-B (PB), Colistin (CL) and Norfloxacin (NX). The zones of inhibition were measured and interpreted as per Clinical and Laboratory Standards Institute guidelines (CLSI)[14]. All dehydrated media and antibiotic discs were procured from HiMedia labs, in Mumbai, India.

RESULTS

A total of 720 samples were processed, out of which 390 (54%) were culture positive with significant growth and 330 (46%) had no growth (culture negative) (Table 1).

Table 1. Percentage of culture positives

Total Number of samples	Number	Percentage
The number of samples tested	720	100%
No. of cultural positives	390	54%
No. of culture negatives	330	46%

Out of the total 390 culture positive samples, 93 (24%) were *Acinetobacter* and 297 (76%) were other organisms (Table 2).

Table 2. Distribution of positive culture

Positive growth	Number of isolates	Percentage
Acinetobacter organisms	93	24%
Other organisms	297	76%

Acinetobacter isolates were obtained from various specimens as well as various wards. The isolation rate of *Acinetobacter* spp. was maximum in ICU 46 (49%) followed by Surgery ward 18 (19%), Medicine ward 15 (16%) and Orthopaedics ward 5 (5%) (Table 3). In the present study, there was a higher incidence of *Acinetobacter* infection in males 64 (69%) than in females 29 (31%) (Figure 1).

In the present study maximum positivity of *Acinetobacter* spp. was seen in patients in the age group of >50 years with an incidence of 61% in males and 55% in females (Figure 2).
The isolation rate of *Acinetobacter* sp was maximum from Endotracheal aspirate sample 25 (27%), followed by pus 21 (23%), blood 21 (23%), urine 14 (15%) and sputum 12 (13%) (Table 4). The most predominant species of *Acinetobacter* isolated was *A. baumannii* 89 (96%) followed by *A. lwoffi* 4 (4%) (Table 5).

Table 3. Distribution of *Acinetobacter* isolates among various wards (n=93)

WARD	Male	%	Female	%	Total	%
ICU	32	50	14	48	46	49
Surgery ward	12	19	6	21	18	19
Medicine ward	13	20	2	7	15	16
PICU	1	2	3	10	4	4
Orthopaedics ward	5	8	0	0	5	5
Paediatric ward	1	2	1	3	2	2
Gynaecology ward	0	0	3	10	3	3
Total	64	100	29	100	93	100

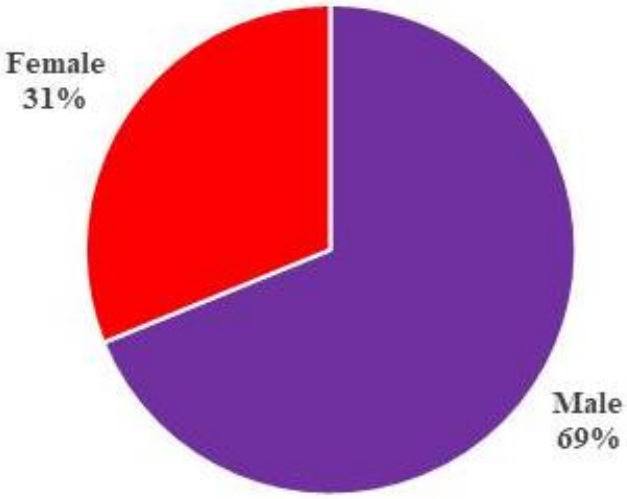

Figure 1. Overall Gender wise distribution of *Acinetobacter* species

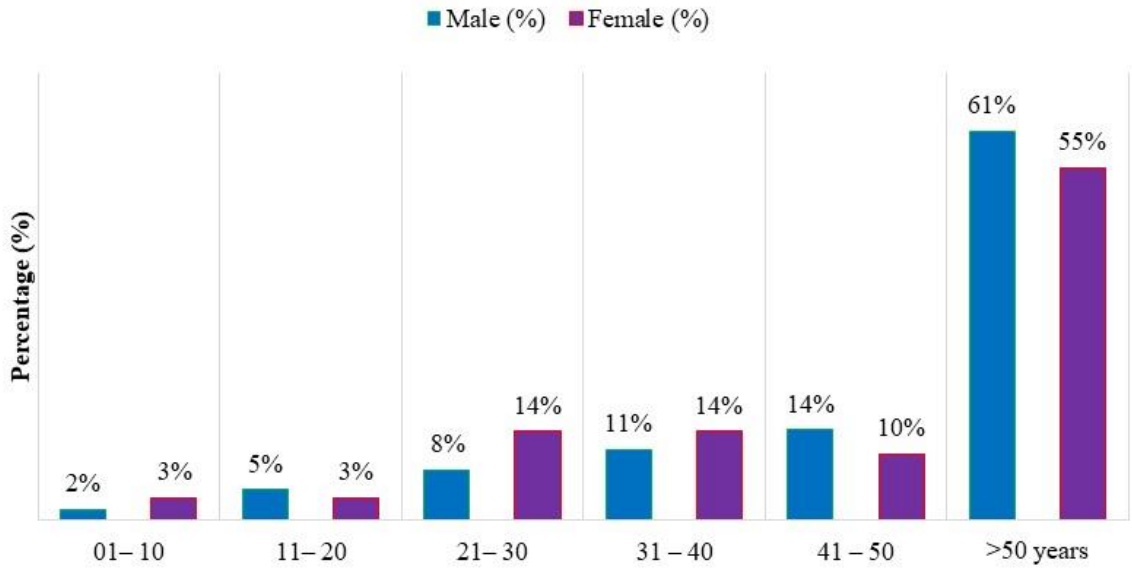

Figure 2. Age and sex-wise distribution of *Acinetobacter* species

Table 4. Distribution of *Acinetobacter* species among various clinical specimens (n=93)

Specimen	Male	%	Female	%	Total	%
Endotracheal aspirate	17	27	8	28	25	27
Sputum	10	16	2	7	12	13
Pus	13	20	8	28	21	23
Blood	14	22	7	24	21	23
Urine	10	16	4	14	14	15
Total	64	100	29	100	93	100

Table 5. Identification of *Acinetobacter* species

Acinetobacter Species	Haemolysis on blood agar	Growth at 37°C	Growth at 44°C	Citrate utilization	Glucose oxidation fermentation	Arginine decarboxylation	Glucose utilization
Acinetobacter baumannii (96%)	-	+	+	+	+	+	+
Acinetobacter lwoffii (4%)	-	+	-	-	-	-	-

Table 6. *In vitro* activity of various antimicrobial agents against *Acinetobacter* isolates

S. No.	Antibiotic Disc	*Acinetobacter* baumannii n = 93			
		Sensitive	%	Resistant	%
1	Gentamicin (GEN)	30	32	63	68
2	Amikacin (AK)	30	32	63	68
3	Levofloxacin (LE)	73	78	20	22
4	Ciprofloxacin (CIP)	33	35	60	65
5	Norfloxacin (NX)	37	40	56	60
6	Ceftazidime (CAZ)	45	48	48	52
7	Cefotaxime (CTX)	15	16	78	84
8	Cefepime (CPM)	27	29	66	71
9	Cefoperazone-Sulbactam (CFS)	68	73	25	27
10	Ampicillin-Sulbactam(A/S)	63	68	30	32
11	Piperacillin-Tazobactam (PIT)	36	39	57	61
12	Meropenem (MRP)	75	81	18	19
13	Polymyxin-B (PB)	89	96	4	4
14	Colistin (CL)	93	100	0	0

* ($p<0.01$)

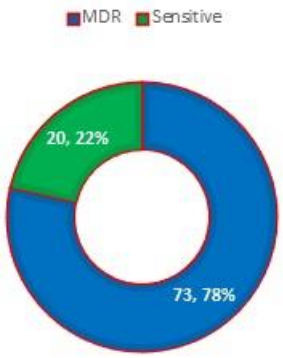

Figure 3. The percentage of drug-resistant *Acinetobacter* isolates

Among the 93 isolates of *Acinetobacter*, all showed 100% sensitivity to colistin and 96% to polymyxin B. High levels of resistance were seen for Cefepime (84%) and Cefotaxime (71%). The p-value was found to be statistically significant for these resistant antibiotics (Table 6).

The percentage of drug-resistant *Acinetobacter* isolates which were multi-drug resistant (MDR) was 58 (62%). All MDR isolates were resistant to at least one agent in three or more antimicrobial categories; penicillin, cephalosporins, aminoglycosides, fluoroquinolones and carbapenems (Figure 3).

DISCUSSION

Acinetobacter spp has emerged as the main cause of ICUs infection. *Acinetobacter baumannii* is regarded as a life-threatening pathogen associated with community-acquired and nosocomial infections, mainly seen in ventilator associated pneumonia. Multi drug resistant *Acinetobacter spp* are threat in ICU set-up. Their ubiquitous nature in the ICU environment and inadequate infection control has raised the incidence of *Acinetobacter* infection in the past two decades. A number of risk factors enhance the spread and persistence of *Acinetobacter* spp like mechanical ventilation, admission to ICUs, underlying chronic debilitating conditions and prolonged hospital stay.

Rangel *et al*., (2021) stated that the COVID-19 pandemic has generated an overuse of antimicrobials in critically ill patients and *Acinetobacter baumannii* frequently causes nosocomial infections, specially in intensive care units has increased over time[16].

In this present study, 93 (24%) *Acinetobacter* spp were isolated from all the clinical samples like Endotracheal aspirate (27%), pus (23%), blood (23%), urine (15%), sputum (13%) which is higher to the study conducted by Rajkumari *et al*., (2020)[13] in which the prevalence of *Acinetobacter baumannii* is 11%

In our present study, Maximum *Acinetobacter* spp. was isolated from ICU 46 (49%) followed by Surgery ward 18 (19%), Medicine ward 15 (16%) and Orthopaedics ward 5 (5%) while a lower percentage of isolation were observed from other wards. Another study by Ababneh *et al*., (2021) reported *Acinetobacter baumannii* isolates were recovered, mostly from surfaces in the internal medicine ICUs[18].

In the present study, the isolation rate of *Acinetobacter* spp was maximum from Endotracheal aspirate sample 25 (27%), followed by Pus 21 (23%), Blood 21 (23%), Urine 14 (15%) and Sputum 12 (13%) which is similar to Rajkumari *et al*., (2020)[13]. This varies in the study by Gupta *et al*., (2015)[19] where *Acinetobacter* isolation was predominant in urine and tracheobronchial secretions. Another study by Mohammed *et al*., (2022)[20] found that the predominant isolation of *Acinetobacter* was from blood (36.9%) followed by Pus (22.5%), respiratory samples (14.4%), urine (11.7%), and other body fluids (9%). An increase in *Acinetobacter* occurrence in blood cultures was reported in the same hospital Mohammed *et al*., (2022)[20].

In this study, the most predominant species of *Acinetobacter* isolated was *Acinetobacter baumannii* 89 (96%) followed by *Acinetobacter lwoffii* 4 (4%) which was similar to studies conducted by Albayrak *et al*., (2021) and Rajkumari *et al*., (2020)[13]. The predominance of *Acinetobacter baumannii* isolated from various samples was observed by Raina *et al*., (2016) [(11)]. Two major factors for the persistence of *Acinetobacter baumannii* in hospital environments and ICUs are resistance to antibiotics and resistance to disinfectants.

Among the 93 isolates of *Acinetobacter*, all showed 100% sensitivity to colistin and 96% to polymyxin B followed by Meropenem (81%), Levofloxacin (78%), Cefoperazone-Sulbactam (73%), Ampicillin-Sulbactam (68%), Ceftazidime (48%), Norfloxacin (40%), Piperacillin-Tazobactam (39%), Ciprofloxacin (35%), Gentamicin (32%), Amikacin (32%), Cefepime (29%), Cefotaxime (16%) which is more or less similar to Rajkumari *et al*., (2020) [13.] This study shows 100% sensitivity to colistin and 96% to polymyxin B which is similar to Raina *et al*., (2016) [11] and Dash *et al*., (2013) [22].

High levels of resistance were seen for Cefotaxime (84%) and Cefepime (71%). The p-value was found to be statistically significant for these resistant antibiotics which are similar to Dash *et al*., (2013). In their study it is reported that most of the *Acinetobacter* spp were highly resistant to ceftazidime (93%), cefepime (89%).

Among a total of 93 *Acinetobacter* isolates, 68 isolates were MDR. All MDR isolates belonged to *Acinetobacter baumannii* which is Similar to the studies by Rajkumari *et al*., (2020) [13]. Norfloxacin was tested only in urine isolates and 60 % of isolates were resistant to this antibiotic. The percentage of multi drug-resistant *Acinetobacter* isolates was 73 (78%) whereas Rajkumari *et al*., (2020) [13] showed 80% MDR. All eight (5.8%) PDR isolates were 100% sensitive to colistin [22].

Susceptibilities of *Acinetobacter* against antimicrobials are considerably different among countries, centres and even among wards of the same hospital. Therefore, such types of

local surveillance studies and around are important in deciding the most adequate therapy for *Acinetobacter* infections.

CONCLUSION

This study concluded that *Acinetobacter* spp indicates its role as a nosocomial pathogen, especially in critically ill patients admitted to ICUs. It is a great challenge for physicians to treat MDR *Acinetobacter* spp. In our study, *Acinetobacter* was resistant to the most commonly used antibiotics. The emergence of carbapenem resistance is worrisome. In our study, colistin and polymyxin B was the most sensitive antibiotics. Appropriate and rational use of antibiotics is necessary to prevent microbial resistance. Though the organism has developed multidrug resistance, it has largely remained susceptible to disinfectants and antiseptics. Strict control of the hospital environment, hand hygiene and optimizing/ judicious use of antibiotics is recommended to reduce the resistance rates and also to reduce the MDR frequency in the hospital.

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