ORIGINAL ARTICLE

Prevalence and Antimicrobial Susceptibility Pattern of *Acinetobacter baumannii* Complex in Clinical Samples Among Patients at a Tertiary Care Hospital, Jaipur

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Abstract: Aims and objectives: *Acinetobacter causes a wide spectrum of infections, including nosocomial pneumonia, secondary meningitis, surgical wound infections, skin and soft tissue infections, urinary tract infections, bacteraemia, and transmission via the hands of hospital personnel. The study aimed to determine the prevalence of Acinetobacter baumannii complex isolates and the antimicrobial susceptibility pattern of isolated A. baumannii complex. in clinical samples among patients at Mahatma Gandhi Medical College and Hospital.* ***Introduction:*** *In recent decades, Acinetobacter baumannii (A. baumannii) infections have also occurred outside the ICU or in trauma patients after natural disasters, and they have even affected patients after co-morbidities in the community.* ***Materials and methods:*** *All A. baumannii complex isolates (non-repetitive) from different clinical samples received in a clinical microbiology laboratory from inpatients and outpatients at Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, were included in the study. Routine microscopy of the samples was done. Gram's staining was done on all samples except urine. All clinical samples were inoculated on blood agar and MacConkey agar and incubated at 370 °C for 18–24 hours. Antimicrobial susceptibility testing of the isolated A. baumannii complex was done by the VITEK2-AST Compact system.* ***Results:*** *Among 6483 samples, 157 (2.42%) A. baumannii complex isolates were culture-positive, 68.37% were sterile, and 29.19% were other culture-positive. The maximum sensitivity of A. baumannii isolates was seen to be Tigecycline (70%), followed by Minocyclin (29.9%), while maximum resistance was observed for Piperacillin/Toazobactam (97%), followed by Imipenem, Meropenem (96.8%), Ceftazidime (96%), Cefepime (91.7%), Ciprofloxacin (88%), and Gentamycin (87%).* ***Conclusion:*** *Based on this study, it could be concluded that, as antibiotic resistance increases, hardships will be experienced in A. baumannii complex treatment unless the necessary precautions are taken and new antibiotics are discovered. In order to prevent the spreading of resistant Acinetobacter strains, infection control measures should be taken, clinicians and laboratory workers should cooperate during antibiotic use, and hospital hygienic rules should be observed.*

Keywords: *Acinetobacter baumannii, antibiotic, susceptibility, resistance.*

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INTRODUCTION

The genus Acinetobacter species is a non-fermentative and non-motile, Gram-negative coccobacillus, which comprises 27 known and several unnamed provisional species. Clinically, we most often identify Acinetobacter baumannii (A. baumannii) as the cause of infection. A. baumannii is a typically short, almost round, rod-shaped (Coccobacillus) Gram-negative bacterium. It is named after Paul Baumann, a bacteriologist [1,2]. It can be an opportunistic pathogen in humans, affecting people with compromised immune systems,

and is becoming increasingly important as a hospital-derived infection. Despite its occasional discovery in environmental soil and water samples, its natural habitat remains unknown [3,4]. The Acinetobacter species cause infections and are associated with increased morbidity and mortality rates.

Acinetobacter causes a wide spectrum of infections, including nosocomial pneumonia, secondary meningitis, surgical wound infections, skin and soft tissue infections, urinary tract infections, and bacteremia. Outbreaks of infections are often associated with the spread of a unique strain and have been linked to the contamination of respiratory therapy equipment, intravascular access devices, bedding materials, and transmission via the hands of hospital personnel [5–9]. A. baumannii is part of the ACB complex (A. baumannii, A. calcoaceticus, and Acinetobacter). It is difficult to determine the specific species of members of the ACB complex, and they comprise the most clinically relevant members of the genus [10]. Strains of A. baumannii have started to acquire resistance to newly developed antimicrobial drugs and have become prevalent in many hospitals. A. baumannii was found to be multi-drug resistant against antimicrobial drugs such as aminopenicillin, cephalosporins, first and second-generation cephalosporins, cephamycins, aminoglycosides, ureidopenicillins, chloramphenicol, and tetracyclines.

Material & Methods

A prospective study was conducted at Mahatma Gandhi Medical College and Hospital (India) on patients presenting with signs and symptoms of A. baumannii complex infection for a period of 6 months. All A. baumannii complex isolates (non-repetitive) were obtained from different clinical samples received in the clinical microbiology laboratory from inpatients and outpatients of Mahatma Gandhi Hospital (MGH), Sitapura, Jaipur, Rajasthan.

Collection and transportation of specimens:

Various types of samples, including urine, blood, pus, discharge from the skin and soft tissue sites, sputum, ET, fluids (including cerebrospinal fluid (CSF), ascitic fluid, pleural fluid, etc.), miscellaneous swabs (including ear swabs, throat swabs, vaginal swabs, wound swabs, etc.), tissue, and central line tips, were collected. Samples were collected with universal precautions using prescribed sterile techniques and transported to the laboratory as soon as possible, maintaining optimum transportation conditions. A detailed relevant history was taken, including age, sex, the history of any in-dwelling medical devices used, and the duration of wards and ICU stays. All the samples were collected from various patients and outpatient wards.

Transport and storage of specimens:

After the collection of the sample, the container was properly labeled with the patient's name, ID number, etc. The specimens were then transferred to the laboratory as quickly as possible, usually within 1 hour after collection, and processed as soon as possible. When processing was delayed, they were stored at 4 °C.

Processing of Specimen

The specimen underwent routine microscopy. All samples, with the exception of urine, underwent Gram's staining. We conducted wet microscopy on urine samples to identify bacteria and pus cells. We inoculated all clinical samples on blood agar and MacConkey agar, then incubated them at 37 °C for 18–24 hours. Only non-lactose fermentative (NLF) Gram-negative bacilli were oxidase-negative. We used the VITEK-2 Compact Method to finalize the identification of Gram-negative bacteria. The VITEK2-AST Compact system conducted antimicrobial

susceptibility testing on the isolated A. baumannii complex.

Results

The present study was carried out at Mahatma Gandhi Medical College & Hospital, Jaipur, in the Department of Microbiology. We processed a total of 6483 samples for bacterial culture. Among 6483 samples, 157 (2.42%) A. baumannii complex isolates were culture-positive, 68.37% were sterile, and 29.19% were other culture-positive, as shown in Table 1.

Table 1. Distribution of culture-positive isolates.

Total samples	Number of isolate samples	(%) age
Total *Acinetobacter baumannii* complex isolate	157	2.42%
Total positive culture	1893	29.19%
Sterile	4433	68.37%
Total	6483	100%

Out of total culture-positive cases, the maximum number were from inpatient department (IPD) 154 (98.08%), followed by outpatient department (OPD) 3 (1.91%). Out of the total 157 culture-positive cases, males accounted for 135 (85%) and females for 22 (14%), as shown in Table 2 and Figure 1.

Table 2. Distribution of positive culture cases among patient attending OPD wards

IPD/OPD	Number of isolates	% age
IPD	154	98.08%
OPD	3	1.91%
TOTAL	157	100%

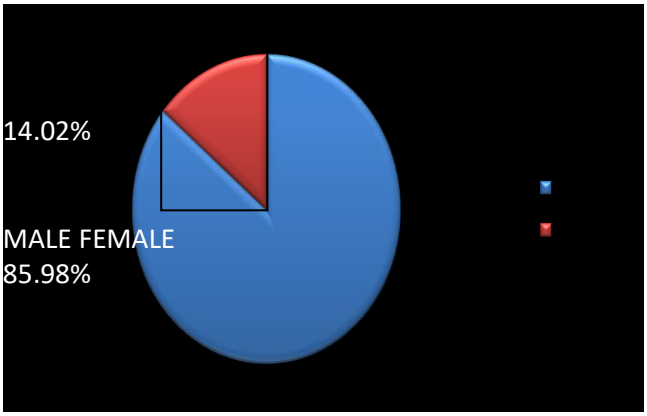

Figure 1. Sex distribution of culture-positive cases.

In the present study, a total of 157 culture-positive cases were observed. Most positive cases were observed in the age group of more than 50 years, which is 56.05% (n = 88), as shown in Table 3. On the basis of the distribution of isolates from different wards or ICU's in the hospital, it was found that the maximum number of isolates were from MICU, while the least common isolates were from PICU. Most of the isolates were obtained from ET (74) samples, followed by blood (40) samples, and the least number

of ACB complex samples were isolated from pus (1), urine (1), and CSF (5) in the present study (Table 4).

Table 3. Age-wise distribution of culture-positive cases of the ACB complex (n-157).

S. No.	Age	Total
1	0-10	5
2	11-20	7
3	21-30	12
4	31-40	16
5	41-50	25
6	>50	88

Table 4. Distribution of isolates according to the specimen (n=157).

Specimen	No of Isolates	% Age (%)
Blood	**40**	**25.4%**
Urine	2	1.27%
Pus	1	0.63%
E.T. secretion	**74**	**47.1%**
Swab	9	5.73%
CSF	5	3.18%
Body fluids	10	6.36%
Sputum	16	10.1%
TOTAL	**157**	100%

As shown in Table 5, the current study found that tigecycline (TGC) had the highest sensitivity at 70%, followed by minocycline (29.9%) and cotrimoxazole (20%), while piperacillin/toazobactam had the maximum resistance at 97%, followed by imipenem, meropenem (96.8%), ceftazidime (96%), cefepime (91.7%), ciprofloxacin (88%), and gentamycin (87%).

Table 5. Antimicrobial susceptibility pattern of ACB complex isolates (n=157).

S/. No.	ANTIBIOTICS	Resistant		Sensitivity		Intermediate	
		n	%	N	%	N	%
1	Cefoperazone/Sulbactam(CPZ/S)	118	75%	19	12%	20	12.7%
3	Piperacillin/Tazobactam (PIT)	153	97%	4	2.5%	--	--
4	Ceftazidime(CAZ)	152	96%	4	2.5%	1	0.63%
5	Colistin (CL)	11	7%	2	1.2%	144	91.7%
6	Cefepime (CPM)	144	91.7%	7	4.4%	6	3.82%
7	Imipenem (IPM)	152	96.8%	5	3.1%	--	--
8	Meropenem (MRP)	152	96.8%	5	3.1%	--	--
9	Gentamicin (GEN)	137	87.26%	12	7.6%	8	5.09%
10	Netilmycin (NET)	117	74.5%	25	15.9%	15	9.93%
11	Cipropfloxacin (CIP)	139	88.5%	11	15.9%	7	4.45%
12	Cotrimoxazole (COT)	120	76.4%	32	20.3%	5	3.18%
13	Minocycline (MIN)	88	56%	47	29.9%	22	14.0%
14	Tigecycline (TGC)	15	9.5%	110	70%	32	20.3%
15	Levofloxacin (LE)	125	79.6%	11	7%	21	13.7
16	Ticarcilin/Clavunic (TCC)	135	86.%	5	3.1%	17	10.8%

Discussion

Multidrug-resistant Gram-negative pathogens are associated with high morbidity and mortality. Multidrug-resistant *Acinetobacter spp*. has been reported worldwide and has now emerged as one of the hardest healthcare-associated infections to control and treat. Patients admitted to the burn unit, ICU, and those wards with central intravenous catheters and respiratory devices are the main targets of this organism [11,12]. Delay in receiving adequate empirical antimicrobial therapy has an adverse effect on clinical outcomes in hospital-acquired infections caused by *A. baumannii [13]*. *Acinetobacter*-associated nosocomial infections in critically ill patients are on the rise [14,15]. Its MDR phenotype can acquire new mechanisms of resistance and nosocomial outbreaks [16]. Resistance to antibiotics poses a serious and growing problem because such resistant infectious diseases are becoming more difficult to treat. Resistant bacteria do not respond to the antibiotics and continue to cause infections [17]. In the last few decades, there has been a general trend of increasing incidences of infection due to this pathogen around the globe [18].

Acinetobacter spp. were considered to be quiet bystanders until their role in hospital-acquired infections was described. Of the many species of *Acinetobacter* that have been described, *Acinetobacter calcoaceticus, a species from the Acinetobacter baumanii* complex (Acb complex), is clinically the most important. In the current study, a total of 6483 specimens were received in the microbiology laboratory for culture and sensitivity. Out of these samples, 1893 were positive cultures, and *A. baumannii* complex isolates were 157 (2.42%). In the study of Sabir *et al.,* the percentage of positive culture was 87.17%, which is much higher than the present study [19]. A similar study by Sharma et al. showed that the maximum frequency of *A. baumannii* isolates was recovered from ICUs (63.04%) compared with wards, which is found to be similar to the studies done by Xia et al. [11,20]. In the present study, it was concluded that out of 157 *A. baumannii* complex isolates, 3 were OPD and 154 were IPD patients. The current study coincides with the study of Leung *et al***.** in 2019, who found that out of 284 *A. baumannii* complex isolates, 8 had OPD and 276 had IPD [21].

The current investigation identified Acinetobacter isolates most frequently from respiratory tract intubated patients (ET samples: 47%), followed by blood (25%) sputum (10%), and body fluids (6.36%). This finding is consistent with the results reported in previous studies by Markogiannakis et al., Chim et al., and Alvarez-Lerma et al. [22-24]. As determined by the present study, A. baumannii complex can affect individuals of any age; however, the age group >50 years exhibited the highest incidence, followed by 41-50 years and 61-70 years. An analogous result was documented by Guckan et al. (2015), who discovered that the majority of cases involving A. baumannii complex were observed in individuals aged 50 years and older [25].

The findings of the present study revealed that males exhibited a higher prevalence of A. baumannii complexes (85%) in comparison to females (13%). These findings were strikingly comparable to those of Alamghrabi et al. [26]. These authors identified 88.02 percent males and 11.08 percent females among clinically suspected cases in 2018 [26]. Ferdous et al. (2017) reported comparable results, indicating that a greater proportion of clinically suspected cases (79.04%) occurred in males than in females (20.96%) [27]. An additional study conducted in India documented 33% resistance to carbapenems, while a study conducted in Korea found 55.8% resistance [28-29]. A comparable level of resistance to carbapenems was observed among Acinetobacter spp. isolates at the Aga Khan University Hospital in Karachi [30].

A study conducted in Norway identified A. baumannii in approximately 9% of the isolates, of which 95.6% were resistant to gentamicin, ciprofloxacin, nalidixic acid, trimethoprim/sulfamethoxazole, and trimethoprim/sulfamethoxazole, and intermediately susceptible to amikacin [31]. These findings corroborate the results reported in the present study. An additional investigation conducted in Saudi Arabia unveiled that *A. baumannii* isolates exhibited substantial resistance to the following antibiotics: cefotaxime (75%), ticarcillin, ampicillin, and tetracycline (76.4% each), and aztreonam (80.5%) [32]. Only amikacin showed a low rate of resistance compared with other antibiotics (40.3%) [32]. Resistance to antibiotics routinely used treating *A. baumannii* has increased globally, including piperacilin/tazobactam, imipenem, cefoperazone/sulbactam, meropenem, and gentamicin. Additionally, the resistance rate to colistin, which was uncommon in prior years, was 5.5%. This finding indicated that there is potential for a greater proportion of colistin resistance to emerge in the future.

87% of *Acinetobacter spp*. were resistant to third-generation cephalosporins, aminoglycosides, and quinolones, according to another study from India, indicating a high prevalence of MDR [33]. An investigation conducted in the United States also highlights a concerning circumstance: 18% of *A. baumannii* isolates recovered from patients undergoing solid organ transplants have colistin resistance [34]. The susceptibility of this pathogen to antimicrobials differs considerably between regions and centres. Hence, it is imperative to conduct local surveillance studies in order to identify the most appropriate empirical treatment.

To develop effective therapies against *A. baumannii*, it's crucial to understand how different resistance mechanisms interact. The treatment of A. baumannii complex will become more challenging due to the rising antibiotic resistance, unless appropriate measures are implemented and novel medications are developed. To mitigate the dissemination of drug-resistant Acinetobacter strains, it is imperative to implement infection control measures, foster collaboration between doctors and laboratory workers in antibiotic administration, and adhere to hospital hygiene protocols.

Conclusion

Acinetobacter species is an emergent and global nosocomial pathogen. The concerning resistance pattern exhibited by *A. baumannii* in healthcare settings necessitates the implementation of judicious antibiotic usage and effective infection control measures. Additionally, clinical guidance concerning the potential hazards of therapeutic failure is critical.

Author contributions:

M.A., V.P.M., S.K., and R.S. conceived the original draft preparation. M.A., V.P.M., S.K., and R.S. was responsible for the data acquisition**,** collection and assembly of the articles. M.A., V.P.M., S.K., and R.S. was responsible for the conception and design. R.S. was responsible with the supervision of the manuscript.

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