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Clinical Profile of Patients and Antibiogram of *Acinetobacter baumannii* Isolates in a Tertiary Care Hospital, Central India

Prashanth K. Guddeti*, Harshada Shah, Ramanath Karicheri and Luxmi Singh

Department of Microbiology, Index Medical College, Hospital & Research Centre, Malwanchal University, Indore, Madhya Pradesh, India.

Abstract

***Acinetobacter baumannii* (*A. baumannii*) is a remarkable opportunistic pathogen responsible for a great proportion of hospital-associated infections and the high prevalence of resistance towards many classes of antibiotics makes the treatment challenging. The present cross-sectional study was conducted in the Department of Microbiology, IMCHRC, Indore. The study was approved by IEC and conducted from October 2019 to September 2021. A total number of 168 *Acinetobacter* species including 143 *A. baumannii* were isolated from the various clinical specimens, the majority of the isolates were obtained from the respiratory system (66%), followed by urine, pus/wound swab, blood, fluids and other samples. The majority of the patients who had underlying/diagnosed with a disease such as aspiration pneumonia/pneumonia (35%), cerebrovascular accident/haemorrhagic shock (30.7%), respiratory failure (24%), accelerated HTN/HTN(18%), and less common were septicemia (8.4), acute kidney injury/ chronic kidney diseases (7.7%) and trauma/burns (5.5%). The antibiotic susceptibility testing showed higher antibiotic resistance to cefotaxime (94%), ceftazidime (93%), cefepime (92%), imipenem (92%), meropenem (90%) and the resistance was low to doxycycline (39%) Polymyxin B (8%). The association between antibiotic resistance and the clinical profile of patients was found significant (p-value < 0.05). In our study, a remarkably high antibiotic resistance pattern was observed in the classes of antibiotics in *A. baumannii* isolates, mostly MDR and XDR. To address infection caused by antibiotic-resistant *A. baumannii*, appropriate antibiotic administration in a clinical setting is essential. Moreover, local and national surveillance data, stringent infection control, and antimicrobial stewardship are required.**

Keywords: *Acinetobacter baumannii*, Antibiogram, Clinical Profile and MDR

***Correspondence:** prashanth8687@gmail.com

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INTRODUCTION

Acinetobacter baumannii (*A. baumannii*) is a remarkable opportunistic pathogen responsible for a great proportion of hospital-associated infections such as ventilator-associated pneumonia, urinary tract infections, septicemia, endocarditis, meningitis and wound infections.[1] Patients in ICUs who are kept on life support for an extended period usually get *A. baumannii* infections, and treatment failures are common.[2] Endotracheal intubation, intravenous (I.V.) catheters, prostheses, prior antibiotic therapy, and underlying illnesses like aspiration pneumonia/ pneumonia, respiratory failure, cerebrovascular accident, hypertension, diabetes mellitus, acute kidney injury, chronic kidney disease, and cancer are risk factors for *A. baumannii* infections. Due to widespread antibiotic resistance and the persistence of microorganisms in hospital settings, such infections are extremely difficult to treat.[3]

According to the World Health Organization (WHO) published report *A. baumannii* has been identified as a lead pathogen for the development of newer antibiotics and anti-infectives.[4] *A. baumannii* isolates have developed resistance to the majority of antibiotic classes during the past three decades as a result of both acquired and innate resistance mechanisms.[5] The antimicrobial agent *A. baumannii* has developed resistance to a variety of antibiotics from several classes, including aminoglycosides, cephalosporins, fluoroquinolones, carbapenems, tetracyclines and lipopeptides.[6] The main cause of antibiotic resistance is the transmission of resistance genes through the mutation of target genes and plasmids. Given its incredible ability to acquire antibiotic resistance determinants, *A. baumannii* may leave us with few useful therapeutic alternatives.[7]

The initiation of effective empiric treatment is challenging due to the high prevalence of resistance towards many classes of antibiotics. *As a result of its present antibiotic resistance, A. baumannii has become a "superbug" in hospitals, particularly in intensive care units (ICUs).*[8] The last-resort medications polymyxins, such as polymyxin B or colistin and tigecycline, or a combination of one of these classes with a second agent, are available as alternative treatments. There are additional reports of *Acinetobacter* spp. isolates that are resistant to colistin or polymyxin B worldwide. In some situations of infections with pandrug-resistant bacteria, there are no therapeutic choices available because of the rising levels of antibiotic resistance in isolates. *Acinetobacter* spp. are hard to eliminate because they have adapted to persist in hospital settings.[9] In India, studies have reported the antimicrobial resistance pattern and associated with pre-disposing factors in *A. baumannii* infections.[3,6] However limited data are available from India related to antimicrobial resistance pattern of *A. baumannii* and clinical profile of patients infected with *A. baumannii* infections.

The present study was based hypothesis that there is association between the antimicrobial resistance pattern and clinical profile (predisposing factors, underlined diseases and diagnosed diseases). The study was undertaken with the objectives to isolate *A. baumannii* from various clinical samples and evaluate the antibiotic resistance pattern of *A. baumannii* and clinical profile of patients.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Microbiology, Index Medical College, Hospital & Research Centre (IMCHRC), Indore, from October 2019 to September 2021. A total of 168 non-duplicate, consecutive isolates of *Acinetobacter* were obtained from different clinical samples such as blood, urine, pus, wound swab, aspirated fluids, sputum, endotracheal- tube/aspirate/ and BAL, etc., from patients admitted in the hospital and who given written informed consent for the study. All the isolates of *Acinetobacter* were identified by using standard microbiological procedures, including Gram staining, characteristics of the colony on culture media, catalase test, oxidase test and motility.[10] Speciation of *Acinetobacter* (Table 1) was performed based on biochemical tests; urease, citrate, OF glucose, nitrate reduction test, hemolysis, gelatine hydrolysis, growth at 44°C, chloramphenicol sensitivity test and arginine hydrolysis test.[3,11,12]

The disk-diffusion method was used for the antimicrobial susceptibility testing. The

Table 1. Speciation of Acinetobacter species

Acinetobacter species	Phenotypic Tests											
	Catalase	Oxidase	Motility	Urease	Citrate	OF glucose	Arginine decarboxylase	Nitrate reduction test	Gelatine hydrolysis	Haemolysis	Growth at 42°C	Chloramphenicol sensitivity
A. baumannii	+	-	-	V	+	+	+	-	-	-	+	R
A. calcoaceticus	+	-	-	V	+	+	+	-	-	-	-	R
A. lwoffii	+	-	-	V	-	-	-	-	-	-	-	S
A. haemolyticus	+	-	-	-	+	V	+	-	+	+	-	R
A. junii	+	-	-	-	+	-	+	-	-	-	-	R
A. radioresistens	+	-	-	-	-	-	+	-	-	-	-	R

A. baumannii: Acinetobacter baumannii, A. calcoaceticus: Acinetobacter calcoaceticus, A. lwoffii: Acinetobacter lwoffii, A. haemolyticus: Acinetobacter hemolyticus, A. junii: Acinetobacter junii, A. radioresistens: Acinetobacter radioresistens, -: Negative Reaction, +: Positive Reaction, V: Variable Reactions, *S*: Sensitive, R: Resistant, OF: Oxidation-fermentation.

test isolates were inoculated in peptone water broth and incubated at 37°C for 2-3 hours. Then turbidity of inoculated broth was compared with 0.5 McFarland standard. Lawn culture was made by streaking the swab evenly in 3-planes onto the surface of the petri dish containing Mueller-Hinton Agar. The isolates were tested by using the following antibiotic discs (Hi-Media) ampicillin-sulbactam (A/S, 10/10 mcg), piperacillin-tazobactam (PIT, 100/10 mcg), ceftazidime (CAZ, 30 mcg), cefepime (CP, 30 mcg), cefotaxime (CTX, 30 mcg), amikacin (AK, 30 mcg), gentamicin (GEN, 10 mcg), ciprofloxacin (CIP, 5 mcg), levofloxacin (Le, 5 mcg), imipenem (IMP, 10 mcg), meropenem (MRP, 10 mcg), polymyxin B (PB, 300 unit), doxycycline (DO, 30 mcg), tetracycline (TC, 30 mcg) and trimethoprim-sulfamethoxazole (TS, 1.25/23.75 mcg). After the application of antimicrobial discs, the MHA plates were kept for overnight incubation at 37°C in ambient air for 16-18 hours and after overnight incubation, the zone diameters (including the 6mm disc) were measured with a ruler on the under-surface of the petri dish and interpreted as sensitive, intermediate and resistant according to Clinical and Laboratory Standards Institute standards (CLSI 2018) guidelines. Quality Assurance Every culture medium utilized in this investigation underwent sterility and performance testing. Using the *E. coli* ATCC 25922 control strains, the AST's quality was ensured.[13]

Statistical Analysis

Descriptive statistical methods like frequency and percentage distribution and graphical presentation were used for the analysis of categorical variables in the study. The Chi-square test was used to test the association between antibiotic resistance and the clinical profile of patients.

RESULTS

A total number of 168 *Acinetobacter* species, such as 143(85%) *A. baumannii*, 12 (7%) *A. calcoaceticus*, 09 (5%) *A. lwoffii*, 03 (2%) *A. haemolyticus*, 01 (1%) *A. junii* were isolated from various clinical specimens. Among the 143 *A. baumannii*, most of the isolates 95 (66%) were obtained from the respiratory samples, such

as endotracheal tube/secretions, sputum and bronchoalveolar lavage, followed by urine 17 (12%) pus/wound swab14 (10%), blood 10 (7%), fluids 4(3%) and other samples3(2%) (Figure 1). A remarkably higher percentage, 76% (109) of *A. baumannii* isolates were found in ICU compared with general wards24% (34). The average age of the patients was (54.36 ± 16.80) years, *A. baumannii* infection was more common in patients in the age group 61-86 and 41-60 years 55 (38%) and less common in the 19-40 years age group 33 (23%). There was a higher incidence of infection among the males observed at 86 (60%) as compared with females at 57 (40%).

The majority of *A. baumannii* isolates from patients who had underlying/diagnosed diseases such as aspiration pneumonia/ pneumonia 45 (35%), cerebrovascular accident/ haemorrhagic shock 44 (30.7%), respiratory failure 35 (24%), accelerated HTN/HTN 26 (18%), and less common were septicemia 12 (8.4), acute kidney injury/chronic kidney diseases 11 (7.7%) and trauma/burns 8 (5.5%). The most common pre-disposing factors observed in patients with mechanical ventilation/endotracheal tube 83 (58%), urinary catheter 76 (53%), extended hospital stay (> 7 days) 70 (49%), and less common factors were diabetes mellitus 26 (18%), surgical site infections and COPD 14 (9.7%), central venous catheter and cancer 03 (2%) (Figure 2).

The antibiotic susceptibility testing showed higher antibiotic resistance to cefotaxime 135 (94%), ceftazidime 133 (93%), cefepime 132 (92%), imipenem 131 (92%), meropenem 130 (90%), amikacin 126 (88%), ciprofloxacin 126 (88%), trimethoprim-sulfamethoxazole 126 (88%), gentamicin 125 (87%), levofloxacin 125 (87%), piperacillin-tazobactam 117 (81%), ampicillin-sulbactam 105 (73%), tetracycline 11 (64%) (applied to urine samples only) and the resistance was low to doxycycline 56 (39%) Polymyxin B 11 (8%) (Figure 3). Of 143 *A. baumannii* isolates, 123 (86%) were multidrug-resistant (MDR) and 122 (85%) extensively resistant (XDR); the isolates resistant to at least one agent in three or more antimicrobial categories- penicillin's, cephalosporins, aminoglycosides, β-lactam combination, fluoroquinolones, and carbapenems.

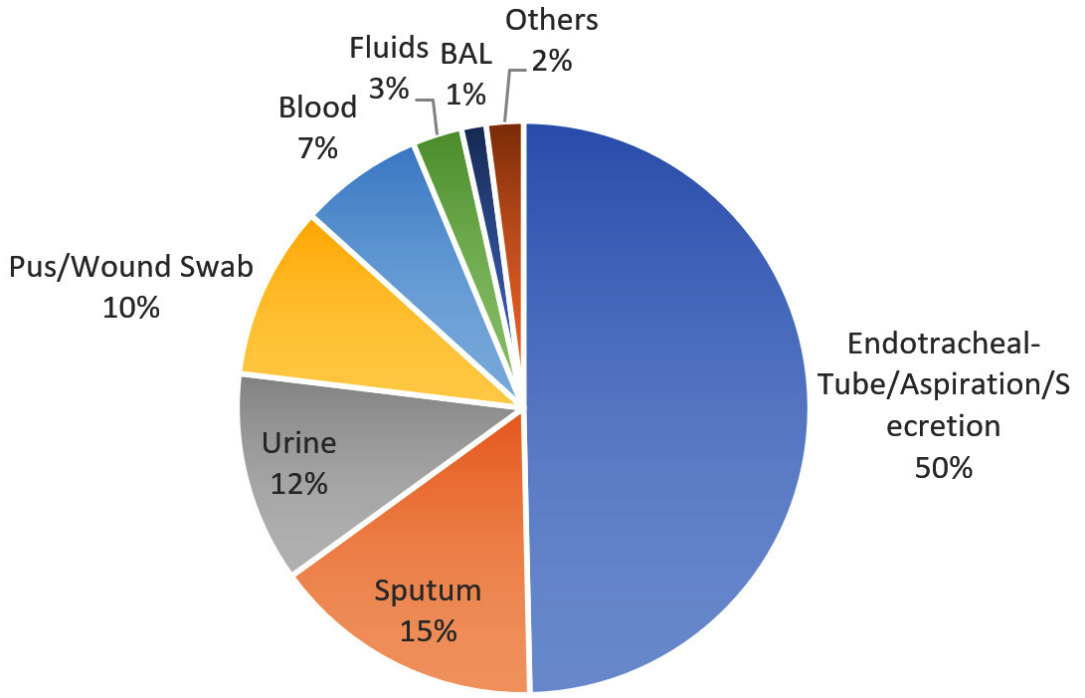

Figure 1. Frequency Distribution of *A. baumannii* in Clinical Samples (n=143)

Table 2. Association of Clinical Profile of patients and Antibiotic-resistance in *A. baumannii* isolates

Hospital associated infections	Clinical Profile of patients	*A. baumannii* n=143 (%)	β-lactam combin-ations	Cephalo-sporins	Aminogly-cosides	Fluoroqu-inolones	Carba-penems	Lipo-peptides	Tetra-cyclines	Folate pathway antagonists
Ventilator associated pneumonia	Aspiration pneumonia/ pneumonia	45(31)	38	42	37	39	40	5	18	38
		p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001
	COPD	14 (9.7)	10	12	12	12	12	1	7	11
		p-value	0.090	0.006	0.006	0.006	0.006	0.001	1	0.029
	Respiratory	35 (24)	32	35	35	35	35	1	13	34
	Failure	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001
	Mechanical	83 (58)	77	82	80	80	81	7	39	78
	Ventilation	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.745	<0.001
Septicemia	CVA/Hemor-rhagic Shock	44 (30.7)	42	44	44	44	44	3	21	43
		p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001
	Septicemia	12 (8.4)	10	12	9	9	11	0	2	9
		p-value	0.039	<0.001	0.146	0.146	0.006	0.000	0.039	0.146
	Central line	03 (2.0)	3	3	3	3	3	0	0	3
		p-value	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
	Accelared	26 (18)	22	24	24	23	23	5	14	24
	HTN/HTN	p-value	0.001	<0.001	<0.001	<0.001	<0.001	0.002	-	<0.001
UTI/CAUTI	CKD/AKI	11 (7.7)	7	9	8	9	9	3	2	9
		p-value	0.549	0.065	0.227	0.065	0.065	0.227	0.065	0.065
	Catheterization	76 (53)	66	73	70	71	73	7	43	69
		p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Surgical Site Infection	Post-surgical/SSI	14 (9.7)	9	12	13	12	12	2	7	11
		p-value	0.212	0.006	0.001	0.006	0.006	0.006	1	0.029
	Trauma/Burns	8 (5.5)	7	8	7	8	7	0	3	7
		p-value	0.070	<0.001	0.070	<0.001	0.070	<0.001	0.727	0.070
	Diabetes	26 (18)	23	24	25	24	26	3	12	24
	mellitus	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Prosthesis	05 (3.5)	4	5	5	5	5	0	1	5
		p-value	0.187	0.031	0.031	0.031	0.031	0.031	0	0.031
Others	Extended	70 (49)	59	65	63	64	65	8	29	61
	Hospital stay (> 7 days)	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0	<0.001
	Cancer	03 (2.0)	2	3	3	2	2	0	2	2
		p-value	1.000	0.250	0.250	1.000	1.000	0.250	1	1.000

(If p-value < 0.05 is significant by Chi–Square Test)

DISCUSSION

A. baumannii has become an important hospital pathogen in recent years. It is notorious for developing antibiotic resistance to most widely administered antimicrobials and has alarmingly high rates of AMR.[4] The present study was conducted to evaluate the antibacterial resistance pattern and clinical profile of patients. Out of 143 isolates of *A. baumannii,* a significantly higher percentage (76%) of *A. baumannii* isolates were found in ICU as compared with general wards (24%) which is similar to other studies.[14,15]A study from Pune which is showed quite contrasting results such as 62% and 38% ICU and wards respectively.[12]

Our study results showed that in ICU the majority of patients acquired aspiration pneumonia/pneumonia usually which is after hospital admission and was attributed to the

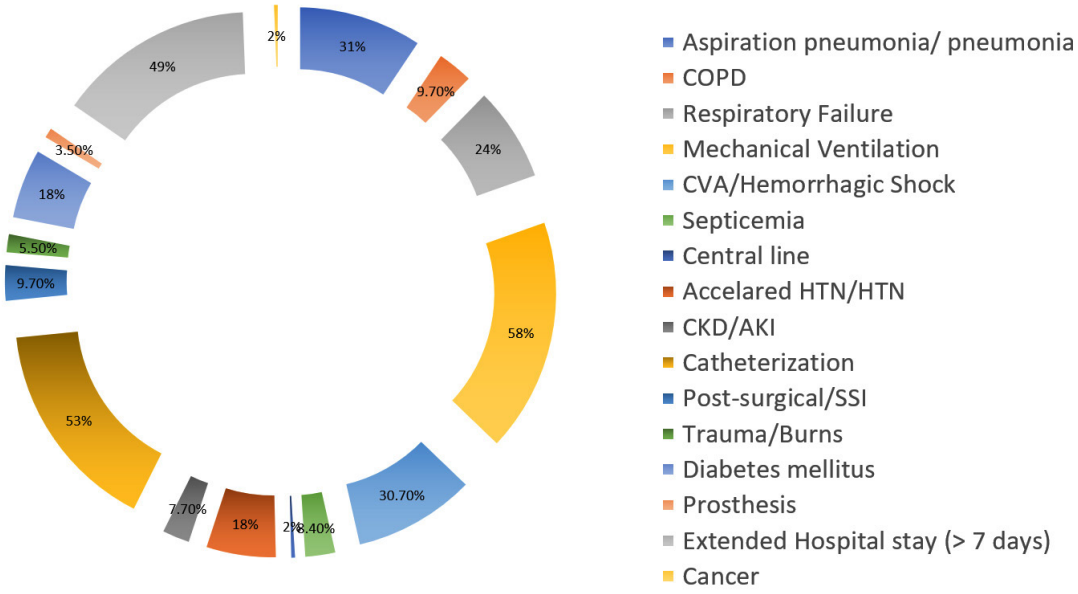

Figure 2. Distribution of *A. baumannii* isolates with Clinical Profile of patients (n=143)

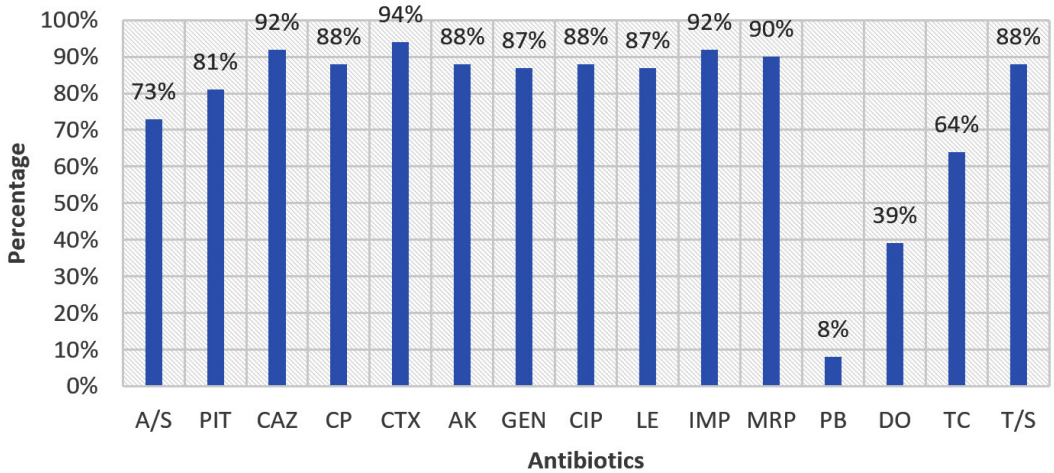

Figure 3. Antibiotic resistance patterns in *A. baumannii* (n=143)

use of mechanical ventilation. Few studies from Maharashtra, India and Turkey, have also reported similar findings.[3,16] Patients with cerebrovascular accident/hemorrhagicshock were 30.7% observed in our study which is consistent with other studies conducted by Konca C *et al.* and Ryu *et al.*[16,17] In the present study, respiratory failure was documented in 24% patients, whereas in other studies reported 16.3% and 3.2% respectively Ryu *et al.* and Mathai A.S. *et al.*, which is not following with present study.[17,18] In our observation 18% of patients were suffering from hypertension and septicemia (8.4%) while, acute kidney injury, chronic kidney diseases (7.7%), trauma or burns (5.5%)were evident.

The incidence of *A. baumannii* isolates was more common in the patients who had catheterization (53%) in our study, almost analogous to the study by Tripathi *et al.*[3] We observed extended hospital stay in 49% of patients in our study which is not matching with the study reported by Tripathi *et al.*[3] The previously published studies have accounted for the usage of central-line in 21.5% and 51% of the patients but our results showed less percentage (2%) of usage.[3,17] One of the recent studies showed surgical site infections 37.5% which is higher as compared with our result (9.7%).[3] The remaining findings like prosthesis, COPD, cancer and diabetes mellitus of our study were consistent with previously published results.[3,17] the majority of the *A. baumannii* isolates were obtained from the respiratory samples (66%), which is parallel to the results of studies previously conducted,[1,9,16] but as compared with other studies this sample size is higher.[19, 20, 21]In previously published results reported that 37% of *A. baumannii* isolates were from blood samples, but in our study, a smaller number of isolates were from blood.[12,16,21] Few studies conducted in Iran and Karnakata reported 46% and 30%, respectively, of *A. baumannii* isolates from pus/wound samples which were similar to other studies also with slight variation in the number, but in our study very less number (14) of isolates from pus/wound samples.[20,22] Next to this 12% of the samples was from UTIs, and the remaining 3% and 2% were body fluids and others, respectively. These results were consistent with other studies.[12,20,21]

The high proportions of resistance patterns to 3rd and 4th generation cephalosporins such as cefotaxime 94%, ceftazidime 93%, and cefepime 92%, the results following recent studies conducted by Banerjee T *et al.*, Raut *et al*. and Rajkumari *et al.*[15,23,24] This study showed a relative abundance of carbapenems resistance; imipenem 92%, and meropenem 90% which is consistent with the previous study conducted in Iran but our results were highest than previous studies.[15,23] The increasing frequency of resistance patterns of aminoglycosides showed amikacin at 88%, and gentamicin at 87%, these findings concord with previous studies conducted in Varanasi and Iran.[15,23]Trends of resistance patterns fluoroquinolones; ciprofloxacin 88%, levofloxacin 87%, penicillins/β-lactamase inhibitors 77%, folate pathway inhibitors 88% and tetracycline 64%.[15,24] There were little variations in antibiotic resistance patterns rate when compared with other studies conducted by Khoshnood *et al.*, M. Moosavian *et al.* and Pattanaik A. *et al.*[1,20, 22]

The trends of resistance patterns of doxycycline 39% Polymyxin B 8% were observed low which is in concord with recent studies and observed raised compared with previous studies.[1, 4,14,24]*A. baumannii* have a natural MDR phenotype and high proportions of MDR and XDR isolates were found in our study, and increasing and high proportions of MDR and XDR are also been reported globally.[9] The association between antibiotic resistance and the clinical profile of patients was found significant as shown in Table 2.

CONCLUSION

In our study, remarkably high antibiotic resistance to classes of antibiotics was observed in *A. baumannii* isolates, especially MDR and XDR strains. The emergence of MDR and XDR *A. baumannii* is a serious global threat to public health. Currently, there is no effective drug to fight against multidrug resistance isolates, apart from polymyxins. To address the infections caused by antibiotic-resistant *A. baumannii*, appropriate antibiotic administration in a clinical setting is essential. Moreover, local and national surveillance data, stringent infection control, and antimicrobial stewardship are required. Molecular methods have been developed for the accurate identification of *Acinetobacter* species. The speed, accuracy, ability to detect outbreaks, and

interpretation of changing trends in technology of molecular methods are enabling further scope of research.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

HS and RK conceptualized the study. HS, RK and PKG designed the study. PKG and LS performed the experiments and wrote the manuscript. HS and RK guided and revised the manuscript. All authors read and approved the final manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This study was approved by the ethics committee of Index Medical College, Hospital & Research Centre affiliated with Malwanchal University, Indore (MP) (MU/Research/EC/Ph.D/2019/44(a)).

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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