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ORIGINAL RESEARCH

Molecular detection of *acinetobacter baumannii* in the nosocomial infections of intensive care units with special emphasis on clinical types and antimicrobial resistance pattern in a tertiary care hospital

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ABSTRACT

Introduction - For more than a century, nosocomial infections, often known as hospital-acquired illnesses (HAI), have been acknowledged as a serious issue. Non-fermentative gram negative bacteria (NFGNB) have been linked to human illness more and more in recent years. A. baumannii is the most commonly encountered NFGNB, and it is frequently linked to high morbidity and mortality, including infections like meningitis, respiratory tract infections, urinary tract infections, bacteremia, and skin and soft tissue infections, particularly in patients with severe device-associated illness. **Method-** From June 2022 to December 2024, this study was carried out at the Index Medical College Hospital & Research Centre in Indore, Madhya Pradesh. The study included non-repeating samples taken from various intensive care unit patients, which were then forwarded to the microbiology lab for sensitivity testing and culture identification. Included were all patients who had been in intensive care units for more than 48 hours. The study excluded patients with less than 48 hours and those not in the intensive care unit. Every sample underwent microbiological processing. In accordance with CLSI standards 2020, bacterial identification and antibiotic resistance patterns were carried out. PCR was also used to validate the presence of the intrinsic blaOXA-51like gene in A. baumannii. **Result -** Twenty-eight *Acinetobacter baumannii* were identified from 200 patients in this prospective research. A. baumannii infections were discovered in 14% of cases (28 acquired infections out of 200) based on microbiological and clinical correlation. 18 (64.2%) respiratory samples had the highest percentage of isolates, followed by blood 7 (25%), urine 2 (7.1%), and pus 1 (3%) samples. The majority of the antimicrobial drugs did not affect the isolates at all. According to assessments of the isolates' antibiotic susceptibility, penicillin and cephalosporin resistance were the most prevalent, followed by gentamicin resistance (83%), and imipenem resistance (79%). Every Acinetobacter that was isolated was vulnerable to polymyxins and tigecycline.It was determined that all 28 isolates of A. baumannii carried the blaoxa-51-like gene. **Discussion-** The current investigation highlights *Acinetobacter baumannii's* increasing clinical importance as a leading nosocomial infection pathogen, especially in intensive care units. When it came to correctly identifying A.baumannii in a variety of clinical specimens, such as respiratory, bloodstream, and wound infections, molecular detection techniques proved to be successful. The presence of A. baumannii was found to be strongly associated with multidrug resistance, particularly to carbapenems and cephalosporins, which presents significant treatment problems.

Key words: Nosocomial infection, *Acinetobacter baumanii*, blaoxa-51-like gene, MDR

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INTRODUCTION

Nosocomial infections are those that develop in a hospital or health care facility and manifest 48 hours or more after hospitalisation, or within 30 days after the patient's discharge [1]. Health care-associated infections (HAIs) are the most common complications affecting hospitalized patients. In India it is estimated that 10-30% of admitted patients acquire a HAI. Device associate hospital acquired infections are serious cause of concern for hospitals [2].

Most hospital infection outbreaks are most likely to be caused by this behaviour. This opportunistic pathogen is known to cause nosocomial pneumonia, bacteremia, urinary tract infections, and infections of the skin and soft tissues, particularly in individuals with serious medical conditions [3,4]

Gram positive bacteria like Staphylococcus spp., Streptococcus, Enterococcus spp., and yeast like Candida spp., as well as gram negative bacilli like Escherichia coli and Klebsiella spp., were responsible for major nosocomial infections observed in the intensive care unit. The pathogenic potential of non fermentative gram negative bacilli has been proven beyond a reasonable doubt due to their frequent isolation from clinical specimens and their correlation with the disease [4]. *A. baumannii* is the most clinically significant due to its high resistance to antibiotics and association with hospital-acquired infections, such as pneumonia, bloodstream infections, and wound infections. Other species in the *Acinetobacter complex* may also cause infections but tend to be less virulent and resistant.

Acinetobacter baumannii is distinguished from other *Acinetobacter complex* species by glucose oxidation**,** growth at 44°C, and the presence of the blaOXA-51-like gene**.**

Acinetobacter baumannii isolates are often resistant to high concentrations of antimicrobial drugs because of both intrinsic and acquired mechanisms, such as increased production of multidrug resistance (MDR) efflux pump proteins [5].

The current study was founded on the concept that the clinical profile (predisposing variables, highlighted diseases, and diagnosed diseases) and the pattern of antibiotic resistance are related. The study's goals were to separate A. baumannii from different clinical samples and assess the bacteria's pattern of antibiotic resistance as well as the clinical characteristics of the patients.

MATERIAL AND METHODS

The current prospective cross-sectional study was carried out from June 2022 to December 2024 at the Department of Microbiology at Index Medical College, Hospital & Research Centre (IMCHRC), Indore. Twenty-eight isolates of A.baumannii were isolated from various clinical samples from hospitalised patients, including sputum, endotracheal tubes, aspirated fluids, blood, urine, wound swabs, pus. All the isolates of A.baumannii were identified by using standard microbiological procedures. Antimicrobial susceptibility testing (AST) on Muller-Hinton agar plates was conducted using the Kirby Bauer disk-diffusion method using the following antibiotic discs (Hi Media, Mumbai, India). All of the isolates of A.baumannii were tested, and the results were interpreted in accordance with the Clinical and Laboratory Standards Institute standards (CLSI 2020). These included ampicillin-sulbactam (A/S, 10/10 mcg), ceftazidime (CAZ, 30 mcg), cefotaxime (CTX, 30 mcg), cefepime (CP, 30 mcg), gentamicin (GEN, 10 mcg), amikacin (AK, 30 mcg), levofloxacin (Le, 5 mcg), ciprofloxacin (CIP, 5 mcg), meropenem (MRP, 10 mcg), imipenem (IMP, 10 mcg), polymyxin B (PB, 300 unit), tetracycline (TC, 30 mcg), doxycycline (DO, 30 mcg), piperacillin-tazobactam (PIT, 100/10 mcg), and trimethoprim-sulfamethoxazole (TS, 1.25/23.75 mcg). Focus on the analysis of the carbapenem resistant genome, including class D blaoxa51 by molecular methods.

RESULT

In this investigation, 200 samples were taken from patients who were admitted to various intensive care units and processed microbiologically. 78 (39%) of the 200 clinical test samples were isolated microorganisms. Of these, 58 (79.45%) were gram negative bacilli and nineteen (17.33%) were gram positive budding yeast cells, two (2.7%) were gram positive cocci, and There was mention of the distribution of various clinical samples.

Table -1 Distribution of clinical samples of test from ICUs

S.N.	Sample	Total sample
1.	Foley’s urine	83
2.	ET.Tube	54
3.	Central line (+ Blood)	19
4.	Blood	18
5.	Suction tip	10
6.	ET aspirate	6
7.	Pus	5
8.	Sputum	3
9.	Tracheotomy tip	2
	Total	**200**

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DEMOGRAPHIC DETAILS OF PATIENTS ADMITTED TO ICUs

The age distribution of patients admitted to the intensive care unit is displayed in Table 2.1. Critically sick people older than 60 years were admitted to intensive care units. 54.59±18.71 was the average age distribution

Table .2.1: Age wise distribution of patients admitted to ICU

S.N.	Age	No. of Patients
1.	**<20**	15(7.5%)
2.	**21-40**	32(16%)
3.	**41-60**	73 (36.5%)
4.	**≥61**	80 (40 %)
Mean		50 ±15.61

Out of the 200 cases, 128 were male, and 72 were female. The ratio was 1.7:1 male to female. Table 2.2

Table 2.2: Gender wise distribution of patients admitted to ICU

S.N.	Gender	No. of Patients	Percent
1.	Male	128	64%
2.	Female	72	36%
Total		200	100%

Table 2.3: Distribution of *A. baumannii* from different clinical sample

S.N.	Sample	Test	
		Total sample	**Total Acinetobacter isolate**
1.	ET.Tube	54	11(39.28%)
2.	ET aspirate	6	3(10.7%)
3.	Central line tip (+ Blood)	19	5(17,8%)
4.	Suction tip	10	4(14,2%)
5.	Blood	18	2(7,1%)
6.	Foley'sUrine	83	2(7,1%)
7.	Pus	5	1(3,5%)
8.	Sputum	3	0
9.	Tracheastomy tip	2	0
	Total	**200**	**28**

DEMOGRAPHIC DETAILS OF PATIENTS WITH *A.BAUMANNII* INFECTION/COLONIZER

The age distribution of patients infected or colonised with A. baumannii was displayed . A. baumannii was identified from 46.4% of patients in this study who were between the ages of 40 and 60. Between 44.7747 to 58.8653 is the population's mean age.

Table 3.1: Age wise distribution of patients with *A.baumannii* infection /colonization

S.N.	Age	No. of Patients with A. baumannii infection/colonization
1.	<20	4 (14.2%)
2.	21-40	2 (7.14%)
3.	40-60	13(46,4%)
4.	<61	09 (32.14%)
Total		28
Mean±SD		+51.82 _20.96

Table 3.2: Gender wise distribution of patients with *A.baumannii* infection/colonization

	Gender	No. of Patients
1.	Male	21 (75%)
2.	female	7(25%)

Table 3.3: Distribution of *A. baumannii* associated with infection and colonization

Infection/colonizer	No. of *A. baumannii*
Associated with infection	17
Associated with colonizer	11

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CLINICAL MANIFESTATIONS

Four nosocomial infections were the primary cause of A. baumannii in our investigation. wound infections, urinary tract infections linked to catheter use, nosocomial bacteremia, and ventilator-associated infections **[Table 3.4]**.

Table 3.4. Infections associated with *A.baumannii*

S.N.	Infections	No. of *A.baumannii* n=17
1.	Ventilator associated Infections	10
2.	Nosocomial bacteremia	5
3.	Catheter associated urinary tract infection	1
4.	Wound infection	1

Genotypic Identification of *A.baumanii*

There were 28 isolated of *A.baumannii* also gave the positive result in PCR with OXA 51 primer. Genotyping of *A.baumannii isolates***.** Presence of a 350 bp band indicates *bla*$_{oxa\ 51}$.These results were confirmed by DNA sequencing. *blaOXA 51* gene sequence showed similarity (99%) with known sequence of NCBI.

Table 3.5 displays the antimicrobial resistance pattern of the isolated A. baumannii. The only drugs that shown 100% susceptibility were tigecycline and polymyxins.

Table 3.5 Antibiotic resistance pattern of isolated *A.baumannii* (Infection/Colonizer)

S.N.	Antibiotics	Antibiotics	No. of Strains	Percent
1.	Piperacillin(100µg)	PI	28	100.0
2.	Ampicillin/Sulbactum(10/10µg)	A/S	28	100.0
3.	Ticarcillin(75µg)	TC	28	100.0
4.	Piperacillin/Tazobactum(100/10µg)	PIT	23	83
5.	Cefotaxime(30µg)	CTX	28	100.0
6.	Ceftriaxone(30µg)	CTR	28	100.0
7.	Ceftazidime(30µg)	CAZ	28	100.0
8.	Cefepime(30µg)	CPM	28	100.0
9.	Cefepime/Tazobactum(30/10 µg)	CPT	26	96
10.	Cefoperazone/Sulbactam	CFS	24	86
11.	Aztreonem	AT	28	100
12.	Amikacin(30µg)	AK	22	79.4
13.	Gentamycin(10µg)	GEN	23	83
14.	Tobramycin(10µg)	TOB	26	96
15.	Netilimicin	NET	26	95
16.	Ciprofloxacin(5µg)	CIP	24	85
17.	Ofloxacin(1 µg)	OF	25	85.3
18.	Levofloxacin(5µg)	LE	22	82.4
19.	Tetracycline(30µg)	*TE*	25	85.3
20.	Cotrimoxazole(1.25/23.75µg)	*COT*	26	96
21.	Imipenem(10µg)	IMP	22	79.4
22.	Meropenem(10µg)	MRP	22	79.4
23.	Polymyxin B	PB	0	0.0
24.	Colistin	CL	0	0
25.	Tigecycline	TGC	0	0.0

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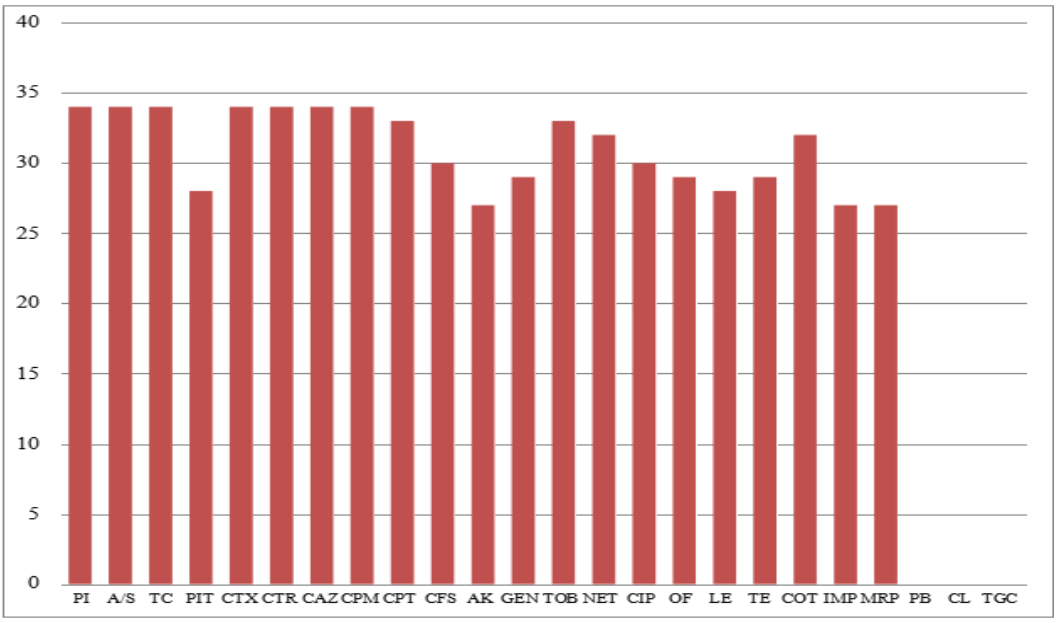

Antibiotic resistance pattern of isolated A.baumannii (Infection/Colonizer)

DISCUSSION

From June 2022 to December 2024, 200 test cases from Indore's tertiary care facilities were examined for this study. A total of 200 samples were taken from patients who were admitted to various intensive care units and processed microbiologically.

Out of the strains of A. baumannii, only 17 exhibited clinical signs and symptoms that are considered indicative of infection, whereas the remaining 11 did not exhibit any of these symptoms that are considered in colonizer. Only 17 strains of A. baumannii were linked to infection out of the 28 strains that were isolated in this investigation, according to microbiological and clinical analysis. A. baumannii colonised the remaining 11. Ten percent of people in the Indore region had an A. baumannii infection. 8.5 out of every 1000 intensive care unit days had a rate of Acinetobacter infection.. In a comparable study, Omer et al. found that 9.5% of the participants had Acinetobacter infections [6]. In Rajasthan, according to certain Indian studies, A. baumannii was the most prevalent nosocomial infection, particularly in intensive care units, with an 83.2% prevalence [7]. Similarly, Acinetobacter was the most prevalent ICU isolate in Pune [8]. The current study is lower than previous studies that were published in Pakistan, Japan, and Kuwait, which revealed Acinetobacter levels of 24.85%, 18%, and 22.1%, respectively. [9,10,11]. Variations in research populations and localities, resource availability, overcrowding or a lack of nurseries, antibiotic use, and monitoring techniques for nosocomial infection identification can all be blamed for the discrepancies across the studies. [12].

28 A.baumannii were identified based on their phenotype. The molecular approach was also used for bacterial confirmation. The presence of a gene similar to blaOXA-51 verified the existence of every isolated A. baumannii. Numerous variations of blaOXA have been identified. According to Turton JF et al., all strains of A. baumannii have blaOXA-51like genes, however some are linked to ISAba1..[13,14]. All 28 Acinetobacter in the current investigation have blaOXA-51-like genes. Additionally, earlier research found that every A. baumannii had a blaOXA-51like gene. [15,16]. Thus, it is possible to identify A. baumannii phenotypically. If Acinetobacter spp. were accurately identified down to the species level, the true clinical relevance of these organisms may be better recognised. [17].

A. baumannii was commonly isolated from respiratory samples, such as endotracheal tips (11 out of 54) and secretions (3 out of 6), and 28 isolates were identified from a variety of clinical samples. Urine (21-27%) and tracheobronchial secretions (24.8-48.8%) have been found to contain the majority of Acinetobacter isolates in a number of studies. [18][19]

CONCLUSION

The current investigation highlights Acinetobacter baumannii's increasing clinical importance as a leading nosocomial infection pathogen, especially in intensive care units.. The presence of A. baumannii was found to be strongly associated with multidrug resistance, particularly to carbapenems and cephalosporins, which presents significant treatment problems. Furthermore, tertiary care hospitals can improve patient care results and design tailored therapeutic strategies with the help of an understanding of the clinical spectrum and resistance profiles.

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