

## CARBAPENEM RESISTANCE AND BIOFILM PRODUCTION IN CLINICAL ISOLATES OF ACINETOBACTER BAUMANNII IN A TERTIARY CARE HOSPITAL, CENTRAL INDIA

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### Abstract

**Background:** Carbapenems is the last-resort antibiotic to treat *A. baumannii* infections. The emergence of carbapenem resistance is extremely alarming and biofilm production capability plays an important role in the survival of *A. baumannii* and its persistence in hospital settings. **Materials and Methods:** 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. **Result:** Out of 143 *A. baumannii* isolates, 92% of carbapenem resistant *A. baumannii* (CRAB) isolates were found in various clinical specimens. the majority of isolates were obtained from endotracheal tube/secretions (53%) followed by sputum and bronchoalveolar lavage (16%), urine (11%) pus/wound swab (9%), blood (8%), fluids and others (3%). A significantly higher percentage 77% of *A. baumannii* isolates were found in ICU compared with general wards 23%. Carbapenem-resistant *A. baumannii* (111) isolates were further tested for detection biofilm production, 44% of CRAB isolates were strong positive, 32% moderate positive and 8% weak positive, while 16% were negative. The association between carbapenem resistance and biofilm production was analyzed statistically and the p-value was found to be significant (0.002). **Conclusion:** In our study, we found a remarkable increase in carbapenem resistance and biofilm production in clinical isolates of *A. baumannii* and a significant association between biofilm production and carbapenem resistance.

## INTRODUCTION

*Acinetobacter baumannii* (*A. baumannii*) has been identified as a "critical priority" organism for the research and development of novel antibiotics and anti-infectives, according to a report published by World Health Organization (WHO).<sup>[1,2]</sup> *A. baumannii* causes a wide variety of infections, mostly acquired in clinical settings, and is commonly associated with elevated rates of morbidity and mortality (26–60%).<sup>[3-5]</sup> Antimicrobial resistance (AMR) is considered to be a silent pandemic, many outbreaks have been reported globally and the mortality rate for *A. baumannii* infections caused by multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *A. baumannii* is significant.<sup>[3]</sup> The rise of MDR and XDR strains of *A. baumannii* has been a serious concern for healthcare practitioners globally since

the early 2000s, with an alarmingly low number of antibiotics still suitable for their treatment.<sup>[6]</sup> The WHO has classified MDR *A. baumannii* as a priority 1 pathogen among antibiotic-resistant bacteria because of its serious effects on public health.<sup>[7]</sup> Carbapenems is the last-resort antibiotic to treat MDR *A. baumannii* infections. The emergence of carbapenem resistance is extremely alarming. Several studies had reported a range of 40–75 percent carbapenem resistance in *A. baumannii* across India.<sup>[8,9]</sup>

*A. baumannii* infections are common in ICU patients, due to patients on life support systems, prolonging their hospital stay, and treatment failures are frequently encountered.<sup>[3]</sup> Furthermore, *A. baumannii* infections in critical care units may be related to a lack of environmental surface cleanliness as well as the continuous use of medical devices such as endotracheal tubes and urinary and

intravascular catheters, which make patients more vulnerable to intensive handling by healthcare workers.<sup>[10]</sup> Biofilm production is a well-known pathogenic mechanism in device related infections in hospitals. The ability of *A. baumannii* to produce biofilm on abiotic surfaces may facilitate or enhances its survival and persistence in hospital environments, which in turn contributes to the extensive spread of *A. baumannii* infection throughout the world.<sup>[7,11]</sup>

The relationship between antibiotic resistance and biofilm-production capacity has drawn the curiosity of medical researchers, who believe that these two features might considerably influence infection outcomes.<sup>[12]</sup> As a result, the present study was conducted on *A. baumannii* clinical isolates to explore the frequency of the production of biofilm and its association with carbapenem resistance in *A. baumannii*.

## 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 132 isolates of carbapenem-resistant *A. baumannii* (CRAB) were obtained from different clinical samples such as sputum, endotracheal-tube/aspirate/ and BAL, urine, blood, wound swab, pus, aspirated fluids and others, from patients admitted in the hospital. All the isolates of *A. baumannii* were identified by using standard microbiological procedures.<sup>[13,14]</sup>

The Kirby Bauer disk-diffusion method was used for the antimicrobial susceptibility testing (AST) on Muller-Hinton agar plate by using following antibiotic discs (Hi Media, Mumbai, India) 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), all the isolates of *A. baumannii* were tested and the results were interpreted as per

Clinical and Laboratory Standards Institute standards (CLSI 2018). The AST's quality control was ensured by *E. coli* ATCC 25922 strain.<sup>[15]</sup>

### Biofilm Detection

Biofilm production was determined by using microtiter plate method, *A. baumannii* isolates were cultured in Brain Heart Infusion (BHI) Broth with 0.2% glucose and incubated overnight at 37°C. 200 µl of cell suspension made with overnight growth was diluted in a ratio of 1:40 with sterile BHI -0.2% glucose and added to sterile 96-well polystyrene microtiter plates wells and then incubated. After being incubated for 24 hours, the wells were gently cleaned three times with 200 µl of phosphate-buffered saline (PBS), dried inverted, and stained for 15 minutes with 1% crystal violet. To solubilize crystal violet, the wells were washed once more in 200 microliters of an ethanol-acetone solution (80:20 v/v). A microplate reader was used to determine the optical density at 620 nm (OD 620) [Table 1].<sup>[16,17]</sup>

### 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 carbapenem resistance and biofilm production frequency if the p-value < 0.05 is considered significant.

## RESULTS

Out of 143 *A. baumannii* isolates, 132 (92%) carbapenem-resistant *A. baumannii* were found in various clinical specimens. The majority of the isolates were obtained from endotracheal tube/secretions 70 (53%) followed by sputum and bronchoalveolar lavage 21 (16%), urine 15 (11%) pus/wound swab 12 (9%), blood 10 (8%), fluids and others (deep tissue, etc.) 4(3%). The average age of the patients was (54.36 ± 16.80) years, *A. baumannii* infection was more common in patients in the age group years 61-86; 54 (41%), 41-60; 48 (36%) and less common in 19-40; 30 (23%). There was a higher incidence of infection among the males observed at 78 (59%) as compared with females at 54 (41%). A significantly higher percentage 77% (102) of *A. baumannii* isolates were found in ICU compared with general wards 23% (30).

**Table 1: Grading of biofilm production according to microplate reader OD values**

Mean OD values (620nm)	Biofilm production	Results
< 0.275	None	Negative
0.275 - 0.55	Weak	Positive- Weak
0.56- 0.825	Moderate	Positive- Moderate
≥0.826	Strong	Positive- Strong

**Table 2: Distribution of Biofilm producing carbapenem-resistant *A. baumannii* isolates in various clinical samples**

Clinical Sample	Positive for Biofilm production n (%)	Negative for Biofilm production n (%)	n=132 (%)
Endotracheal Tube/ Secretion	62 (88)	8 (12)	70 (53)
Sputum & BAL	15 (71)	6 (29)	21 (16)
Urine	13 (86)	2 (14)	15 (11)

Pus/Wound Swab	10 (83)	2 (17)	12 (9)
Blood	8 (80)	2 (20)	10 (8)
Fluids & Others	3 (75)	1 (25)	4 (3)

**Table 3: Association of Biofilm production and Carbapenem Resistance in A. baumannii**

	Carbapenem Resistance	Carbapenem Sensitive	Chi-Square	p-value
Biofilm production – Positive	111	5	9.89	0.002
Strong	58	3		
Moderate	43	1		
Weak	10	1		
Biofilm production - Negative	21	6		

(If the p-value is < 0.05, show the significant association)

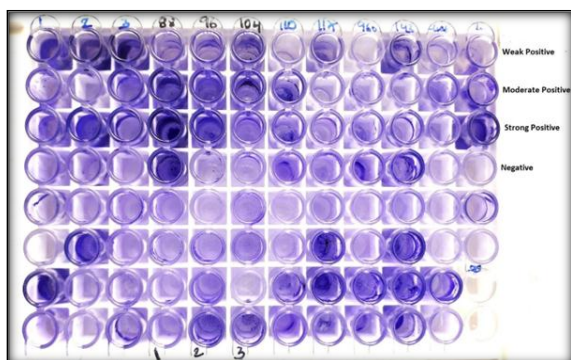
**Table 4: Results of Biofilm Production by Microtiter method documented in previous studies**

S. No.	Study	Country	Year	Strong %	Moderate %	Weak/None %
1	Pattanaik et al. <sup>[25]</sup>	India	2019	63.7	-	36.3
2	Khamari et al. <sup>[28]</sup>	India	2019	71.4	-	28.6
3	Yang CH et al. <sup>[26]</sup>	Taiwan	2019	45.4	32.4	15.6
4	Ranjbar et al. <sup>[23]</sup>	Iran	2019	70.6	12.2	17.2
5	Celik et al. <sup>[29]</sup>	Turkey	2020	90	-	10
6	Shekutie AM et al. <sup>[7]</sup>	China	2020	25	14.4	60.6
7	Asaad et al. <sup>[27]</sup>	Egypt	2021	20.2	34	45.8
8	M.M.Al-Shamiri et al. <sup>[11]</sup>	China	2021	51.4	41.4	4.3
9	Present Study	India	2023	44	32	24

**Table 5: Association of Biofilm production and XDR A. baumannii**

	n (%)	Biofilm formation- Positive	Biofilm formation- Negative	Z value	p-value
XDR	122 (92)	101	21	7.24	<0.001

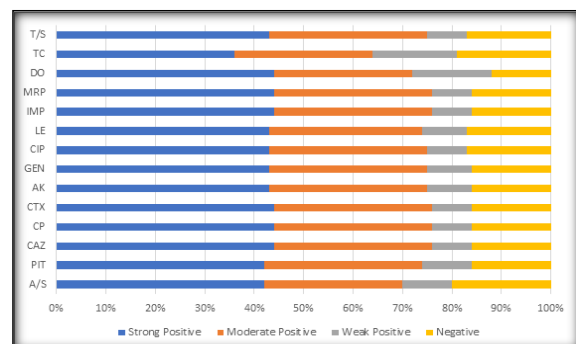
The carbapenem resistant A. baumannii isolates were further tested for biofilm production in this most of biofilm producing CRAB were from endotracheal tube/secretions samples showing 88% (62), followed by 86%, 83%, 80%, 75% and 71% from urine, pus/wound swab, blood, fluids & others and sputum/BAL samples respectively [Table 2]. A greater number of biofilm-producing CRAB isolates was found in ICUs 87 (85%) from that 48% were strong positive, 36% moderately positive and 1% weak positive for biofilm production, and 15% were negative. Twenty-four (80%) CRAB isolates gave positive results for biofilm production, while 6 (20%) were negative from different wards [Figure 1].



**Figure 1: Assay of Biofilm production by Microtiter plate method**

Among the 132 CRAB isolates, one hundred eleven (84%) were positive (44% strong positive, 32% moderate positive and 8% weak positive) for biofilm production, while 16% (21) were negative. The

association between carbapenem resistance and biofilm production was analyzed statistically and the p-value was found to be significant (p-value = 0.002) [Table 3]. The biofilm production association was also observed with other classes of antibiotics, which were showing almost similar to CRAB isolates results, the comparison of the resistance pattern of biofilm-forming A. baumannii isolates with non-biofilm formers was shown in [Fig. 2]. Of 132 CRAB, 122 (92%) were XDR and 10 (8%) were Non-XDR, in this 83 % (101) were XDR A. baumannii positive for biofilm production, while 17% were negative. In Non-XDR A. baumannii shows 100% isolates positive, non-biofilm-producing isolates were not found.



**Figure 2: Antibiotic resistance and Biofilm Production in Carbapenem Resistant A. baumannii**

## DISCUSSION

Carbapenem-resistant A. baumannii has recently spread over the globe. The treatment of A.

baumannii emerged as an unmet medical need, and it is a crucial part of our struggle against these bacteria.<sup>[18]</sup> The present study was conducted to evaluate the carbapenem resistance and association of biofilm production.

Infections with MDR *A. baumannii* were traditionally treated with carbapenems, however recent use of these drugs has increased the prevalence of carbapenem resistance.<sup>[19]</sup> The resistance pattern towards the carbapenems shows 92% in the present study which is exactly correlating results (92%) of a recently conducted study by Khoshnood et al (2023),<sup>[18]</sup> and there was a slightly reduced resistance ranges in the previous study reports 86% Odsbu et al,<sup>[20]</sup> and 74% M. Moosavian et al.<sup>[21]</sup> The resistance patterns of carbapenems were low in previous years as compared with the present.<sup>[2,7,22]</sup> A study conducted by Ranjbar R et al (2019),<sup>[23]</sup> from Iran in clinical isolates of *A. baumannii* recovered from burn wound infections, in that authors reported that 94% of isolates were resistant to imipenem and meropenem, as compared this study our study results related to carbapenem resistance was less.

Due to the high incidence of antimicrobial treatment failure in patients admitted to the ICU wards, the rise in XDR infections greatly concerns medical professionals. In this present study, we found 92% were XDR and 8% were Non-XDR, which is the most similar study conducted in Iran by Zighami et al,<sup>[24]</sup> reported that 91% of *A. baumannii* isolates were XDR, compared to that less XDR 78% were observed by Ranjbar R et al (2019),<sup>[23]</sup> but a recent study conducted by Khoshnood et al,<sup>[18]</sup> it was reported that 26% XDR, indicating different frequencies in various geographical regions of the country and their study, the sample size was relatively small (13/50).<sup>[18]</sup>

Biofilm production is one of the virulence factors of *A. baumannii* associated with prior antibiotic usage; the presence of foreign devices; prolonged hospitalization; residence in an intensive care unit (ICU); high colonization pressure; and prolonged mechanical ventilation.<sup>[3]</sup> Eighty-four percent of carbapenem-resistant *A. baumannii* isolates were positive for biofilm production but other studies reported 62.5% Badave GK et al, 63.7% Pattanaik A. et al and 90% Donadu MG et al.<sup>[2,16,25]</sup> In the present study, the association between carbapenem resistance and biofilm production was analyzed statistically and the p-value was found to be significant (p-value =0.002) There was a significant association found between carbapenem resistance and biofilm production (p-value = 0.002). This was in concordance with studies conducted by Yang CH et al. (2019) and Asaad et al. (2021). The development of biofilms on surfaces reduces the effectiveness of antibiotics and complicates the clinical management of infections [Table 4].<sup>[3,26,27]</sup>

Among all XDR *A. baumannii* isolates, 83% were positive for biofilm production, while 17% were negative. In Non-XDR *A. baumannii* shows 100%

isolates positive, non-biofilm producing isolates were not found [Table 5]. Recently a study was undertaken by Khoshnood et al,<sup>[18]</sup> to determine the biofilm formation among the clinical isolates of *A. baumannii* which were associated with XDR, they found 92% of isolates found biofilm production which is exactly similar to our study results (92%).<sup>[7]</sup> There was a significant association seen between XDR and biofilm formation (p-value <0.001). This was in concordance with studies conducted by Ranjbar et al. (2019)<sup>[23]</sup> and Asaad et al. (2021).<sup>[27]</sup> The development of persister cells, population heterogeneity, antibiotic tolerance, and infections caused by biofilms should all be taken into account as significant risk factors when selecting an effective treatment, particularly in the case of *A. baumannii*. It is disputed what kind of relationship there is between *A. baumannii* biofilm production and antibiotic resistance. According to numerous studies, the production of biofilms by CRAB is stronger than that of resistant bacteria, suggesting a link between the two.<sup>[3,23,27]</sup>

## CONCLUSION

In our study, we found a remarkable increase in carbapenem resistance and biofilm production in clinical isolates of *A. baumannii* and a significant association between biofilm production and carbapenem resistance. The increase of carbapenem resistance in *A. baumannii* is one of the main problems in the treatment of infections caused by *A. baumannii*. Rationale use of antibiotics is important and necessary to prevent microbial resistance catastrophe. To lower the rates of antibiotic resistance, it is advised to strictly regulate the hospital environment, practice good hand hygiene, and utilize antibiotics judiciously and to their maximum potential. Further studies will deepen our comprehension of this organism. Treatment strategies in the future may be strengthened by the clinicians' knowledge gained from treating patients with *A. baumannii* infections.

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