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Biofilm Formation of *AcinetobacterBaumannii* in Clinical Samples from Tertiary Care Hospital

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Extended Abstract

Introduction:*Acinetobacter sp*., particularly *A. baumannii*, is emerging bacterial pathogens that have become prevalent worldwide. *A. baumannii* is the second most common Gram-negative pathogen isolated from clinical samples after *Pseudomonas aeruginosa*. Multidrug-resistant *Acinetobacter baumannii* (MDRAB) is often associated with co-infection by other pathogenic organisms, which can make it challenging to determine its attributable mortality. In addition to antibiotic resistance, biofilm formation is another characteristic that contributes to the survival of *A. baumannii* in the presence of antibiotics and high stresses.*A. baumannii* has become a significant concern for healthcare systems worldwide because of its ability to rapidly develop antibiotic resistance. In recent years, it has been increasingly associated with hospital-acquired infections, especially in intensive care units (ICUs), where patients are at increased risk of developing infections due to their weakened immune system and invasive procedures. MDRAB infections are difficult to treat, and this problem is compounded by the ability of *A. baumannii* to form biofilms. Biofilms are complex communities of microorganisms embedded in a matrix of extracellular polymeric substances (EPS). The EPS matrix provides a protective environment for the microorganisms, making it difficult for antibiotics to penetrate and reach the bacterial cells. This mechanism of protection allows *A. baumannii* to survive in hostile environments and resist the effects of antibiotics and disinfectants.

Background and Objectives: The present study was conducted at Pt. BDS, PGIMS Rohtak, to investigate the prevalence of multidrug-resistant *Acinetobacter baumannii* and its biofilm-forming capacity. The study was conducted for a period of one year from January 2022 to December 2022. The samples were collected from patients admitted to the hospital during this period. The samples were collected from various sources such as blood, urine, wound, and respiratory secretions. The samples were processed using standard microbiological methods to isolate and identify the bacterial strains. The isolates were then subjected to antimicrobial susceptibility testing to determine their resistance patterns.The Modified Tissue Culture Plate Method to assess the biofilm-forming capacity of the multidrug-resistant *Acinetobacter baumannii* isolates was used. This method is a quantitative assay that measures the amount of biofilm formed by the bacterial isolate. The absorbance value is proportional to the amount of biofilm formed. The biofilm-forming capacity of the isolates was categorized as strong, moderate, or weak, based on the absorbance values.

Result: Among 57,137 clinical samples, 1099 samples were identified to contain *Acinetobacter spp*. Upon further identification, it was found that out of the 1099 *Acinetobacter spp*. isolated, 558 of them were *Acinetobacter baumannii*.The study focused on multidrug-resistant (MDR) *Acinetobacter baumannii* isolates, and it was found that out of the 558 *Acinetobacter baumannii* isolated, 201 (36%) were identified as MDR organisms. This is a significant finding as MDR Acinetobacter baumannii is a major public health concern due to its limited treatment options and potential for high morbidity and mortality rates.Additionally, the study assessed the biofilm-forming capabilities of the isolated Acinetobacter baumannii strains. The results showed that 42.7% of the strains were strong biofilm producers, 33.3% were moderate biofilm producers, and 23.8% were weak biofilm producers. This finding is important as biofilm formation is a major virulence factor that contributes to the survival and persistence of *Acinetobacter baumannii* in healthcare settings, leading to an increased risk of infection transmission and antibiotic resistance.

Conclusion:The findings suggest that antimicrobial testing should be done to identify the most effective treatment options for infections caused by *A. baumannii*. Since biofilm formation can protect the bacteria from the effects of antibiotics, it is essential to identify the antimicrobial agents that are most effective against biofilm-producing strains of *A. baumannii*. Overall, the results of this study emphasize the importance of understanding the role of biofilm formation in the persistence and resistance of *A. baumannii* infections. Healthcare providers should be aware of the potential for biofilm formation and consider this factor in the management and treatment of infections caused by this path.

Keywords: AcinetobacterBiofilms, *Acinetobacter baumannii*, MultiDrug Resistance(MDR), Nosocomial, Biofilm production.

INTRODUCTION

The importance of Acinetobacter, opportunistic gram-negative bacteria, in medical settings is growing. It was once thought to be an ambient saprophyte, but it is now recognized as a significant nosocomial infection that primarily affects immunocompromised people.

Acinetobacter species are gram-negative coccobacilli that are short, stout, and have DNA G+C contents that range from 39 to 47 mol% (1). Some of the species in the genus include *A. baumannii, A. calcoaceticus, A. lwoffii, A. hemolyticus, A. johnsonii, and A. junii. A. calcoaceticus* and *A. baumannii* are difficult to differentiate from one another. The *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex causes 80% of clinical diseases caused by Acinetobacter species (2,3).

Many illnesses, including Septicemia, meningitis, pneumonia, urinary tract infections, and super-infections in burn victims, have been linked to Acinetobacter sp. *A. baumannii* that is multidrug resistant (MDR) is not a recent problem, but its antibiotic resistance and capacity to acquire resistance determinants make it a well-known pathogen. Production of -lactamases, reduced expression of outer membrane proteins, and increased expression of efflux pumps all affect antibiotic resistance. Many β-lactam antibiotic resistance mechanisms, such as those produced by Acinetobacter spp. against cephalosporins, carboxypenicillins, and carbapenems, are possible. Extended-spectrum β-lactamase (ESBL) enzymes are a major factor in this resistance. By hydrolyzing the β-lactam ring, ESBLs can hydrolyze broad-spectrum cephalosporins and monobactams (2,3).

Antibiotic resistance is brought on by numerous subtypes of β-lactamases, such as ESBL, AmpC, and carbapenemases. Many antibiotics, including penicillins, cephalosporins, carbapenems, and monobactams, can be impacted by these enzymes. Most of these antibiotics' carbonyl groups are reacted with by MBLs, a kind of class β-lactamase, employing zinc ions. MBLs include enzymes like VIM and IMP, and acquired carbapenemases also include enzymes that aren't MBLs (3).

The therapeutic importance of AmpC β-lactamases, which confer resistance to specific cephalosporins and are unaffected by β-lactamase inhibitors already on the market(4). Carbapenems are usually needed to treat infections brought on by Acinetobacter bacteria since these bacteria frequently develop resistance to other antibiotics. However, over the past ten years there have been more reports of Acinetobacter strains that are resistant to carbapenems. Another way for germs to survive in the presence of antibiotics is by producing biofilms, and *Acinetobacter baumannii* is a well-known culprit for bacterial biofilm discharge pollution of medical equipment (5). The global issue of antibiotic resistance in bacterial illnessesis facilitated by the biofilm development. Antibiotic abuse and errors have contributed to the growth of multi-drug resistant pathogens like *Acinetobacter baumannii*. Due to this bacteria's high level of innate resistance to antibiotics and propensity to pick up antibiotic resistance genes, new illnesses that are challenging to treat have started to appear (6, 7). The text states that because of its clinical importance and capacity to acquire resistance determinants, *Acinetobacter baumannii* is one of the most harmful bacteria in the current antibiotic era. In many nations and locations, surveillance study demonstrates that this bacteria has increased its resistance to imipenem and meropenem by 42.5% and 43.4%, respectively (8).

Antibiotic-resistant *A. baumannii* has become more common overall, with different locations reporting differing levels of resistance. The degree of antimicrobial resistance among Acinetobacter spp. has been described using the words "multidrug-resistant" (MDR), "extensive drug resistant" (XDR), and "pan drug resistant" (PDR). Currently, the most effective medications against drug-resistant *A. baumannii* are tigecycline and polymixins B and E. Production of carbapenemases, increased efflux pump expression, genetic modifications to penicillin binding proteins (PBP) and decreased expression of porins are the causes of resistance to carbapenems. In smaller laboratories, automation in bacterial identification and antibiotic susceptibility testing (AST) is becoming more and more crucial(8).

Acinetobacter is a genus of strictly aerobic, non-motile, catalase-positive, and oxidase-negative gram-negative coccobacilli. It grows well in complex media between 20°C and 30°C without the requirement for growth stimulants. The most typical carbon and energy sources utilised by the majority of Acinetobacter strains are acetate, lactate, or pyruvate. The genus contains 12 DNA groupings, or genospecies, and ten new species, including three with human origins, have been found. The *A. calcoaceticus baumannii* complex, which includes *A. baumannii*, *A. calcoaceticus*, and two additional closely related species, is linked to nosocomial and community-acquired illnesses (9–11).

MATERIALS AND METHODS:

Sample Processing And Identification: This study includes a variety of clinical samples that were received at the microbiology lab, including sputum, bronchial secretions, endotracheal tubes, urine, blood, pus, wound swabs, etc.

Processing of sample: Processing of the sample involved collecting approximately 5 to 10 ml of blood from adults and 2 ml from children under the age of 18. The collected samples were then transferred to brain heart infusion broths of 50 ml and 10 ml, respectively. Blood culture bottles were incubated aerobically at 37 °C for 24 hours. Following the incubation, they were sub-cultured on blood agar and MacConkey agar. Blood culture bottles that did not show any signs of growth (turbidity or hemolysis) were re-incubated for 36, 48 and 72 hours at 37 °C aerobically, and after the seventh day, the results were declared negative (12).

Modified Tissue Culture Plate Method: By inoculating the isolates into brain heart infusion (BHI) broth with 2% sucrose, biofilm generation will be evaluated. The culture will be diluted 1:100 in new BHI broth with sucrose medium after an overnight incubation at 37 °C. 200 ml of a diluted solution will be put to 96 wells of flat-bottom tissue culture plates, where it will be cultured for 24 hours at 37 °C. After that, phosphate buffered saline will be used to wash the wells four times. The biofilms that are still stuck to the wells will be dyed with crystal violet (0.1%) and preserved with 2% sodium acetate. Wells will undergo one more rinse. After that, 200 ml of an 80:20 ethanol/acetone mixture will be poured to each well to dissolve the crystal violet. Using an ELISA reader with a 570nm wavelength, the optical densities (OD) of stained adherent biofilms will be assessed after drying. As a positive

control, ATCC strains of *A. baumannii* (ATCC 19606) will be utilized, and sterile, uninoculated BHI broth will be used as a negative control. Strong, moderate, and weak biofilm producers will be defined as strains having an OD value [> 0.24], [0.12-0.24], and [< 0.120], respectively. The photos of the experimental setup, petri dishes, test tubes, and plates are shown in figure 1– 5.

Figure 1: Preparation for detection of biofilm by modified tissue culture plate method.

Figure 2: Preparation for detection of biofilm by modified tissue culture plate method.

Figure 3: After preparing a plate incubated for 24 hours.

Figure 4: A plate after 24 hours of incubation.

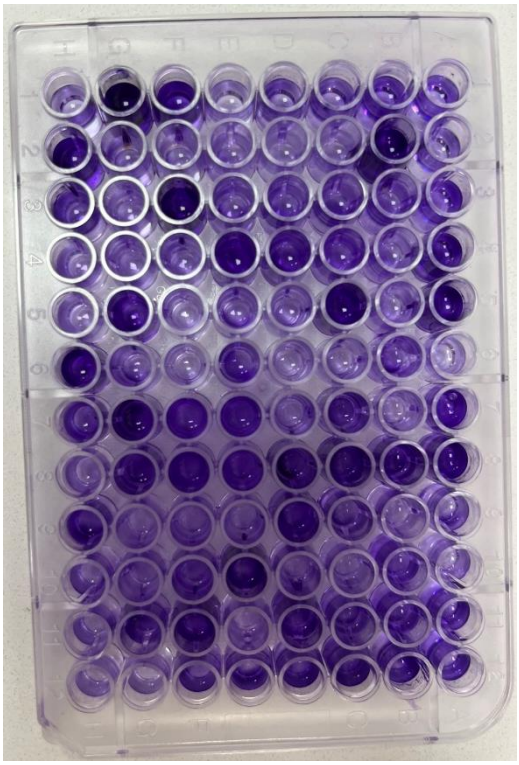

Figure 5: A plate after final reading.

RESULT AND DISCUSSION

The current study was carried out at Pandit Bhagwat Dayal Sharma's Post Graduate Institute of Medical Sciences in Rohtak, Haryana, and the National Institute of Medical Sciences & Research in Jaipur, Rajasthan. The study included a variety of laboratory samples including blood, urine, pus, cerebrospinal fluid, pleural fluid, drain, sputum, tracheal, endotracheal aspirate, and bronchoalveolar lavage. They were handled in accordance with standard microbiological procedures.

These samples yielded organisms that were recognized and speciated. *Acinetobacter baumannii* complex was isolated from 1099 out of the total 57137 samples that were received. There were 558 of them that were *Acinetobacter baumannii*.

The antimicrobial susceptibility testing was done by using Kirby-Bauer disk diffusion method on Mueller-Hinton Agar as per Clinical and Laboratory Standard Institute (CLSI) guidelines (13).

The antibiotics used were Gentamicin (10Ug), Amikacin (30ug), Ciprofloxacin (5ug), Levofloxacin (5ug), Amoxiclav, Ampicillin (10ug), Piperacillin-Tazobactam (100ug/10ug)), Cefepime (30ug), Cefotaxime (30ug), Ceftazidime (30ug), Imipenem (10ug), Meropenem (10ug). Organisms which were resistant to 3 or more than 3 were taken MDR of 558 *Acinetobacter baumannii* 201 *Acinetobacter baumannii* were MDR.

GENDER SPECIFIC DISTRIBUTION OF MDR *ACINETOBACTER BAUMANNII* ISOLATES

Among the 201 isolates, 136 (68%) were patients who were male, and 65 (32%) were patients who were female (Table2,Figure6).

Table 1: Distribution of isolates from MDR *A. baumannii* by sex

Sr. No.	Gender	No. of Patients	Percentage
1.	Female	65	32 %
2.	Male	136	68 %

Males outnumbered females by a margin of 68% to 32%, as shown in Table above.

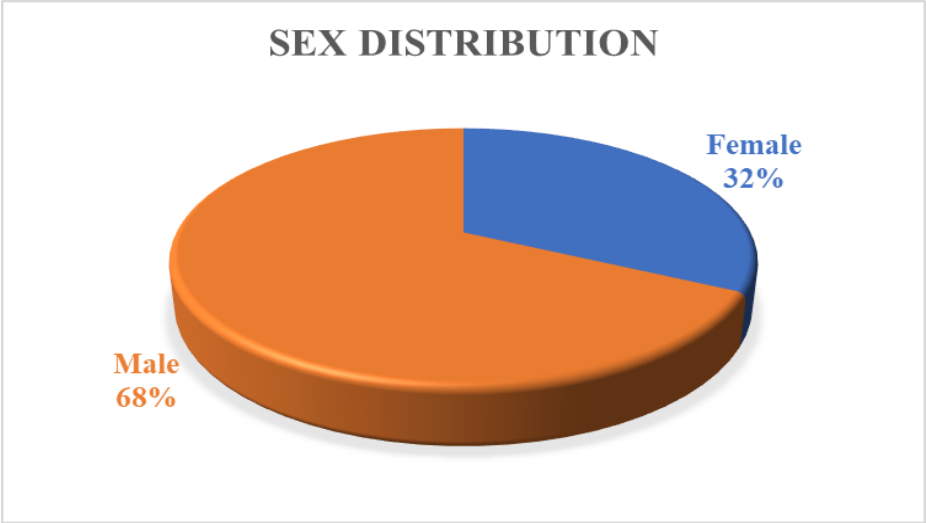

Figure 6: Sex distribution of isolates among MDR *A. baumannii*.

AGE DISTRIBUTION AMONG THE MDR *ACINETOBACTER BAUMANNII* ISOLATES (Table, Figure7)

Table 2: Patients' overall age distribution and its percentage.

Sr. No.	Age (years)	Patients	Percentage
1.	0-10	53	59.05%
2.	10-20	13	11.97%
3.	20-30	32	31.56%
4.	30-40	28	23.8%
5.	40-50	19	19.59%

6.	50-60	23	21.73%
7.	60-70	25	25.61%
8.	70-80	5	4.48%
9.	80-90	3	2.21%
10.	**Total**	**201**	

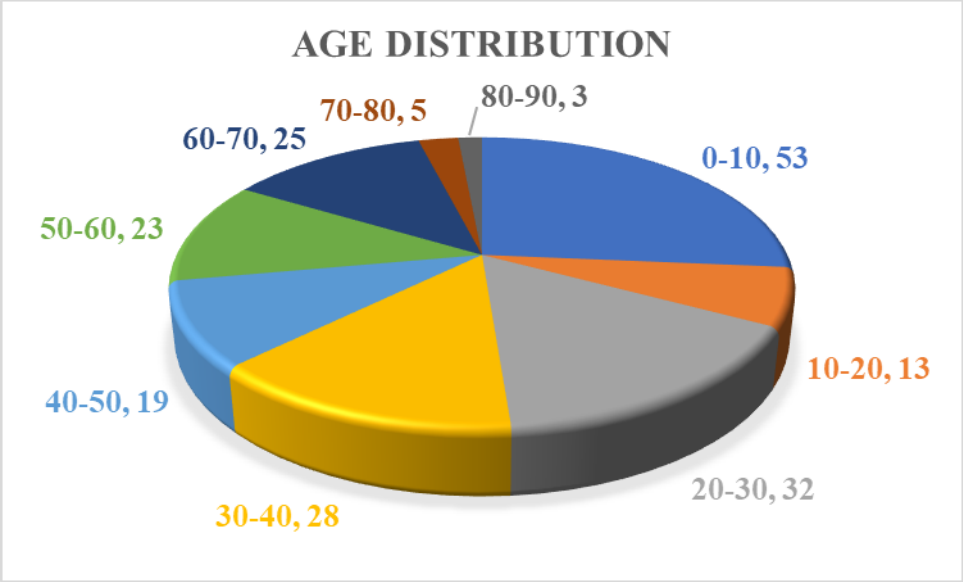

Figure 7: Age distribution of total patients among MDR *A. baumannii* isolates.

Maximum male patients with *A. baumannii* infections were in the age range of 0 - 10 years (20.59%), followed by 30 - 40 years (17.65%). Additionally, 16.18% of the male patients were in the 20 - 30 year age range, 12.50% were in the 50 - 60 year range, 11.76% were in the 60 - 70 year range, 8.82% were in the 40 - 50 year range, 7.35% were in the 70 - 80 year range, and 2.21 % of the age group 80 – 90 years (

Table, Figure).

In females, 38.46 % of *A.baumannii* were isolated in the age group of 0 – 10 years and 15.38 % belonged to 20 – 30 years followed by13.85 % in the age group 60 – 70 years, 10.77 % of the age group 40 – 50 years, 9.23 % of the age group 50 – 60 years, 6.15 % of the age group 30 – 40 years, 4.62 % of the age group 10 – 20 years,1.54 % of the age group 70 – 80 years (

Table4, Figure8).

DISTRIBUTION OF ISOLATES ACCORDING TO SAMPLES

Among the 201 MDR *A. baumannii* isolates, 79 (39.30 %) were found in Blood samples followed by 41 (20.40 %) from Tracheal samples, 29 (14.43 %)from Pus samples, 20

(9.95 %) from Urine samples, 11 (5.47 %) from Endotracheal Aspirate samples, 9 (4.48 %) from Sputum samples, 6 (2.99 %) from Cerebrospinal fluid samples, 3 (1.49 %) from Broncho alveolar Lavage samples, 2 (1 %) from Pleural fluid samples, 1 (0.50 %) from Drain sample (

Table5 Figure 9).

Table 3: Age Distribution of Male, Female, and their Percentage.

Sr. No.	Age (Years)	Female	Percentage	Male	Percentage
1.	0-10	25	38.46 %	28	20.59 %
2.	10-20	3	4.62 %	10	7.35 %
3.	20-30	10	15.38 %	22	16.18 %
4.	30-40	4	6.15 %	24	17.65 %
5.	40-50	7	10.77 %	12	8.82 %
6.	50-60	6	9.23 %	17	12.50 %
7.	60-70	9	13.85 %	16	11.76 %
8.	70-80	1	1.54 %	4	2.94 %
9.	80-90	0	0.00 %	3	2.21 %

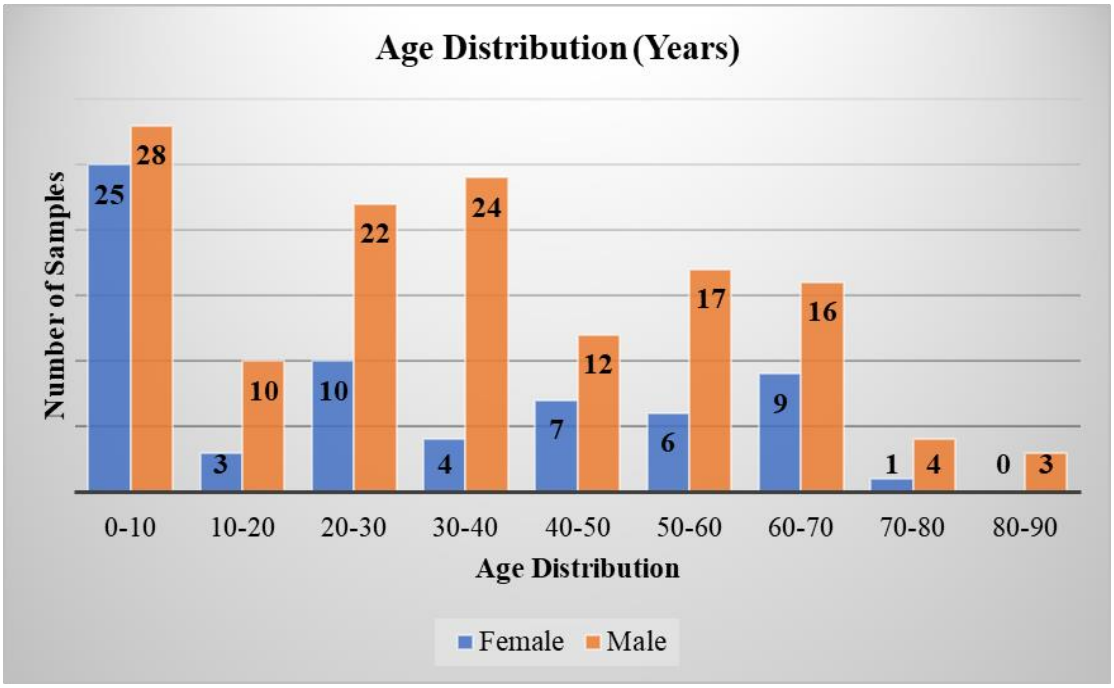

Figure 8: Age distribution of patients (Male, Female) among MDR *A. baumannii* isolates.

Table 4: Sample Distribution and their Percentage

Sr. No.	Sample	Count of Sample Type	Percentage
1.	Bronchoalveolar Lavage	3	1.49%
2.	Blood	79	39.30%
3.	Cerebrospinal Fluid	6	2.99%
4.	Drain	1	0.50%
5.	Endotracheal Aspirate	11	5.47%
6.	Pleural Fluid	2	1.00%
7.	Pus	29	14.43%
8.	Sputum	9	4.48%
9.	Tracheal	41	20.40%
10.	Urine	20	9.95%

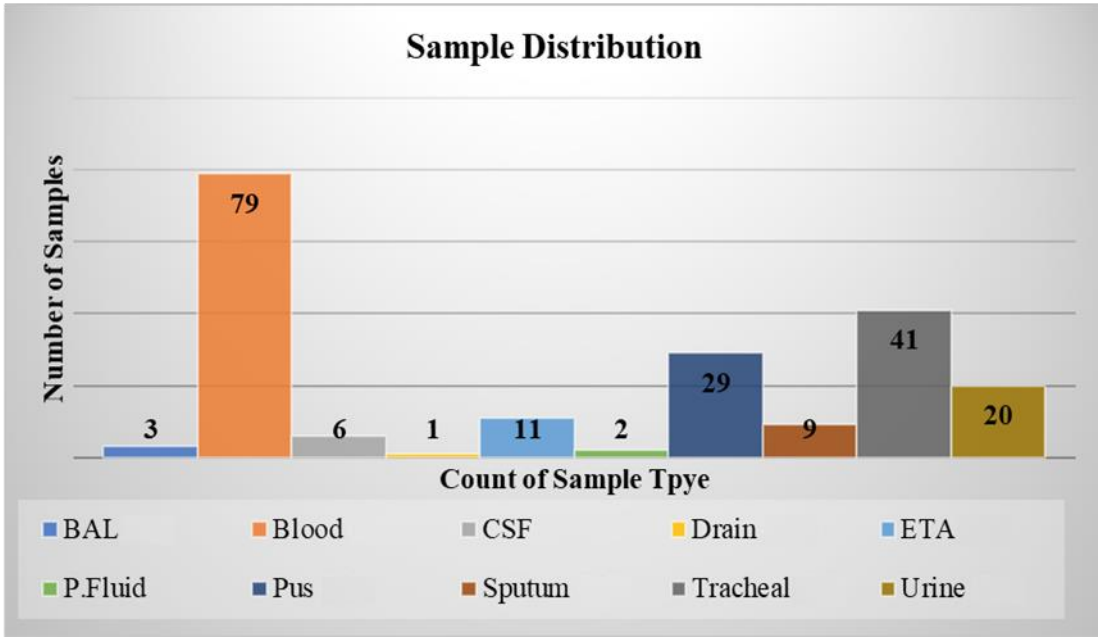

Figure 9: Sample Distribution and Number of Samples

Table 5: Distributionof Patients according to report and their Percentage

Sr. No.	Department	Number of patients	Percentage
1.	A&E	4	1.99
2.	GOD	12	5.97
3.	POD	6	2.99
4.	NICU	44	21.89
5.	URO	11	5.47
6.	SOD	10	4.98
7.	BPS	13	6.47
8.	CTVS ICU	7	3.48
9.	TICU	24	11.94
10.	MICU	30	14.93
11.	ENT	2	1.00
12.	ICU	13	6.47
13.	MOD	22	10.95
14.	SKIN	2	1.00
15.	OOD	1	0.50
16.	**Total**	**201**	

The largest numbers of isolates were found in NICU, followed by MICU and TICU, as shown in the aforementioned Table6 and Figure10.

ANTIBIOTIC SENSITIVITY PROFILE OF MDR *ACINETOBACTER BAUMANNII*

Cefepime (96.02%) had the highest rate of resistance, followed by Cefotaxime (95.02%), Ampicillin & Piperacillin + Tazobactam (86.57%), and Cefotaxime.

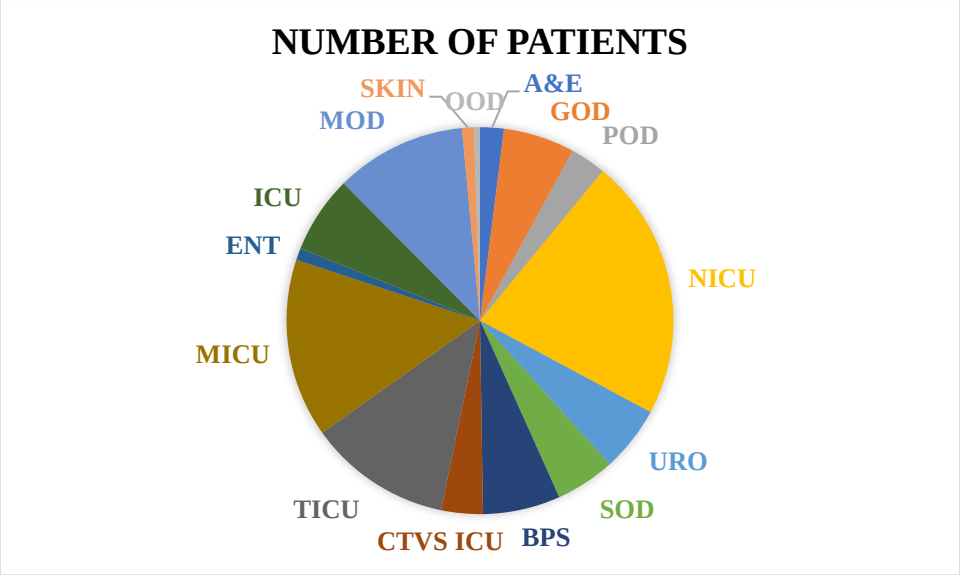

Figure 10: Distribution of isolates according to the ward.

Table 6: Percentage of antibiotics that are sensitive and resistant.

Sr. No.	Antibiotics	Resistant in percentage	Sensitive in percentage
1.	Gentamicin	60.70%	39.30%
2.	Amikacin	58.21%	41.79%
3.	Ciprofloxacin	85.07%	14.935%
4.	Levofloxacin	82.59%	17.41%
5.	Amoxiclav	61.69%	38.31%
6.	Ampicillin	86.57%	13.43%
7.	Piperacillin + Tazobactam	86.07%	13.93%
8.	Cefepime	96.02%	3.98%
9.	Cefotaxime	95.02%	4.98%
10.	Ceftazidime	84.58%	15.42%
11.	Imipenem	80.60%	19.40%
12.	Meropenem	78.61%	21.39%

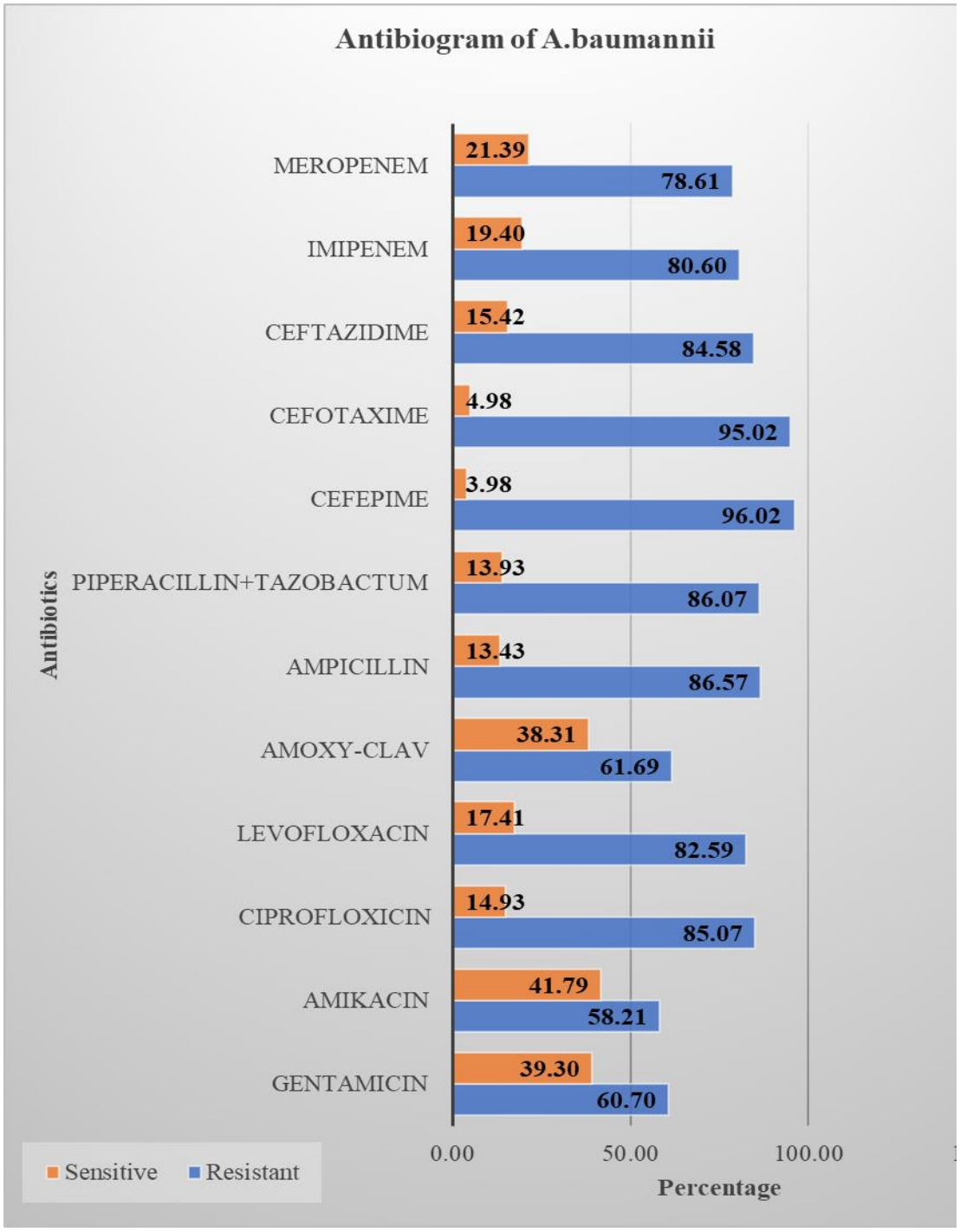

Figure 11: Profile of MDR *Acinetobacter baumannii* Antibiotic Sensitivity.

The strains were resistant to Ciprofloxacin and Ceftazidime in amounts of 85.07% and 84.58%, Levofloxacin and Imipenem in amounts of 82.59% and 80.60%, Meropenem, Amoxiclav, Gentamicin, and Amikacin in amounts of 78.61%, 61.69%, 60.70%, and 58.21%, respectively (Table7 and Figure11).

BIOFILM FORMATION IN MDR *ACINETOBACTER BAUMANNII*

A total of 201 (36%) of the 558 *Acinetobacter baumannii* isolates were MDR organisms. Antimicrobial resistance demonstrated by a type of microbe to at least one antimicrobial medication in three or more antimicrobial categories is known as multidrug resistance (MDR).

Positive controls will be provided by ATCC strains of *Acinetobacter baumannii* (ATCC 19606), and negative controls will be provided by sterile, uninoculated BHI broth.

Acinetobacter baumannii MDR biofilm development was detected in 201 cases using the modified tissue culture plate method are (Table8):

42.7 % (86 isolates) Strong biofilm producer (> 0.24)

33.33 % (67 isolates) Moderate biofilm producer (0.12 – 0.24)

23.97 % (48 isolates) Non biofilm producer (< 0.120)

Table 7: Comparison of Biofilm formation in Different Countries.

Biofilm formation	**Saudi Arabia** (146)	**China** (147)	**Italy** (148)	**Taiwan** (149)	**Iran** (150)	**India** (151)	**Present Study**
Strong biofilm producer	53.6%	51.4%	55.35%	45.4%	43%	62%	42.7%
Moderate biofilm producer	39.3%	41.4%	22.97%	32.5%	32%	32%	33.33%
Non biofilm producer	7.1%	4.3%	9.39%	6.4%	25%	10%	23.97%

COMPARISON OF BIOFILM PRODUCTION & DRUG RESISTANCE

MDR *Acinetobacter baumannii* has 201 isolates, and 153 (76.1%) of those isolates produced biofilm (Table9).

Table 8: Biofilm Production in MDR *Acinetobacte*r and their Percentage.

Sr. No.	**Biofilm production**	**MDR *Acinetobacter baumannii* (201)**	**Percentage**
1.	Positive (Strong + Moderate)	153 (86 + 67)	76.1 %
2.	Negative	48	23.8 %

CONCLUSION

The findings suggest that antimicrobial testing should be done to identify the most effective treatment options for infections caused by *A. baumannii*. Since biofilm formation can protect the bacteria from the effects of antibiotics, it is essential to identify the nanoparticles as the antimicrobial agents (14-20) that are most effective against biofilm-producing strains of *A. baumannii*. Overall, the results of this study emphasize the importance of understanding the role of biofilm formation in the persistence and resistance of *A. baumannii* infections.

In addition, it is also important to draw attention to the antibiotics problem, which has arisen because of the advent of several bacterial genera that are resistant to numerous antibiotics. *Acinetobacter* is a significant nosocomial infection with a propensity for cross-transmission, especially in intensive care units, and severe treatment choices limitations. As a result, there is an urgent need to restrict the spread of MDR strains in hospitals.Healthcare providers should be aware of the potential for biofilm formation and consider this factor in the management and treatment of infections caused by this path.

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