Journal of Medical Microbiology and Infectious Diseases

JoMMID
ISSN: 2345-5349
eISSN: 2345-5330

Correlation between Biofilm Formation and Multi-Drug Resistance among Clinical Isolates

Rubina Shrestha[1], Pooja Ghaju[1], Dilip Kumar Chaudhary[1], Raushan Kumar Karn[1], Rajesh Kumar Thakur[1], Suresh Jaiswal[2], Ram Krishna Shrestha[1*]

[1]Department of Microbiology, Modern Technical College, Pokhara University, Kathmandu, Nepal; [2]School of Health and Allied Sciences, Pokhara University, Pokhara, Nepal

ARTICLE INFO

Original Article

Keywords: Biofilm, Resistance, Pathogens, Chronic infection, Multidrug resistance, Antimicrobial agents

Received: 30 Jul. 2022
Received in revised form: 05 Nov. 2023
Accepted: 28 Oct. 2023
DOI:

***Correspondence**
Email: rknasika008@gmail.com
Tel: +9779841423483
Fax:

ABSTRACT

Introduction: Biofilms are often found in communities of microorganisms in chronic and persistent infections, exhibiting high resistance against antimicrobial agents. Biofilm serves as a barrier, impeding the penetration of drugs and constraining their effectiveness. Multiple methods, such as the Tissue Culture Plate method, Congo Red Agar method, Tube method, bioluminescent assay, and fluorescent microscopic examination, can be used to evaluate biofilm production. **Methods**: The study included a total of 300 clinical isolates representing a range of bacterial species, including *Acinetobacter baumannii* (n=9), Coagulase Negative Staphylococcus (n=7), *Enterobacter aerogenes* (n=7), *Enterococcus faecalis* (n=15), *Escherichia coli* (n=137), *Klebsiella pneumoniae* (n=23), *Proteus mirabilis* (n=4), *Pseudomonas aeruginosa* (n=16), *Salmonella typhi* (n=11), and *Staphylococcus aureus* (n=68). Associations among isolates capable and incapable of biofilm formation and their multidrug resistance phenotypes were evaluated. **Results**: Among the 300 clinical isolates tested, 289 isolates (96.3%) exhibited biofilm formation. The most prevalent biofilm-forming organisms were *A. baumannii* (n=9), *Citrobacter koseri* (n=1), Coagulase Negative Staphylococcus (CONS) (n=7), *E. aerogenes* (n=7), *E. faecalis* (n=15), *E. coli* (n=137), *Klebsiella oxytoca* (n=1), *K. pneumoniae* (n=23), *P. mirabilis* (n=4), *P. aeruginosa* (n=16), *S. typhi* (n=11), *S. aureus* (n=68), and *Streptococcus pneumoniae* (n=1). The biofilm-forming isolates demonstrated increased resistance compared to isolates that did not form biofilms. **Conclusion**: Antimicrobial resistance represents a critical characteristic of infections involving biofilms. The study identified biofilm production in 92.7% of the isolates tested via TCP and in 72.3% of the isolates using the CRA. Furthermore, it was observed that pathogens with multidrug resistance (MDR) exhibited a higher biofilm production tendency than non-producing pathogens.

INTRODUCTION

Microbial communities known as biofilms are encased within their own self-produced extracellular polymeric matrix [1]. Bacteria can adopt two distinct growth modes, the first being planktonic cells, while the second involves sessile aggregates known as biofilms [2]. Biofilms are microbial communities in which microbes generate extracellular polymeric substances (EPS), including proteins (less than 1-2%, which may include enzymes), DNA (less than 1%), polysaccharides (comprising 1-2% of the total), and RNA (less than 1%). In addition to these components, water accounts for up to 90%, playing a crucial role in the nutrient flow within the biofilm matrix [3]. Biofilm architecture comprises two primary elements: a network of water channels for nutrient transport and dense cell clusters lacking prominent pores. Microbial cells within biofilms exhibit distinctive arrangements and display pronounced variations in physiology and physical properties [4]. Pathogenic microorganisms exploit biofilm formation to adhere to implants and prosthetic devices. [5]. Microorganisms adhere to surfaces and secrete polysaccharides, forming biofilms that create an optimal environment for genetic material exchange, which, in turn, contributes to the emergence of drug-resistant

[DOI: 10.61186/JoMMID.11.3.148]

pathogens [6, 7]. Within the biofilm matrix, bacteria communicate through the production of chemotactic particles or pheromones, known as quorum sensing [8]. The availability of essential nutrients, chemotactic response towards the substrate, bacterial motility, surface adhesion mechanisms, and surfactant presence are among the factors influencing biofilm formation [8]. Microorganisms within biofilms exhibit inherent resistance to antimicrobial agents compared to their planktonic counterparts [9]. Significantly higher antimicrobial concentrations are required to effectively inhibit the growth of biofilm-associated organisms due to the potential for antibiotic resistance to increase by up to 1,000-fold [9]. During biofilm growth, adaptive mechanisms of resistance, as opposed to genetic alterations, contribute to the development of antibiotic resistance. This allows drug-resistant pathogens to evade host defenses and antibiotic treatments, resulting in persistent infections [10]. Drug-resistant biofilm-producing isolates pose a significant challenge in human diseases [11]. Biofilm formation is commonly associated with infections affecting human surfaces, including teeth, skin, and the urinary tract [12]. Biofilm formation is related to various medical conditions, such as upper respiratory tract infections, endocarditis, thrombophlebitis, and urinary tract infections (UTIs). It is particularly prevalent in the presence of medical devices [13]. Numerous bacterial species can form biofilms, including *P. aeruginosa, S. epidermis, E. coli, S. aureus, E. cloacae*, and *K. pneumoniae* [14].

Biofilms have significant implications for public health as microorganisms within biofilms display reduced susceptibility to antimicrobial agents. Susceptibility to antimicrobial agents can arise due to biofilm growth or can be acquired, resulting from the transfer of extrachromosomal elements to susceptible organisms within the biofilm. Antibiotic-resistant pathogens primarily originate in healthcare settings, resulting in extended hospitalizations, increased healthcare expenses, and elevated mortality rates. Multidrug-resistant (MDR) organisms have the potential to spread to additional healthcare facilities and within the community [15, 16, 17]. Biofilms formed by bacteria exhibit infectious properties, which can lead to nosocomial infections. Hospital-acquired infections contribute to a substantial burden of morbidity and mortality. Hospital environments are susceptible to contamination with bacterial pathogens that can grow as biofilms on surfaces. Frequently encountered nosocomial pathogens such as *P. aeruginosa* synthesize exopolysaccharides, forming intricate biofilm structures that facilitate adherence to abiotic surfaces, thus protecting the action of antibiotics [18, 19, 20, 21]. Several methods are available for detecting biofilm production, including the Tissue Culture Plate (TCP), Tube method (TM) assay, Congo Red Agar method (CRA), bioluminescent assay, piezoelectric sensor technology, and fluorescent microscopy examination [22, 23, 24, 25, 26, 27].

This study includes biofilm formation and comparing antibiotic sensitivity patterns between biofilm producers and non-producers.

MATERIAL AND METHODS

A cross-sectional study conducted in a laboratory setting aimed to investigate the comparative antibiotic susceptibility among biofilm-producing and non-producing strains. The study was conducted at Star Hospital, with sample processing performed at the Microbiology Department, Modern Technical College, Pokhara University, Kathmandu, Nepal, between October and December 2018. The convenience sampling method was employed, and only specimens with positive cultures were included, while samples exhibiting mixed growth were excluded. A total of 300 bacterial isolates were obtained. The bacterial isolates were from different clinical specimens, with a prevalence rate of 61.4%, as reported by Sanchez *et al*. [12, 32]. Ethical approval was obtained from the Institutional Review Board (IRB) of the Nepal Health Research Council (NHRC), Kathmandu, Nepal (Ref. no.: 821). Written informed consent was obtained from the patients receiving care at Star Hospital.

Three hundred bacterial isolates were selected randomly from various clinical specimens obtained from Star Hospital and transferred to the Microbiology Laboratory at Modern Technical College. For subsequent analysis, the isolates were cultured on MacConkey Agar and Blood Agar plates. Biofilm detection was conducted using the tissue culture plate method and the Congo red agar technique. For the tissue culture plate method, bacterial isolates were obtained from freshly prepared agar plates and then inoculated into 10 mL Trypticase Soy Broth supplemented with 1% glucose. Subsequently, the broths were then incubated at 37 °C for 24 h. Next, the cultures were diluted at a dilution ratio 1:100 using fresh medium. Later, individual wells of sterile 96-well polystyrene plates with a flat-bottom surface treated for tissue culture were filled with a volume of 200 µL. A control organism was similarly incubated, diluted, suspended, and introduced into the corresponding wells of the tissue culture plate. For negative control, wells were filled with sterile broth inoculated with the control organism. Subsequently, the plates were incubated at 37 °C for 24 h, followed by gentle tapping to remove the contents of each well. Then, the wells were washed four times using 0.2 mL of phosphate-buffered saline (pH 7.2) to remove free-floating bacteria. The biofilm formed by bacteria that adhered to the wells was subsequently fixed using a 2% sodium acetate solution and then stained with a 0.1% crystal violet solution. The excess stain was

[DOI: 10.61186/JoMMID.11.3.148]

removed using deionized water. Later, an ethanol and acetone solution in a ratio of 80:20 was applied to each well to achieve a uniform biofilm layer. The optical density of the stained biofilm adhered to the surface was measured using a microplate reader at a 550 nm wavelength. Each experiment was performed in triplicate and repeated three times. Interpretation of biofilm production was based on the criteria outlined by Stepanovic *et al*. [15, 28]. For the Congo red agar method, the CRA medium was prepared with 37 g/L of brain heart infusion broth powder, 50 g/L of sucrose powder, 10 g/L of agar No.1 powder, and 8 g/L of Congo red indicator powder. Initially, the Congo red agar was prepared as a concentrated solution in water and subjected to autoclaving at 121 °C for 15 min, independently from the other components. Subsequently, the answer was added to the autoclaved brain heart infusion agar containing sucrose at 55 °C. Test organisms were inoculated onto the CRA plates and incubated under aerobic conditions at 37 °C for 24 h. Biofilm production was indicated by black colonies with a dry crystalline consistency. The Congo red agar method was performed in triplicate [17].

Table 1. Interpretation of biofilm production

Average OD value	Biofilm production
≤ ODc / ODc < ~ ≤ 2x ODc	Non/ weak
2x ODc< ~ ≤ 4x ODc	Moderate
> 4x ODc	Strong

Optical density cut-off value (ODc) = average OD of negative control + 3× of Standard deviation (SD) of negative control.

Antibiotic susceptibility testing. Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) [28]. The inoculum was directly applied to Muller-Hinton Agar (MHA). The following antibiotics were used: Amoxycillin, Cefotaxime, Nitrofurantoin, Norfloxacin, Ofloxacin, Gentamycin, Cefixime, Cotrimoxazole, Cephalexin, Amikacin, Meropenem, Piperacillin-tazobactam, Cefoxitin, and Vancomycin. Interpretation of the results was performed according to the criteria established by CLSI.

Statistical analysis: Data entry was performed using Microsoft Excel 2013, followed by analysis using SPSS version 16.

RESULTS

A total of 300 clinical isolates were obtained from various specimens. Of 300 isolates, 173 were *E. coli*, followed by *S. aureus* (n=68), *K. pneumoniae* (n=23), *P. aeruginosa* (n=16), *E. faecalis* (n=15),*S. typhi* (n=11), *A. baumanii* (n=9), Coagulase Negative Staphylococcus (CONS) (n=7), *E. aerogenes* (n=7), *P. mirabilis* (n=4), *S. pneumoniae* (n=1), *K. oxytoca* (n=1), and *C. koseri* (n=1).

The clinical specimens included urine (n=174) followed by pus (n=66), sputum(n=21), blood (n=10), tracheal aspirate (n=8), fluid (n=6), endotracheal tube (ET) (n=2), central venous pressure (CVP) (n=1) and tissue (n=1), as shown in Table 2.

Table 2. Details of collected specimens and identified bacteria

Organisms	Urine	Pus	Sputum	Blood	ET tube	Fluid	CVP Tube	Tissue	Total
E. coli	109	24	1	0	1	1	0	1	137
S. aureus	25	28	8	2	0	5	0	0	68
P. aeruginosa	17	1	5	0	0	0	0	0	23
K. pneumoniae	5	4	4	0	2	0	1	0	16
E. faecalis	5	8	1	0	1	0	0	0	15
S. typhi	0	0	0	11	0	0	0	0	11
A. baumanii	1	2	1	0	5	0	0	0	9
CONS	5	1	0	0	1	0	0	0	7
E. aerogenes	5	0	2	0	0	0	0	0	7
P. mirabilis	4	0	0	0	0	0	0	0	4
K. oxytoca	1	0	0	0	0	0	0	0	1
S. pneumoniae	1	0	0	0	0	0	0	0	1
C. koseri	1	0	0	0	0	0	0	0	1
Total	179	68	22	13	10	6	1	1	300

Biofilm-producing bacteria. Of the 300 clinical isolates, 289 (96.3%) were biofilm producers, as determined by the TCP and Congo red agar methods. TCP revealed 150 isolates as strong biofilm producers, 128 as moderate biofilm producers, and 22 as none or weak biofilm producers — meanwhile, using CRA 217 isolates were biofilm producers (Table 3).

[DOI: 10.61186/JoMMID.11.3.148]

Table 3. Frequency of biofilm production by different methods

Method	Biofilm production	No. of isolates
Biofilm production	Yes	289 (96.3%)
	No	11 (3.3%)
	Strong	150 (49.5 %)
TCP method	Moderate	128 (42.2%)
	Non / Weak	22 (7.3%)
CRA method	Positive	217 (71.6%)
	Negative	83 (27.4 %)

Among the clinical isolates obtained from the specimens, 289 were identified as the major predominant biofilm producers. All *K. pneumonia* (n=23), *S. aureus* (n=68), *CONS* (n=7), *E. faecalis* (n=15), *P. aeruginosa* (n=16), *E. aeruginosa* (n=7), *P. mirabilis* (n=4), *K. oxytoca* (n=1), *S. pneumoniae* (n=1) and *C. koseri* (n=1) isolates were biofilm producer. Among the 137 *E. coli* isolates, 131 exhibited biofilm production. Similarly, of the 11 *S. typhi* isolates, 8 demonstrated biofilm production, and of the 9 *A. baumannii* isolates, 7 showed biofilm production.

Most of the biofilm-producing bacteria were derived from urine (60.2%), followed by pus (22.8%), sputum (7.3%), blood (3.5%), tracheal aspirate (2.8%), fluid (2.1%), endotracheal tube (ET) (0.7%), central venous pressure (CVP) tube (0.3%), and tissue (0.3%) (Table 4).

Table 4. Biofilm formation of isolates from various clinical specimens

Organisms	Urine	Pus	Sputum	Blood	ET tube	Fluid	CVP Tube	Tissue	Total
E. coli	104	23	1	0	1	1	0	1	131
S. aureus	25	28	8	2	0	5	0	0	68
P. aeruginosa	17	1	5	0	0	0	0	0	23
K. pneumoniae	5	4	4	0	2	0	1	0	16
E. faecalis	5	8	1	0	1	0	0	0	15
S. typhi	0	0	0	8	0	0	0	0	8
A. baumanii	1	1	0	0	5	0	0	0	7
CONS	5	1	0	0	1	0	0	0	7
E. aerogenes	5	0	2	0	0	0	0	0	7
P. mirabilis	4	0	0	0	0	0	0	0	4
K. oxytoca	1	0	0	0	0	0	0	0	1
S. pneumoniae	1	0	0	0	0	0	0	0	1
C. koseri	1	0	0	0	0	0	0	0	1
Total	174	66	21	10	10	6	1	1	289

Association of biofilm production and MDR. Compared to non-biofilm-producing organisms, biofilm-producing organisms exhibited significantly higher levels of drug resistance to all tested antibiotics, as shown in Table 5.

Table 5. Biofilm formation and MDR

		Biofilm Producer		*P*-value
		Yes	No	
MDR	Yes	151	4	0.366
	No	138	7	

Distribution of MDR among clinical isolates. Among the 289 biofilm-producing isolates, 151 (52.2%) exhibited multidrug-resistant (MDR) phenotypes among biofilm-positive and biofilm-negative organisms. Among the 11 biofilm-negative organisms, only 4 (36.4%) exhibited MDR phenotypes (Table 6).

Antibiogram of Gram-positive isolates. The predominant Gram-positive isolates were found to be MDR. Forty-seven percent of the *S. aureus* isolates were identified as MRSA. None of the MRSA isolates exhibited resistance to Vancomycin. CONS isolates showed higher resistance than *S. aureus* (Table 8).

[DOI: 10.61186/JoMMID.11.3.148]

Table 6. MDR in different biofilm-producing organisms

Biofilm-producing organisms	MDR
E. coli	61 (40.4%)
S. aureus	32 (21.2%)
K. pneumonia	14 (9.3%)
E. faecalis	12 (7.9%)
P. aeruginosa	11 (7.3%)
A. baumanii	7 (4.6%)
CONS	4 (2.6%)
E. aeruginosa	3 (2.0%)
P. mirabilis	2 (1.3%)
S. typhi	2 (1.3%)
C. koseri	1 (0.3%)
S. pneumonia	1 (0.3%)
K. oxytoca	1 (0.3%)

Table 7. Susceptibility pattern rates of Gram-negative bacteria producing biofilms

Organisms		Antibiotic (%)									
		AK	CTX	CTR	MRP	OF	COT	PIT	NIT	NX	AMX
E. coli	Sensitive	45.8	32.1	32.1	58	39.6	33.6	45	49	35.1	16
	Resistant	54.2	67.9	67.9	42	60.3	66.4	55	51	64.9	84
K. pneumoniae	Sensitive	39.1	26.1	21.7	73.9	26.1	47.8	21.7	13	21.7	-
	Resistant	60.9	73.9	78.3	26.1	73.9	52.2	78.3	87	78.3	-
P. aeruginosa	Sensitive	25	-	-	50	25	-	43.8	0	50	-
	Resistant	75	-	-	50	75	-	56.2	100	50	-
P. mirabilis	Sensitive	75	50	75	75	0	50	50	0	50	0
	Resistant	25	50	25	25	100	50	50	100	50	100
A. baumanii	Sensitive	0	0	0	14.3	28.6	0	0	0	0	0
	Resistant	100	100	100	85.7	71.4	100	100	100	100	100
E. aerogenes	Sensitive	57.1	57.1	57.1	71.4	57.1	57.1	71.4	42.8	42.9	28.6
	Resistant	42.9	42.9	42.9	28.6	42.9	42.9	28.6	57.2	57.1	71.4
S. typhi	Sensitive	NT	75	75	100	100	75	100	-	-	62.5
	Resistant	NT	25	25	0	0	25	0	-	-	37.5

Table 8. Antimicrobial susceptibility testing patterns of biofilm-producing Gram-positive bacteria as a percentage

Organisms		Antibiotic (%)									
		AK	CTX	CTR	CN	OF	PIT	NIT	VA	CX	COX
S. aureus	Sensitive	64.7	27.9	42.6	32	56	78	56	100	53	42
	Resistant	35.3	72.1	57.4	68	44	22	44	0	47	58
CONS	Sensitive	100	42.8	42.8	-	42.8	42.8	0	28.6	57.2	28.6
	Resistant	0	57.2	57.2	-	57.2	57.2	100	71.4	42.8	71.4
E. faecalis	Sensitive	20	13.3	20	44.4	46.7	40	33.3	13.3	13.3	20
	Resistant	80	86.7	80	55.6	53.3	60	66.7	86.7	86.7	80

DISCUSSION

A laboratory-based cross-sectional study was conducted in the Microbiology laboratory from October to December 2018. Various clinical specimens were included, and antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method. Biofilm production was assessed using both Congo red agar and tissue culture methods. Most microorganisms (99.9%) demonstrated the ability to produce biofilm on various surfaces, including biological and inert surfaces [29, 30].

[DOI: 10.61186/JoMMID.11.3.148]

In our study, Biofilm formation was detected in 289 isolates (96.3%), while Verma *et al*. (2016) reported 66%) [31]. Sanchez *et al*. (2013) found that of 205 clinical isolates, 126 (61.4%) were biofilm production [32]. Biofilm production in the present study was higher compared to those previous studies. This difference may be attributed to the inclusion of largely multidrug-resistant hospital.

Biofilm-producing organisms mainly were from urine samples (60.2%), followed by pus samples (22.8%), sputum samples (7.5%), blood samples (3.5%), tracheal aspirate samples (2.8%), fluid samples (2.1%), endotracheal tube samples (0.7%), central venous catheter (CVP) samples (0.3%), and tissue samples (0.3%). In a similar study, biofilm-producing organisms were most commonly found in urine samples (30%), followed by urinary catheter tips (25.7%), pus samples (12.8%), sputum samples (11.4%), intravenous catheter tips (10%), and naso-bronchial lavage samples (10%) [29

In our study, of the 137 *E. coli* isolates 131were biofilm producers, including all *S. aureus* (N=68), *K. pneumoniae* (n=23), *P. aeruginosa* (n=16), *E. faecalis* (n=15), *E. aerogenes* (n=7), *P. mirabilis* (n=4), CONS (n=7), *C. koseri* (n=1), *K. oxytoca* (n=1), and *S. pneumonia* (n=1) isolates. Of the 11 *S. typhi* isolates, 8 were biofilm producers, and of the 9 *A. baumanii* isolates, 7 exhibited biofilm production. According to Sanchez *et al*. (2013), among 23 *S. aureus* isolates, 21 strains (91%) tested positive, and among the 54 *K. pneumoniae* isolates, 41 tested positive [32]. Similarly, 36 *P. aeruginosa* and 53 *A. baumanii* isolates, 30 and 29 were biofilm producers. Among the 39 *E. coli* isolates, only 5 were biofilm producers. The clinical isolates in our study that demonstrated biofilm production were similar to the findings of Sanchez *et al*. (2013).

In a similar study by Verma *et al*. (2016), among 168 biofilm producer isolates, *E. coli* was the most prevalent pathogen, accounting for 56 out of 96 isolates, followed by *K. pneumoniae* with 30 out of 45 isolates. *A. baumanii* exhibited biofilm formation in all 6 isolates, while *P. aeruginosa* showed biofilm production in 5 out of 7 isolates. Similarly, *E. cloacae*, *P. mirabilis*, *C. diversus*, and *Proteus vulgaris* demonstrated biofilm formation in all 5, 5, 2, and 2 isolates, respectively [31]. Similar findings were observed in a study conducted by Hassan *et al*. (2011), which reported 25 (22.7%) isolates with strong biofilm production and 45 (41%) isolates with moderate biofilm production [33]. The rate of moderate biofilm production was consistent with our study. Only 11 isolates were identified as biofilm producers using the CRA method out of 111. The significant biofilm production observed in this study using the CRA method could be attributed to device-associated pathogens. In a regional survey in India, among 152 isolates, 53.9% of the isolates demonstrated biofilm production, while 46% did not exhibit biofilm production [34].

In a separate study conducted by Verma *et al*. (2016), out of a total of 111 strains, 66% (111 strains) demonstrated biofilm production by the TCP method, while the remaining 34% (57 strains) did not exhibit biofilm production [31]. Nevertheless, 58% of the isolates exhibited biofilm production using the CRA method, while the remaining 42% did not. In contrast, the TCP method demonstrated a sensitivity of 95% and specificity of 100%, surpassing the CRA method, which exhibited a sensitivity of 74% and specificity of 90.9%. The TCP method provides a quantitative and convenient approach for assessing biofilm formation in microorganisms. Likewise, a study by Saha *et al.* (2018) reported 89.7% of the clinical isolates from hospitalized patients as biofilm producers; at the time, in outpatients, only 10.3% exhibited the same feature [35]. Bacterial biofilms account for 65% of nosocomial infections, emphasizing their significant contribution to healthcare-associated conditions.

Organisms that produce biofilms exhibited a higher prevalence of MDR than organisms that did not. Of the 289 organisms that produced biofilms, 151 were identified as having MDR phenotypes. However, among the 11 non-biofilm-producing organisms, only 4 organisms exhibited MDR. In this study, among the 289 biofilm-producing bacteria analyzed, the predominant MDR organism was *E. coli* (40.4%), followed by *S. aureus* (21.2%), *K. pneumoniae* (9.3%), *E. faecalis* (7.9%), *P. aeruginosa* (7.3%), *A. baumannii* (4.6%), coagulase-negative Staphylococci (2.6%), *E. aerogenes* (2.0%), *P. mirabilis* (1.3%), *S. typhi* (1.3%), *K. oxytoca* (0.7%), *C. koseri* (0.7%), and *S. pneumonia* (0.7%).

Likewise, in a study by Summaiya *et al*. (2012), it was found that *A. baumannii* (35.1%) was the most common multidrug-resistant organism, followed by *P. aeruginosa* (18.9%), *K. pneumoniae* (18.9%), *E. coli* (13.5%), and *S. aureus* (10.8) [36]. In the present study, most biofilm-positive clinical isolates resisted cephalosporins, aminoglycosides, carbapenems, fluoroquinolones, nitrofurantoin, and co-trimoxazole. Drug resistance among the biofilm producers was similarly elevated in the study by Sanchez *et al*. (2013), mirroring our research [32].

A concerning rise in the production of biofilms and the development of drug resistance has been observed among isolates of Gram-negative and Gram-positive bacteria obtained from various clinical samples.

ACKNOWLEDGMENT

The authors express their gratitude to all individuals conducting and supporting this study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES

1. Li chen, Yu-mei Wen. The role of bacterial biofilm in persistent infections and control strategies. Int J Oral Sci. 2011; 3 (2): 66-73.
2. Jamal M, Tasneem U, Hussain T, Andleeb S. Bacterial Biofilm: Its Composition. Formation and Role in Human Infections. J Microbio Biotechnol. 2015; 4 (3): 1-14.
3. Oli AK, Javaregowda PK, Jain A, Kelmani CR. Mechanism Involved in Biofilm Formation of *Enterococcus Faecalis*. 2022.
4. Fux C, et al. Survival strategies of infectious biofilms. Trends Microbiol. 2005; 13 (1): 34-40.
5. Patricia M. tille, PhD, MLS (ASCP): Bailey & Scott's Diagnostic Microbiology: 13th Edition: 35.
6. Tayal R, Baveja S, De A. Analysis of biofilm formation and antibiotic susceptibility pattern of uropathogens in patients admitted in a tertiary care hospital in India. Internat J Health Allied Sci. 2015; 4 (4): 247-49
7. Tabasi M, Eybpoosh S, Siadat SD, Elyasinia F, Soroush A, Bouzari S. Modulation of the gut microbiota and serum

[DOI: 10.61186/JoMMID.11.3.148]

biomarkers after laparoscopic sleeve gastrectomy: a 1-year follow-up study. Obes Surg. 2021; 31 (5): 1949-56.

8. Thomas D, Day F. Biofilm formation by plant associated bacteria. Ann Rev Microbiol 2007; 61: 401-22

9. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. Lancet. 2001; 358 (9276): 135-8.

10. Dardi Charan Kaur, Wankhede SV. A study of biofilm formation and Metallo-beta lactamases in *Pseudomonas aeruginosa* in a tertiary care rural hospital. Int J Sci Res Pu. 2013; 3 (10): 1-7.

11. Swarna SR, Supraja, Gomathi S, Radha Madhavan. A study of biofilm formation in multidrug resistant organisms. J Pharma Res. 2014; 8 (5): 660-4.

12. Hatt JK, Rather PN. Role of bacterial biofilms in urinary tract infections. Curr Top Microbiol Immunol. 2008; 322: 163-192

13. Jessica LL, Alexander RH. *Staphylococus aureus* biofilms: Recent developments inbiofilm dispersal. Front Cell Infect Microbiol. 2014; 4: 178.

14. Parsek MR, Singh PK. Bacterial biofilms: an emerging link to disease pathogenesis. Annu Rev Microbiol. 2003; 57: 677-701.

15. Donlan MR. Biofilm Formation: A Clinically Relevant Microbiological Process. Clin Infect Dis. 2001; 33 (8): 1387-92.

16. Luke F. chen, Matthew E Falagas, Anton Y Peleg. Facing the rising tide of multidrug resistant Gram-negative pathogens. Healthcare Infect. 2011; 16: 1-5.

17. Pradeep Kumar SS, Easwer HV, Maya Nandkumar A. Multiple drug resistant bacterial biofilms on implanted catheters – A reservoir of infection. J Assoc Phys India. 2013; 61 (10): 702-7.

18. Abdel-Aziz SM, Aeron A. Bacterial biofilm: dispersal and inhibition strategies. SAJ Biotechnol. 2014; 1 (1): 105.

19. National Audit Office (2004). Improving Patient Care by Reducing the Risk of Hospital Acquired Infection: a Progress Report. London: Stationery Office.

20. Smith K, Hunter SI. Efficacy of common hospital biocides with biofilms of multi- drug resistant clinical isolates. J Med Microbiol. 2008; 57 (Pt 8): 966-73.

21. Zolfaghan PS, Wyncoll DL. The tracheal tube: gateway to ventilator-associated pneumoniae. Crit Care. 2011; 15 (5): 310.

22. Christensen GD, Simpson WA, Younger JA, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase negative Staphylococci to plastic tissue cultures: a quantitative model for the adherence of Staphylococci to medical devices. J Clin Microbiol. 1995; 22 (6): 996-1006.

23. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immun1982; 37 (1): 318-26.

24. Freeman J, Falkiner FR, Keane CT. New method for detecting lime production by coagulase negative staphylococci. J Clin Pathol. 1989; 42 (8): 872-4.

25. Donlan RM, Murga R, Bell M, Toscano CM, Carr JH, Novicki TJ, et al. Protocol for detection of biofilms on needleless connectors attached to central venous catheters. J Clin Microbiol. 2001; 39 (2): 750-3.

26. Aparna MS, Yadav S. Biofilms: microbes and disease. Braz J Infect Dis. 2008; 12 (6): 526-30.

27. Zufferey J, Rime B, Francioli P, Bille J. Simple method for rapid diagnosis of catheter associated infection by direct Acridine orange staining of catheter tips. J Clin Microbiol. 1988; 26 (2): 175-7.

28. Stepanovic S, Vukovi D, Hola V, Di Bonaventura G, Djukic S, Cirkovix I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by *Staphylococci*. APMIS. 2007; 115 (8): 891-9.

29. Costerton J., et al.; Bacterial biofilms: a common cause of persistent infections. Science. 1999; 284 (5418): 1318-22.

30. Khan S, et al.; Isolation of *Shigella species* and their resistance patterns to a panel of fifteenantibiotics in mid and far western region of Nepal. Asian Pacific J Tropical Dis. 2014; 4: 30-34.

31. Verma S, Patil SS. *In Vitro* Biofilm Formation by Uropathogenic Bacteria and their Antibiotic Susceptibility Pattern. JKIMSU. 2016; 5 (3): 2231-4261.

32. Sanchez C, Mende K, Beckius M, Akers K, Romano D, Wenke J, et al. Biofilm formation by clinical isolates and the implications in chronic infections; BMC infect Dis. 2013; 13: 47.

33. Hassan A., Usman J., Kaleem F., Omair M., Khalid A., Iqbal M.; Evaluation of different detection methods of biofilm formation in the clinical isolates; Department of Microbiology, National University of Sciences and Technology(NUST), Islamabad, Army Medical College, Rawalpindi, Pakistan; 2011; 305-311.

34. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of Staphylococci: an evaluation of three different screening methods. Indian J Med Microbiol. 2006; 24 (1): 25-9.

35. Saha S, Mamta DK, Damrolien S, Devi S, Krossnunpuii, Sharma KT. Biofilm production and its correlation with antibiotic resistance pattern among clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital in north-east India. Int J Adv Med. 2018; 5 (4): 964-8.

36. Summaiya AM, Urmi NJ. Assessment of Biofilm Formation by the Causative Organisms of Ventilator Associated Pneumonia at Intensive Care Unit of Tertiary Care Hospital. Nat J Med Res. 2012: 2 (1): 15-8.

Cite this article:
Shrestha R, Ghaju P, Chaudhary DK, Karn RK, Thakur RK, Jaiswal S, Shrestha RK. Correlation between Biofilm Formation and Multi-Drug Resistance among Clinical Isolates. J Med Microbiol Infect Dis, 2023; 11 (3): 148-154.

[DOI: 10.61186/JoMMID.11.3.148]