ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE

Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

ORIGINAL ARTICLE

Biofilm and Antimicrobial Resistance of Clinical and Environmental Isolates of *Pseudomonas aeruginosa*

Abdelraouf A. Elmanama*[1], Renad I Abu-Dan*[1], Suhaila A. Al-Sheboul*[2]

Received: 14/07/2022 ***Accepted:***24/08/2022 ***Published:***01/10/2022

OPEN ACCESS

Doi:
https://doi.org/10.52865/ZMQQ2426

Funding: Nil

Competing Interests:

The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

Affiliation and Correspondence:

1* Islamic University of Gaza, Faculty of Health Sciences Medical Laboratory Sciences Department, Gaza, Palestine.
2 Jordan University of Science and Technology, Faculty of Applied Medical Sciences, Department of Medical Laboratory Sciences, Irbid, Jordan.
*Corresponding Author Email: elmanama_144@yahoo.com

ABSTRACT:

Objectives: To evaluate the clinical and environmental ability of *P. aeruginosa* isolates to make biofilms and to determine their antimicrobial susceptibility patterns.
Methods: Environmental swabs (144), air (28) and water samples (12) were collected from various wards of Gaza strip hospitals. Additionally, 158 clinical *P. aeruginosa* isolates were obtained from the microbiology laboratories of the same hospitals between July 21, 2019 and January 21, 2020. Samples were cultured and bacterial identification was performed using standard microbiological procedures. PCR was used to confirm the identity of *P. aeruginosa*. *P. aeruginosa* isolates were tested for their antimicrobial susceptibility patterns by the agar disk diffusion method. Both qualitative and quantitative methods assessed the biofilm formation by crystal violet and safranin stains.
Results: Among the *P. aeruginosa* isolates (N=150), 90.0% were resistant for ceftazidime, (36.7%) aztreonam, (29.3%) gentamicin, (27.3%) levofloxacin, (22.0%) meropenem, (14.0%) piperacillin, (10.0%) amikacin and (9.3%) imipenem. The results for biofilm formation by tube method showed that 78.0% and 71.3% of the isolates were biofilm producer by crystal violet and safranin methods, respectively. Microtiter plate method demonstrated that 94.0% and 96.0% were biofilm producers by crystal violet and safranin, respectively. In addition, there was a statistical significance between the meropenem resistance and biofilm-forming ability of the isolates.
Conclusions: High resistance rates were detected among *P. aeruginosa* isolates. The lowest rate of resistance was to imipenem and amikacin. As for the biofilm assessment, the tissue culture plate method showed higher detection rates than the tube method.

Keywords: *P. aeruginosa*, biofilm, antimicrobial resistance, detection methods, Gaza- Palestine.

ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE
Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

Introduction

P. aeruginosa is an obligate aerobic Gram-negative bacilli, motile, oxidase positive and non-glucose and non-lactose fermenter (Fujitani, Moffett, and Yu 2017). It is pervasive in the hospital environment and is able to survive in it. *P. aeruginosa* is considered as the major infectious organism in most severe burn victims (Ramos 2011). Many outbreaks of *P. aeruginosa* according to hospital records are linked to environmental contamination, including tap water. There are reports that water systems share in the transition of *P. aeruginosa* in healthcare settings (Loveday et al. 2014).

P. aeruginosa threatens whole patient's attendance process. It is considered as the most complicated health care associated pathogen to be cleared from an infected area. Although there are many novel antimicrobial agents, it is not easy to eradicate wound infections associated with nosocomial strains of *P. aeruginosa* (Lima et al. 2017).

The biofilm-forming ability of *P. aeruginosa* provides protection from host immunity and contributes to antibiotic resistance of this organism (Fujitani, Moffett, and Yu 2017). Microbial biofilms are well-established bacterial communities instilled in a self-created extracellular polymeric matrix (Costerton et al. 1995). They exhibit features that distinguish them from those of their free-floating or planktonic counterpart. Bacterial biofilms exist in variety of environments and niches, For example they may be found on mucosal surfaces, dead or living tissues and, medical devices surfaces in the host like intubation tubes, catheters and artificial heart valves (Donlan and Costerton 2002).

A distinctive feature of biofilms is their increasingly high level of drug resistance, which poses higher risks in clinical settings (Balaban et al. 2004). The major contributor to antibiotic resistance in biofilms is the unusual expression of numerous genes, extracellular matrix, and the metabolic variation of sub-populations within a biofilm (Anderl, Franklin, and Stewart 2000). In addition, the anaerobic conditions within the biofilm due to limited concentrations of oxygen inside the biofilm and modifications, which take place by the intensive congregation of bacteria within biofilms, and the resultant reduction of nutrients, proposes that deep bacteria inside biofilms can live despite the antibiotic treatment. This is due to their decreased metabolic action and minimal rates of cell division than those next to the surface of the biofilms (Anderl, Franklin, and Stewart 2000).

This study shed light on the *P. aeruginosa* isolates' ability to form biofilm from various wards in Gaza strip hospitals. Furthermore, it is a challenge to make a comparison among different isolates from various sources (department, site affected). This study correlated antimicrobial susceptibility patterns of *P. aeruginosa* and its ability to produce biofilms.

Methods

Sample Collection, Handling and Transport

All samples were collected from various wards of four hospitals in Gaza strip (Al-Shifa, Indonesian, Al-Aqsa and European Gaza hospital (EGH)). After obtaining the ethical approval from Palestinian Ministry of Health, clinical isolates were collected from the microbiology laboratories of the same hospitals between 21 of July 2019 and 21 of January of 2020. Isolates were then transported in an icebox within two hours to the Medical Microbiology Laboratory, Islamic School of Gaza (IUG) for processing. Plates containing bacterial isolates were autoclaved and disposed properly after processing.

Environmental Samples

Environmental swabs were obtained from soaps, beds, beddings, door handles, tap handles, tap mouth and bathtubs. One hundred and forty four swabs were collected from patients' rooms at chosen departments by moistening a cotton swab with transport media to isolate *P. aeruginosa* from the various environmental surfaces. Each swab was placed in its container to preserve the viability of microorganisms (Elmanama et al. 2019).

Air Samples

Air samples (28) were collected from patients' rooms after sterilizing the air sampler`s (FSC-IV (China)) top by 70% alcohol swabs, and setting the air sampler to collect 100 liters of air per minute. A sterile and freshly prepared Cetrimide agar (CA) **(**HiMedia, India**)** plate was placed inside the air sampler, and then 100 liters of air was collected.

Water Samples

ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE
Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

Twelve water samples were collected from patients' rooms after sterilizing the opening of the tap with ignition of alcohol dipped cotton ball; the tap was then opened for 2 to 3 minutes. After that, 100 ml of water was collected in a sterile bottle containing 0.1 ml of 3% sodium thiosulfate.

Clinical Isolates

One hundred and fifty eight *P. aeruginosa* clinical isolates were sub cultured into screw capped Triple Sugar Iron Agar (TSIA) (HiMedia, India) tubes.

Microbiological Analysis

For swab samples, each swab was streaked on CA plates and then incubated at 37±0.5 ^{o}C for 24 hours.[9] While plates from air samples were incubated for 24 hours at 37 ˚C. Meanwhile, membrane filtration technique was used to filter 100 ml of water sample through a sterile filtration assembly containing a sterile membrane filter (pore size 0.65 µ). A vacuum was stratified and the sample was dragged within membrane filter. All organisms were attached to the membrane filter, which is then transferred to a CA culture medium in a petri dish. Afterwards incubated at 37±0.5 ^{o}C for 24 hours Isolates on TSIA obtained from microbiology laboratories, were sub cultured onto CA to get pure isolates.

The isolates were then recognized by colony morphology, characteristic growth, Gram stain, Triple sugar iron agar and manner of biochemical profile (catalase, oxidase, motility, and Simmons citrate) in accordance with the standard methods, and the identification was confirmed by PCR using the primers (PA431CF CTGGGTCGAAAGGTGGTTGTTATC and PA431CR GCGGCTGGTGCGGCTGAGTC that were suggested by (Choi et al. 2013). Presumptively identified clinical isolates obtained from the microbiology laboratories were also sub cultured onto CA and re-identified using the same procedures.

Molecular identification of the recovered isolates

A. DNA Extraction and PCR amplification

A modification of previously described procedure (Dashti et al. 2009), was used to extract bacterial DNA. One to two colonies of bacteria were suspend in 100 µl of sterile distilled water. This was incubated at heating block at 100 ˚C for 10 minutes then transferred on to ice bath for 3 minutes to cool, and the resultant solution was centrifuged at 10,000 rpm for 3 min. the supernatant was transferred to

new eppendorf tube for PCR. The purified DNA was amplified using primers writtem above which targeting O antigen acetylase gene for P. aeruginosa. PCR amplification was carried out with the following conditions: denaturation at 95 oC for 3 min; 35 cycles of denaturation at 95oC for 60 sec., annealing at 63oC for 30 s, and extension at 72 oC for 60s and then final extension step at 72 oC for 10 min (Choi et al. 2013).

Agarose gel electrophoresis (2%)

Polyacrylamide gel electrophoresis was performed to separate amplified PCR products as described by (Najjar 2017). Agarose gel (1.2 g) was dissolved in 80 ml 1x Tris-Acetate-EDTA buffer by heating in a microwave. Then three µl Ethidium Bromide was added and mixed. The gel was casted into a mold, which was fitted with a well-forming comb. The agarose gel sunk in electrophoresis buffer within a horizontal electrophoresis apparatus. After amplification, the PCR products and a DNA ladder size marker were loaded into the sample wells to aid in fragment size determination. The electrophoresis apparatus was attached to an electric power supply at a constant current of 70 volts for 40 min at room temperature, and the DNA bands were visualized and documented using a UV trans-illuminator documentation system (Scie-Plas Ltd, UK 7).

Antimicrobial Susceptibility by Agar Diffusion Method

Growth standardization to all the tested isolates was done by colony suspension method. McFarland standards (0.5) was used to give a final concentration of about 1.5×10^8 CFU/mL (colony-forming units per milliliter) to the growth suspension. The antimicrobial susceptibility testing was done by modified Kirby–Bauer diffusion technique; through swabbing the Mueller-Hinton agar (MHA) (HiMedia, India) plates with the Brain Heart Infusion Broth (HiMedia, India) suspension of each isolate. Different antimicrobial disks were then carefully placed onto the surface of the inoculated plates. Afterwards, plates stood for 30 minutes minimally before being incubated at 37 °C for 24 hours. The tests were performed twice. After 24 hours of incubation, the diameter of the zones of inhibition produced by the antimicrobial were substantiated by CLSI 2019 zone diameter interpretative standards (Wei et al. 2020).

Determination of the *In Vitro* Biofilm Formation of *P. aeruginosa*

ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE
Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

Qualitative determination (Tube Method (TM))

Bacterial culture (0.1 mL that was checked to 0.5 McFarland standard) was transported to glass test tube including 10 mL Brain Heart Infusion with 2% glucose and incubated at 37 °C for 72 hours. The medium was eliminated and each tube was washed with distilled water, air-dried and biofilm formation was assayed with crystal violet (Pour et al. 2011). All tests were performed in triplicate. The same procedures were repeated and biofilm formation was evaluated with safranin.

Quantitative Determination (Microtiter Plate Method (MPM))

Three wells of a sterile 96-well U-bottomed microtiter plate were filled with 200 µl of bacterial suspension diluted 1:100 with fresh medium. Negative control containing broth only was prepared. After incubation for 24 hours at 37°C, 250 µl of distilled water (DW) was used to wash wells. After 15 min, 0.2 ml of 2 % crystal violet was used to stain plates for 5 minutes. Excess stain was washed by placing plates under running tap water. The plate was then air-dried. 160 µl of 95% methanol was used to re-solubilize adherent cells (Christensen et al. 1985) and then transferred to a flat microtiter plate to determine optical density (OD) at 570 nm. The same method was used for safranin.

For analysis of test results, the adherence abilities of the test strains were categorized to four classes depending on their ODs: non-adherent, weakly adherent, moderately adherent, or strongly adherent, as calculated by (Christensen et al. 1985).

The intensity of biofilm formation for *P. aeruginosa* isolates was determined by the following criteria.

OD ≤ 0.1 non-adherent.

OD 0.1 < to ≤ 0.2 weakly adherent.

OD 0.2 < to ≤ 0.4 moderately adherent.

OD 0.4 < strongly adherent.

Results

Microbiological Analysis

From all the collected samples and isolates, only 150 isolates have been identified as *P. aeruginosa*, which is Gram-negative bacilli, motile with yellow green pigmentation on Cetrimide agar and

ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE
Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

alkaline/No Change (K/NC) on TSIA. These isolates were obtained from Al-Aqsa (N= 35), European (N=28), Al-Shifa (N=49) and Indonesian (N=38) hospitals. One hundred and forty-four of these isolates were clinical and six were environmental isolates. The isolates were from different hospital wards. Five isolates (3.3%) were from pediatric, fifty nine isolates (39.3%) were from outpatient, three isolates (2.0%) were from nursery, fifty five isolates (36.7%) were from surgery and twenty eight (18.7%) were from the other wards. Additionally, all isolates were catalase, oxidase, and citrate tests positive. The identification of the species was further confirmed by PCR. Fourteen clinical isolates were excluded from the study since they did not match the identification criteria of *P. aeruginosa.*

Antimicrobial Susceptibility of *P. aeruginosa* Isolates

P. aeruginosa isolates (N=150) were screened for their susceptibilities to 8 different antimicrobial agents and the results showed that 90.0% were resistant to ceftazidime followed by aztreonam (36.7%), gentamicin (29.3%), levofloxacin (27.3%), meropenem (22.0%), piperacillin (14.0%), amikacin (10.0%) and the lowest resistant shown was for imipenem (9.3%). Based on the p-value, table (1) shows only one statistical significance between levofloxacin resistance and clinical isolates of different sources (Pus, body fluids, others).

Table (1): Antimicrobial resistance percentage of clinical isolates distributed by specimen type.

Antimicrobial	Pus	Body fluids	Others	p-value
	N=65	N=59	N=20	
Amikacin	12.3	8.5	5.0	0.784
Aztreonam	35.4	42.4	30.0	0.589
Ceftazidime	89.2	88.1	95.0	0.263
Gentamicin	32.3	22.0	40.0	0.423
Imipenem	10.8	10.2	5.0	0.354
Levofloxacin	29.2	23.7	35.0	0.035
Meropenem	24.6	15.3	25.0	0.316
Piperacillin	10.8	16.9	20.0	0.557

Assessment of Biofilm Formation

ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE
Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

Qualitative Determination (TM)

Crystal violet tube method (CV-TM) and safranin tube method (S-TM) was used to assess the biofilm formation of the isolates (figure 1). The results of CV-TM showed that 78.0% (N=117) of the isolates were biofilm producer. While the results of S-TM showed that 71.3% (N=107) of the isolates were biofilm producer.

Quantitative Method (MPM)

Crystal violet MPM (CV-MPM) and safranin MPM (S-MPM) were also used to assess the biofilm formation of the isolates. CV-MPM showed that 123 (82.0%) were strong biofilm producer, 7 (4.7%) were moderately adherent, 11 (7.3%) were weakly adherent and nine (6.0%) were non-adherent (non-biofilm producer). S-MPM showed that 95 (63.3%) were strong biofilm producer, 34 (22.7%) were moderately adherent, 15 (10.0%) were weakly adherent and six (4.0%) were non-adherent.

In this study, CV-MPM was used in all subsequent comparisons. By the use CV-MPM it was observed that all the isolates from Al-Aqsa hospital were biofilm producers as presented in table (2) and the lowset percentage for the biofilm formation were among isolates recovered from the EGH.

Table (2) : The biofilm forming ability of isolates distributed by hospitals using CV-MPM.

Hospital	Strong		Moderate		Weakely adherant		Total positve		Non adherant	
	N	%	N	%	N	%	N	%	N	%
Al-Aqsa	31	88.6	2	5.7	2	5.7	35	100.0	0	0.0
EGH	18	64.3	2	7.1	5	17.9	25	89.3	3	10.7
Al-Shifa	39	79.6	3	6.1	3	6.1	45	91.8	4	8.2
Indonesian	35	92.1	0	0.0	1	2.6	36	94.7	2	5.3

According to the hospital ward, it was observed that all isolates from pediatric, nursery and the others were biofilm producer table (3).

Depending on the specimen source; body fluids were the highest percentage in CV-MPM and the other group was the highest non-adherent group table (3).

ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE
Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

Table (3) Biofilm production using CV-MPM among hospital departments and specimen source of clinical isolates.

Hospital department	Strong		Moderate		Weakley adherent		Total positive		Non adherent	
	N	%	N	%	N	%	N	%	N	%
Pediatric	4	80.0	1	20.0	0	0.0	5	100.0	0	0.0
OP	48	81.4	1	1.7	5	8.5	54	91.5	5	8.5
Nursery	3	100.0	0	0.0	0	0.0	3	100.0	0	0.0
Surgery	42	76.4	4	7.3	5	9.1	51	92.7	4	7.3
Others	26	92.9	1	3.6	1	3.6	28	100.0	0	0.0
Specimen source	**Strong**		**Moderate**		**Weakley adherent**		**Total positive**		**Non adherent**	
	N	%	N	%	N	%	N	%	N	%
Pus	55	84.6	3	4.6	2	3.1	60	92.3	5	7.7
Body fluids	51	86.4	2	3.4	5	8.5	58	98.3	1	1.7
Other	14	70.0	1	5.0	3	15.0	18	90.0	2	10.0

The following table (4) indicates that there is statistical significance between the MPM and TM with a higher percentage positivity using the MPM.

Table (4) : The correlation between MPM method and TM.

MPM		TM				P-value
		Positive		Negative		
		N	%	N	%	
CV	Positive	113	80.1	28	19.9	0.012
	Negative	4	44.4	5	55.6	
S	Positive	105	72.9	39	27.1	0.036
	Negative	2	33.3	4	66.7	

Biofilm Formation and Antibiotic Resistance

Depending on the P-value in the following table (5) the only statistical significance was between the meropenem resistant and biofilm forming ability as shown in table (5).

ISRAA UNIVERSITY JOURNAL OF APPLIED SCIENCE
Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

Table (5): The biofilm forming ability by CV-MPM with antimicrobial resistance.

Antimicrobial		CV-MPM Positive		CV-MPM Negative		P-value
		No.	%	No	%	
Amikacin	**S**	123	93.9	8	6.1	0.874
	I	4	100.0	0	0.0	
	R	14	93.3	1	6.7	
Azetronam	**S**	82	91.1	8	8.9	0.187
	I	5	100.0	0	0.0	
	R	54	98.2	1	1.8	
Ceftazidime	**S**	12	100.0	0	0.0	0.587
	I	3	100.0	0	0.0	
	R	126	93.3	9	6.7	
Gentamicin	**S**	96	94.1	6	5.9	0.856
	I	4	100.0	0	0.0	
	R	41	93.2	3	6.8	
Impenem	**S**	124	93.9	8	6.1	0.866
	I	4	100.0	0	0.0	
	R	13	92.9	1	7.1	
Piperacillin	**S**	119	93.0	9	7.0	0.439
	I	1	100.0	0	0.0	
	R	21	100.0	0	0.0	
Levofloxacin	**S**	97	94.2	6	5.8	0.773
	I	6	100.0	0	0.0	
	R	38	92.7	3	7.3	
Meropenem	**S**	111	96.5	4	3.5	0.042
	I	2	100.0	0	0.0	
	R	28	84.8	5	15.2	

Discussion

Antimicrobial Susceptibility of *P. aeruginosa* Isolates

Among aminoglycosides, it was observed that the resistance for gentamicin is higher than amikacin. The results in this study are compatible with results obtained by (Juayang et al. 2017). The resistance was (13.5%) for gentamicin and (7.5%) for amikacin. However, some studies have demonstrated that amikacin resistance was higher than gentamicin. For example, (Ali et al. 2015) showed that amikacin resistance was 53% and gentamicin was 51%. Different target population, isolate sources, and use of

these individual antibiotics may explain differences among studies with regard to gentamicin and amikacin.

In this study, *P. aeruginosa* showed 90% resistance to ceftazidime. It was the highest resistant percentage among all antibiotics used. Our result was compatible with results obtained by our previous study (Elmanama, Abu-Dan et al. 2019) in which we reported the highest resistance percentage (100%) with ceftazidime. Although, our results were higher than results obtained by (Sader et al. 2017) wherein, the resistance percentage was (84.3%). The sample size, duration, type, and study settings all are possible causes for the variation in the resistance rate (Saeed et al. 2018).

The resistance for piperacillin in this study was 14.0%. This was higher than the results obtained by (Henwood et al. 2001) where it was (5.0%) and lower than results obtained by Sivanmaliappan et al, (Sivanmaliappan and Sevanan 2011) as the resistance rate was (83.3%). The increase or decrease in the resistance is attributed to the rate of piperacillin usage. The increase in usage increases the percentage of antibiotic resistance and vice versa. In addition, the difference in the time interval of different studies probably included new strains that are resistant to many antibiotics that were not present in earlier studies.

Among carbapenems, our results showed that the resistance percentage for meropenem was higher than imipenem. The same was observed by (Jabalameli et al. 2012) the resistance for meropenem was (17.1%) and (16%) for imipenem. However, other studies showed that the resistance in imipenem is higher than meropenem such as (Henwood et al. 2001) where the reported meropenem resistance was only (4.2%) and imipenem resistance was (8.1%). The entangled interlinkage of multiple techniques involving carbapenemase production, efflux system overproduction and lack of outer membrane porins are probably the causes of resistance to carbapenems. Carbapenem resistant is a dramatic issue because it is correlated to an elevated death rate. (Liu et al. 2015)

Among monobactams, aztreonam was used and its resistance percentage was (36.7%). This was higher than the results obtained by (Bouza et al. 1999)where 23% of resistance was obtained. Among quinolones, levofloxacin was used and its resistance percentage was (27.3%). Our results were lower

than those obtained by (Yayan, Ghebremedhin, and Rasche 2015) were the resistance percentage was (34.6%). Chromosomal genes, including target mutations and active efflux play key roles in fluoroquinolone resistance for *P. aeruginosa* isolates. As other Gram-negative bacteria, DNA gyrase is primary target for the fluoroquinolones in *P. aeruginosa*. (Hooper 2000).

Assessment of Biofilm Formation

Qualitative Method (TM)

P. aeruginosa isolates (150) were recovered from various wards of Gaza strip hospitals and were assessed by TM. (78.0%), (71.3%) of the isolates were biofilm producer using CV-TM and S-TM, respectively.

Our findings matched the results obtained by (Lima et al. 2017) who presented that high percentage of their *P. aeruginosa* isolates were biofilm producer (83.3%). Another study conducted by Rewatkar et al also displayed (77.5%) of the isolates were positive for biofilm formation. (Perez et al. 2011) also showed that biofilm was revealed in (33%) of *P. aeruginosa* isolates.

Quantitative Method (MPM)

Using MPM, it was observed that (94.0%), (96.0%) were biofilm producer by CV-MPM and S-MPM, respectively. Our findings were consistent with (Jabalameli et al. 2012) who reported that 96% of the isolates were biofilm producer.

Varying degrees of biofilm forming abilities of this organism have been disclosed by other studies. (Perez, Costa et al. 2011) showed that 64.1% of the isolates were biofilm-producer, while Neopane et al detected biofilm formation in 76.4% of *P. aeruginosa* isolates. In addition, the percentage obtained for biofilm formation by (Mostafa et al. 2017) was 79.4%. Another study conducted by (Mishra et al. 2015) also detected that 75% of *P. aeruginosa* isolates were biofilm producers, 43.5% by (Heydari and Eftekhar 2015) and 64.7% by (Hassan et al. 2011). The variation in the size and origin of the samples may be the reasons for the diversity of the results (Neopane et al. 2017).

Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

In the current study, it was noticed that the percentage of biofilm production was higher in the MPM. This may lead to an assumption that MPM is a more accurate technique than TM. Despite the usage of TM as a screening test to recognize biofilm-producer isolates, MPM could be a credible and dependable quantitative tool for biofilm determination abilities(Neopane et al. 2017).

Biofilm Formation and Antibiotic Resistance

The existing study exhibited a statistical significance between the meropenem resistance and biofilm-forming ability of the tested *P. aeruginosa* isolates. A study conducted by (Neopane et al. 2017) showed that *P. aeruginosa* biofilm producer has higher antibiotic resistance than non-producers. Therefore, biofilm formation may be one of the crucial factors for increasing resistance toward frequently used antibiotics. [31] According to our results, we found that there is a significant correlation between biofilm formation rate and meropenem resistance. Mutations and the recurrent exposure of the bacteria to elevated levels of antibiotics is the probable reasons for antibiotic resistance of bacteria in biofilms (Ciofu et al. 2022).

Conclusions

Conventional methods for *P. aeruginosa* identification are reliable, important and easy to perform, as it was compared to the confirmatory method for bacterial recognition "the PCR technique". TM is a screening test for biofilm detection while MPM is more accurate. Among specimen sources, body fluids occupied the highest percentage of biofilm formation. There is a statistical significance between the meropenem resistance and the biofilm-forming ability of *P. aeruginosa* isolates.

References

Ali, Zaheer, Nusrat Mumtaz, Sehar Afshan Naz, Nusrat Jabeen, and Maryam Shafique. 2015. "Multi-drug resistant Pseudomonas aeruginosa: a threat of nosocomial infections in tertiary care hospitals." *JPMA* 65 (12):12-16.

Anderl, Jeff N, Michael J Franklin, and Philip S Stewart. 2000. "Role of antibiotic penetration limitation in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin." *Antimicrobial agents and chemotherapy* 44 (7):1818-1824.

Balaban, Nathalie Q, Jack Merrin, Remy Chait, Lukasz Kowalik, and Stanislas Leibler. 2004. "Bacterial persistence as a phenotypic switch." *Science* 305 (5690):1622-1625.

Bouza, E, F Garcia-Garrote, E Cercenado, M Marin, and MS Diaz. 1999. "Pseudomonas aeruginosa: a survey of resistance in 136 hospitals in Spain." *Antimicrobial agents and chemotherapy* 43 (4):981-982.

Choi, Hyeon Jin, Myeong Ho Kim, Min Seok Cho, Byoung Kyu Kim, Joo Young Kim, ChangKug Kim, and Dong Suk Park. 2013. "Improved PCR for identification of Pseudomonas aeruginosa." *Applied microbiology and biotechnology* 97 (8):3643-3651.

Christensen, Gordon D, W A Simpson, JJ Younger, LM Baddour, FF Barrett, DM Melton, and EH Beachey. 1985. "Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices." *Journal of clinical microbiology* 22 (6):996-1006.

Ciofu, Oana, Claus Moser, Peter Østrup Jensen, and Niels Høiby. 2022. "Tolerance and resistance of microbial biofilms." *Nature Reviews Microbiology*:1-15.

Costerton, J William, Zbigniew Lewandowski, Douglas E Caldwell, Darren R Korber, and Hilary M Lappin-Scott. 1995. "Microbial biofilms." *Annual review of microbiology* 49 (1):711-745.

Dashti, Ali A, Mehrez M Jadaon, Abdulsamad M Abdulsamad, and Hussein M Dashti. 2009. "Heat treatment of bacteria: a simple method of DNA extraction for molecular techniques." *Kuwait Med J* 41 (2):117-122.

Donlan, Rodney M, and J William Costerton. 2002. "Biofilms: survival mechanisms of clinically relevant microorganisms." *Clinical microbiology reviews* 15 (2):167-193.

Elmanama, Abdelraouf A, Renad I Abu-Dan, Rewaa N Eqtifan, Aisha A Shomar, and Mariam R Rifi. 2019. "Evaluation of Biofilm Formation of Pseudomonas aeruginosa Isolated from Al-Shifa Hospital and their Susceptibility to Acetic Acid." *IUG Journal of Natural Studies* 27 (1).

Fujitani, Shigeki, Kathryn S Moffett, and VL Yu. 2017. "Pseudomonas aeruginosa." *Antimicrobe, Pittsburgh, PA* 15219.

Hassan, Afreenish, Javaid Usman, Fatima Kaleem, Maria Omair, Ali Khalid, and Muhammad Iqbal. 2011. "Evaluation of different detection methods of biofilm formation in the clinical isolates." *Brazilian journal of infectious diseases* 15:305-311.

Henwood, Caroline J, David M Livermore, Dorothy James, Marina Warner, and the Pseudomonas Study Group. 2001. "Antimicrobial susceptibility of Pseudomonas aeruginosa: results of a UK survey and evaluation of the British Society for Antimicrobial Chemotherapy disc susceptibility test." *Journal of Antimicrobial Chemotherapy* 47 (6):789-799.

Heydari, Samira, and Fereshteh Eftekhar. 2015. "Biofilm formation and β-lactamase production in burn isolates of Pseudomonas aeruginosa." *Jundishapur journal of microbiology* 8 (3).

Hooper, David C. 2000. "Mechanisms of action and resistance of older and newer fluoroquinolones." *Clinical Infectious Diseases* 31 (Supplement_2):S24-S28.

Jabalameli, Fereshteh, Akbar Mirsalehian, Babak Khoramian, Marzieh Aligholi, Seyed Sajjad Khoramrooz, Parisa Asadollahi, Morovat Taherikalani, and Mohammad Emaneini. 2012. "Evaluation of biofilm production and characterization of genes encoding type III secretion system among Pseudomonas aeruginosa isolated from burn patients." *Burns* 38 (8):1192-1197.

Juayang, Alain C, Joseph Peter T Lim, Ann Francis V Bonifacio, Alaica Victoria L Lambot, Sean Maybelle Millan, Vic Zyrus Jeriko N Sevilla, Julien Kate T Sy, Paul John Villanueva, Carmina P Grajales, and Christine T Gallega. 2017. "Five-year antimicrobial susceptibility of Pseudomonas aeruginosa from a local tertiary hospital in Bacolod City, Philippines." *Tropical medicine and infectious disease* 2 (3):28.

Lima, Jailton Lobo da Costa, Lilian Rodrigues Alves, Jussyêgles Niedja Pereira da Paz, Marcelle Aquino Rabelo, Maria Amélia Vieira Maciel, and Marcia Maria Camargo de Morais. 2017. "Analysis of biofilm production by clinical isolates of Pseudomonas aeruginosa from patients with ventilator-associated pneumonia." *Revista Brasileira de terapia intensiva* 29:310-316.

Liu, Qianqian, Xiaoqing Li, Wenzhang Li, Xinmiao Du, Jian-Qing He, Chuanmin Tao, and Yulin Feng. 2015. "Influence of carbapenem resistance on mortality of patients with Pseudomonas aeruginosa infection: a meta-analysis." *Scientific reports* 5 (1):1-10.

Loveday, HP, JA Wilson, Karolyn Kerr, R Pitchers, JT Walker, and Jessica Browne. 2014. "Association between healthcare water systems and Pseudomonas aeruginosa infections: a rapid systematic review." *Journal of Hospital Infection* 86 (1):7-15.

Mishra, Shyam Kumar, Prashant Basukala, Om Basukala, Keshab Parajuli, Bharat Mani Pokhrel, and Basista Prasad Rijal. 2015. "Detection of biofilm production and antibiotic resistance pattern in clinical isolates from indwelling medical devices." *Current microbiology* 70 (1):128-134.

Mostafa, Mervat AM, Alaa A Aly, Walaa Abdel–Latif, and Rania AM Alaam. 2017. "Biofilm production by Pseudomonas aeruginosa Clinical Isolates and its Relationship with Pseudomonas Quinolone Signal (pqsA) Gene and Antibiotic Resistance." *The Egyptian Journal of Medical Microbiology (EJMM)* 26 (4).

Najjar, Mahmoud AR AL. 2017. "Relationship between Gene Polymorphism and Type 2 Diabetes in Males Palestinian Population: a Study of Five Gene Polymorphisms."

Neopane, Puja, Hari Prasad Nepal, Rajendra Gautam, Rama Paudel, Shamshul Ansari, Sony Shrestha, and Sangita Thapa. 2017. "Is there correlation of biofilm formation with multidrug resistance and ESBL production in Pseudomonas aeruginosa." *European Journal of Biomedical* 4 (1):366-372.

Perez, Leandro Reus Rodrigues, MCN Costa, Ana Lucia Peixoto de Freitas, and Afonso Luis Barth. 2011. "Evaluation of biofilm production by Pseudomonas aeruginosa isolates recovered from cystic fibrosis and non-cystic fibrosis patients." *Brazilian Journal of Microbiology* 42:476-479.

Pour, Nadia Kazemi, Devendra H Dusane, Prashant K Dhakephalkar, Farokh Rokhbakhsh Zamin, Smita S Zinjarde, and Balu A Chopade. 2011. "Biofilm formation by Acinetobacter baumannii strains isolated from urinary tract infection and urinary catheters." *FEMS Immunology & Medical Microbiology* 62 (3):328-338.

Ramos, Juan-Luis. 2011. *Pseudomonas: Volume 1 Genomics, Life Style and Molecular Architecture*: Springer Science & Business Media.

Sader, Helio S, Michael D Huband, Mariana Castanheira, and Robert K Flamm. 2017. "Pseudomonas aeruginosa antimicrobial susceptibility results from four years (2012 to 2015) of the international network for optimal resistance monitoring program in the United States." *Antimicrobial agents and chemotherapy* 61 (3):e02252-16.

Saeed, Muhammad, Farhan Rasheed, Raja Kamran Afzal, Shahida Hussain, Saba Riaz, and Adeel Ahmad. 2018. "Pseudomonas aeruginosa: Evaluation of pathogen burden and drug-resistance trends in a tertiary care hospital." *J Coll Physicians Surg Pak* 28 (4):279-83.

Sivanmaliappan, Tamil Selvi, and Murugan Sevanan. 2011. "Antimicrobial susceptibility patterns of Pseudomonas aeruginosa from diabetes patients with foot ulcers." *International journal of microbiology* 2011.

Volume 6 :Issue1, October, 2022 | ISSN: 2523-0522

Wei, Bai, Se-Yeoun Cha, Jun-Feng Zhang, Ke Shang, Hae-Chul Park, JeongWoo Kang, Kwang-Jick Lee, Min Kang, and Hyung-Kwan Jang. 2020. "Antimicrobial susceptibility and association with toxin determinants in Clostridium perfringens isolates from chickens." *Microorganisms* 8 (11):1825.

Yayan, Josef, Beniam Ghebremedhin, and Kurt Rasche. 2015. "Antibiotic resistance of Pseudomonas aeruginosa in pneumonia at a single university hospital center in Germany over a 10-year period." *Plos one* 10 (10):e0139836.