



Comparison of two methods for the detection of *Pseudomonas aeruginosa* biofilm formation isolated from different clinical samples

Nuha Abdulrazaq Hameed AL-Mojamaee¹, Hiyam Adil J. ALtaii²

1. Ph.D. Student, Department of biology, College of Science, University of Mosul, Iraq
2. Prof. Dr. Department of biology, College of Science, University of Mosul, Iraq

Abstract: *Pseudomonas aeruginosa* is implicated in a number of infections in humans, mostly related to medical services. Therapy is made much more difficult in hospitals related to antibiotic resistance. Biofilm-related issues with *P. aeruginosa* infections are one of the main treatment challenges. The complex composition of the *P. aeruginosa* biofilm adds to the pathogenic potential of this microbe, increasing its ability to evade the immune system, causing persistent infections that are challenging to treat and leading to treatment failure. In this study, we will evaluate the ability of *P. aeruginosa* to produce biofilm by two methods: Congo Red Agar (CRA) and Microtiter plate (MTP) Assay method.

Keywords: Biofilm, Congo Red Agar (CRA) and Microtiter plate (MTP) Assay method, *P. aeruginosa*

مقارنة بين طريقتين للكشف عن تكوين الأغشية الحيوية لبكتيريا *Pseudomonas aeruginosa* المعزولة من عينات سريرية مختلفة

نهى عبد الرزاق حميد عبد المجمعى¹ أ.د. هيام عادل ابراهيم الطائي²
1,2 جامعة الموصل/ كلية العلوم/ قسم علوم الحياة

الملخص

تتسبب بكتيريا *Pseudomonas aeruginosa* بالعديد من حالات العدوى لدى الانسان، ومعظمها متعلق بالجوانب الطبية. علاج هذه البكتيريا يزداد صعوبة في المستشفيات بسبب انتشار المقاومة المضادات الحيوية. وتعد المشكلات المتعلقة بالبيوفيلم (Biofilm) مع عدوى *P. aeruginosa* واحدة من تحديات العلاج الرئيسية. يضيف التركيب المعقد للأغشية الحيوية لـ *P. aeruginosa* إلى القدرة المسببة للأمراض لهذا الميكروب، مما يزيد من قدرته على التهرب من الجهاز المناعي، مما يسبب التهابات مستمرة يصعب علاجها وتؤدي إلى فشل العلاج. في هذه الدراسة، سوف نقوم بتقييم قدرة *P. aeruginosa* على إنتاج الأغشية الحيوية (Biofilm) من خلال طريقتين: طريقة أجار كونغو الأحمر (CRA) وطريقة صفيحة المايكوتايتر (MTP).

الكلمات المفتاحية: الأغشية الحيوية، أجار الكونغو الأحمر (CRA) وطريقة فحص لوحة العيار الدقيق (*P. aeruginosa*، MTP)



Introduction

Pseudomonas aeruginosa is widely recognized as one of the most prevalent Gram-negative bacterial infections. *P. aeruginosa* has been identified by the World Health Organization (WHO) as one of the top three priority infections in need of urgent treatment research (Tacconelli *et al.*, 2018; Killough *et al.*, 2022; H Mohamed *et al.*, 2023). *P. aeruginosa* is a significant contributor to both mortality as well as morbidity in immune-compromised patients, especially those with cystic fibrosis or CF, serious burns, or tumours. *P. aeruginosa* poses a significant threat as a nosocomial infection in these patients, mainly due to its ability to produce various virulence factors, including Biofilm (Oren & Garrity, 2021; Killough *et al.*, 2022). One of the most important *P. aeruginosa*'s virulence factors are Biofilm, which is formed of surface-adjusted aggregates of bacteria integrated with self-made extracellular polymeric substances (EPS), which allows the pathogens to attach to diverse surfaces, protecting them from shear forces, dehydration, and the host's immune response, which includes natural killer cells, phagocytes, complement, and reactive oxygen species ROS damage. Also the Biofilm which decreases the probability that the bacteria will escape the immune cells as well as antibiotics within the Biofilm and serves as a useful defense against the host immune system and antibacterial compounds, resulting in continuous colonization leading to treatment failure (Bonomo & Szabo, 2006; Szabó *et al.*, 2008; Behzadi *et al.*, 2021).

Numerous techniques, including tissue culture plate (TCP), tube method (TM), Congo Red Agar method (CRA), modified CRA method (MCRA), bioluminescent test, piezoelectric sensors, and fluorescent microscopic inspection, have been described and used to identify the formation of biofilms. Nevertheless, not every one of these techniques can be applied in standard clinical labs, and research comparing various techniques to identify biofilm development (Panda *et al.*, 2016). The main objective of this study was to compare two methods (MTP and CRA), which can be used in a routine clinical laboratory to detect biofilm formation by *P. aeruginosa*.

Material and method

3.2.3 Study approval

The patients volunteered to participate and received written informed assent. The study received approval from the Nineveh-public health department in Mosul. All samples were obtained and analyzed in accordance with the Ethical Committee regulations.

3.2.4 Bacterial isolation and identification



3.2.4.1 Collection of samples

A total of (122) specimens were obtained from (wound swab, burn swab, sputum, and urine) of patients were hospitalized at: Al-Jamboree Teaching hospital, Al-Salam Teaching Hospital, Burns Specialist Hospital and Public Health Laboratory in Mosul as presented in table (1). Specimens' collection was started from March 2021 to mid-May 2021. All specimens were taken from both sex and different ages, and via disposable sterile cotton swabs (or disposable urine cups) and immediately transferred to the Laboratory for cultivating and identification.

Table (1): The source, number, and places of clinical specimen collection used in the study.

Source of clinical specimens	Number of clinical specimens	place
UTI	48	Al-Jamhoree Teaching hospital, Al-Salam Teaching Hospital, Burns Specialist Hospital and Public Health Laboratory
Burns	26	
Wounds	37	
Sputum	11	
Total	122	

3.2.4.2 Isolation of *Pseudomonas aeruginosa*

The specimens were cultured on Cetrimide, Nutrient, MacConkey, and blood agar media, then incubated for (24) hours at (37C°). The identification of the isolated bacteria was conducted based on their colony morphological characteristics, size, shape, color, odor, and pigment production, then subjected to a different biochemical test for identification to the species level as represented by Bergey Manual for Systematic Bacteriology (Bergey *et al.*, 2001).

3.2.4.3 Identification of *Pseudomonas aeruginosa*

All isolated were identified depending on the morphological, microscopic, biochemical test, API 20E and Vitek 2 system.

3.2.5 Identification and characterization of biofilm-forming bacteria

3.2.5.1 Congo Red Agar (CRA) method

The CRA method was used to detect the bacteria's ability to form biofilms. The medium for this test was prepared using sucrose 50g/L, and Congo red (0.8 g/L) stain was added to Brain Heart Infusion agar. The bacteria were cultured on



the CRA medium and incubated at 37 °C for (24) hours. A positive result reveals either a high production of slime with a rough black color or a moderate production of slime with mild black colonies on CRA, while the biofilm non-producing (negative) isolates produced dark pink or red colonies (Freeman *et al.*, 2010).

3.2.5.2 Microtiter plate (MTP) Assay method

MTP assay is a quantitative method for detection the production of biofilm by a microplate reader (Teodósio *et al.*, 2013). Biofilm formation assay was conducted as follows:

- 1) The bacterial isolates were suspended to match the McFarland No.0.50 turbidity standard and then inoculated into Trypticase soy broth containing 1% glucose. Then incubated at 37°C for 18-24 hours.
- 2) After incubation, the bacterial culture was diluted by mixing (50µl) of bacterial broth with (950µl) of tryptic soy broth (TSB) supplemented with 1% glucose, The first three wells were filled only with TSB with 1% glucose as a negative control, and then each 3 wells replicate were loaded by 200µl bacterial suspension for each isolate of sterile 96-well polystyrene microplates.
- 3) The plate was carefully covered and incubated at (37 °C) for (24) hours.
- 4) After the incubation period, the plates were opened, and the liquid growth was drawn out from every well. Subsequently, the bacteria that did not adhere were eliminated using a series of three washes with 200-250 µl of PBS with pH= 7.2 using a suitable micro pipette and the MTP were drained in an inversed status until dried.
- 5) 200 µl of methanol was used to fix adhesive cells for 10 to 15 minutes.
- 6) To stain the biofilms, a volume of 200 µl of a 0.1% solution of purple crystal was added to each well and allowed to incubate for 15 minutes. Following staining process, the extra dye was eliminated by 2-3 washes washing with 200 µl PBS, and then the plate left to dry.
- 7) Subsequently, a volume of 200µl of 95% ethanol was introduced into each well and allowed to incubate for about (10) minutes. The test was performed in triplicate. The quantification of stain extracted by ethanol in every well was performed immediately using spectrophotometric (OD 630) using a microplate reader (Stepanovic *et al.*, 2007) The quantification of produced biofilm was determined as outlined in Table (2).

Table (2): Quantification of biofilm production (Stepanović *et al.*, 2007).



OD value	Biofilm production
$OD_s \leq OD_c$	Non
$OD_c < OD_s \leq 2 * OD_c$	Weak
$2 * OD_c < OD_s \leq 4 * OD_c$	moderate
$OD_s > 4 * OD_c$	Strong

*Cut off value (ODC) = Mean OD of negative Control +3 (Standard Deviation of control). * (ODs) = Optical density of sample.

3.2.12 Statistical analysis

The results of the study were statistically analyzed using the calculation program (SPSS.25V). By adopting the one-way analysis of variance (ANOVA) to determining significant differences between several groups, based on the identification of significant variation in the data. In this research, statistical tests were employed to analyze data, including the chi-square (X²) test is utilized to conduct a comparison of percentages. Duncan to compare means. The numerical data were represented using (Mean $\pm$ SD). In addition T-test was used to compare between two numeric variables and F-test (ANOVA) was used to compare between more than two numeric variables. * A p-value ≤ 0.05 was deemed significant (Daniel & Cross, 2018; Saleh, 2021).

4. Results

4.1 The isolation of *Pseudomonas aeruginosa*

In the current study, a total of (122) specimen were obtained from patients were hospitalized at Al-Mosul hospitals (Al-Jamboree Teaching hospital, Al-Salam Teaching Hospital, Burns Specialist Hospital) from the period from March 2021 to mid-May 2021 and from both ages and both sex. These specimens were collected from (wound swab, burn swab, sputum, and urine) using cotton swabs containing a carrier medium (transport media swabs) to ensure the viability of isolates during transferring them to laboratory.

Depending on cultural, microscopic, and biochemical identification, 49 (40.16%) of *P. aeruginosa* isolates were obtained, including 23/49 (46.93%) from urine, 12/49 (24.48%) from burns, 10/49 (20.4%) wounds, and 4/49 (8.16%) from Sputum samples. According to the results of this study, the highest percentage of isolation was found in urine samples (46.93%), followed by burn, wound, and sputum samples (24.48%, 20.4%, and 8.16%, respectively) as shown in Figure (1).

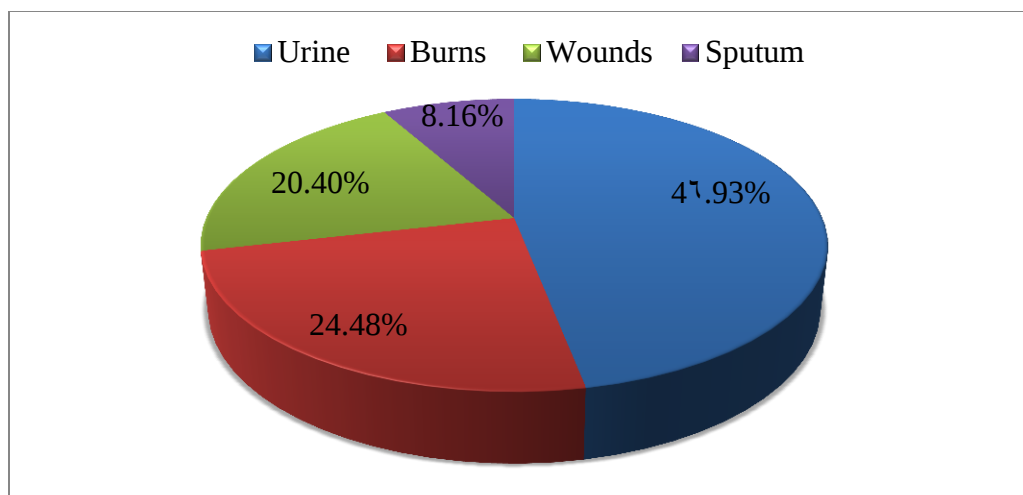


Figure (1): Prevalence *Pseudomonas aeruginosa* isolates in clinical specimens.

4.2 Identification of *Pseudomonas aeruginosa* isolates

The cultural characteristics of all the (49) isolates of *Pseudomonas aeruginosa* isolate on different media such as Nutrient agar (NA), Cetrimide agar, Macconkey agar and blood agar were illustrated in table (3) Cetrimide agar is a selective medium used for the isolating and identifying *P. aeruginosa* and can also be used to inhibit other *Pseudomonas species* (Carroll *et al.*, 2016; Tille, 2014).

Table (3): Cultural Characteristics of *P. aeruginosa*.

Culture media	Growth characteristics
Blood agar	White to gray color colonies, sticky textures bacteria and showed type of beta hemolysis figure (5).
Cetrimide agar	Smooth with flat edges and a high center, mucoid appearance, and fruity odor and the fluorescent green pigment Figure (3).
MacConkey agar	Small pale elevated colonies, non-lactose fermenting Figure (4).
Nutrient agar	Abundant, opaque, shiny, smooth, convex colonies Figure (2).



Figure (2): *P. aeruginosa* colonies on nutrient agar

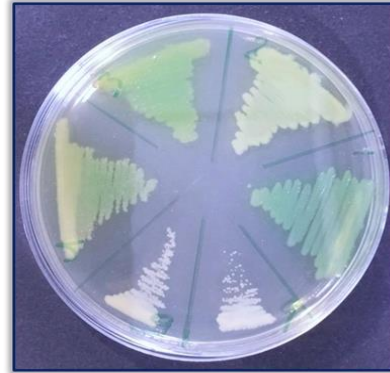


Figure (3): *P. aeruginosa* colonies on Cetrimide agar.

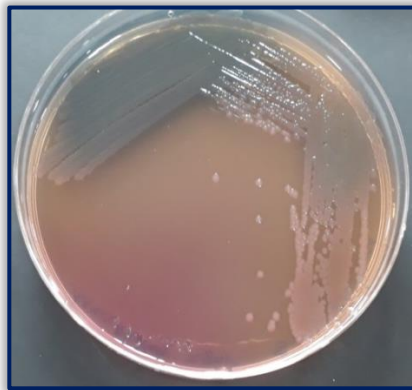


Figure (4): *P. aeruginosa* colonies on MacConkey agar



Figure (5): *P. aeruginosa* colonies on Blood agar.

Bacterial isolates were further identified and characterized by Gram staining. Microscopic examination of *P. aeruginosa* showed gram – negative straight rods - shaped cells, non-spore forming as shown in figure (6) (Brooks *et al.*, 2013).

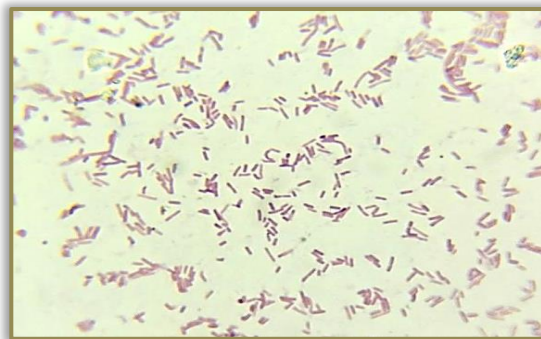


Figure (6): Gram negative *P. aeruginosa* examined by light microscope with 100x magnification power.

For further identification, the biochemical tests used to diagnose bacterial isolates including the catalase test and oxidase test. From the observation of the results of the biochemical tests of *P. aeruginosa* isolates, all isolates gave positive results to the catalase test by forming bubbles, and gave positive reaction to oxidase test indicated when the color changes to dark purple, as shown in table (4).

Table (4): Morphological, Physiological, and Biochemical tests for identification *P. aeruginosa*

Test	Gram stain	Blood hemolysis	Catalase test	Oxidase test
Result	-ve bacilli	+ (Beta)	+	+

4.3 Confirmation of identification of *P. aeruginosa* isolates

Biochemical tests results for identification of *P. aeruginosa* was confirmed by API 20E system. All the (49) isolates of *Pseudomonas aeruginosa* gave a high diagnosis results by using API20E diagnostic kit as shown in figure (7), but for the final diagnosis, the identification of (49) *P. aeruginosa* isolates was confirmed with the more sensitive and accurate VITEK2 System using the GN ID. In this study, diagnosis of Vitek2 Compact gave a probability ratio for the genus and species of *P. aeruginosa* bacteria between 97-99% for all the 49 isolates as shown in figure (8).

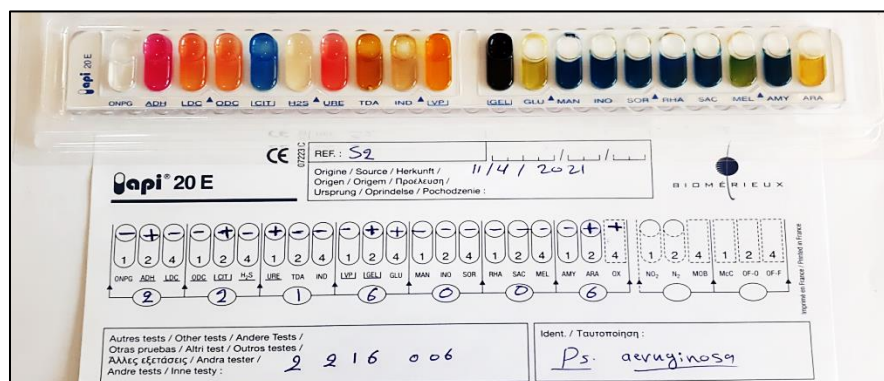


Figure (7): API 20E results for identification of *Pseudomonas aeruginosa*.



bioMérieux Customer: Dr. Radhwan Al-Jammas LAB. Printed January 6, 2022 12:20:09 AM
Microbiology Chart Report GMT-06:00

Patient Name: 15, . Patient ID: 78
Location: Physician:
Lab ID: 78 Isolate Number: 1

Organism Quantity:
Selected Organism : *Pseudomonas aeruginosa*
Source: swab Collected:

Comments:

Identification Information	Analysis Time: 5.80 hours	Status: Final
Selected Organism	99% Probability <i>Pseudomonas aeruginosa</i>	
ID Analysis Messages	Bionumber: 0003453103500252	

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	6	dCEL	-	7	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	24	LIP	+	25	PLE	-	26	TyrA	+	27	URE	-	28	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	38	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	49	IHISa	-	50	CMT	+	51	BGUR	-
58	O129R	+	59	GGAA	-	60	IMLTa	+	61	ELLM	-	62	ILATa	+			

Figure (8): Confirmed test report for the diagnosis of *P. aeruginosa* by VITEK2 system.

4.4 Detection of *P. aeruginosa* biofilm formation

The adhesion of *P. aeruginosa* to the host cells through the production of extracellular polysaccharides promotes biofilm formation and helps hide the bacteria from the host's defenses. Biofilm development increases antibiotic resistance and limits therapy effectiveness during infection (Bhandari *et al.*, 2022). From this point, two methods were applied to examine the potential of the *P. aeruginosa* isolates to produce biofilms:

4.4.1 Congo Red Agar (CRA) method Biofilm production

Congo red agar medium was used to demonstrate the isolates of *P. aeruginosa* capacity to produce biofilm. The isolate were cultured on CRA plates, prepared as mention in material and method. After the incubation for 24 hour at (37 °C), the biofilm formation by the bacterial isolate results was interpreted as in table (5).

Table (5): Biofilm formation results.

Strong biofilm formation	Black color rough colonies
Intermediate biofilm formation	Light black color colonies
No biofilm formation	Red or Pink smooth colonies

CRA test results showed that 31 out of 49 (63.27%) isolates were biofilm producer, whereas 18/49 (36.73%) unproductive isolates as shown in figure (10).

The appearance of colonies for 11/49 (22.45%) isolates were in black color on the Congo red agar (Figure 9) as an indication of their strong biofilm formation, two (14.29%) of them from the urine samples, 6 (66.7%) from burn samples, 1 (20%) from wound samples, and 2 (66.7%) of sputum samples. In terms of intermediate-producer of biofilm which were 20/49 isolates (40.82%), 12 out of 20 (85.71%) isolates from the urine samples, 3 (33.30 %) from burn samples, 4 (80%) from wound and 1 (33.30%) of sputum sample, as described in Table (7) summarizes the ability of the *P. aeruginosa* to produce biofilms based on the sample sources.



Figure (9): Congo red agar method: (A & C) Strong biofilm production; (B) Non biofilm production.

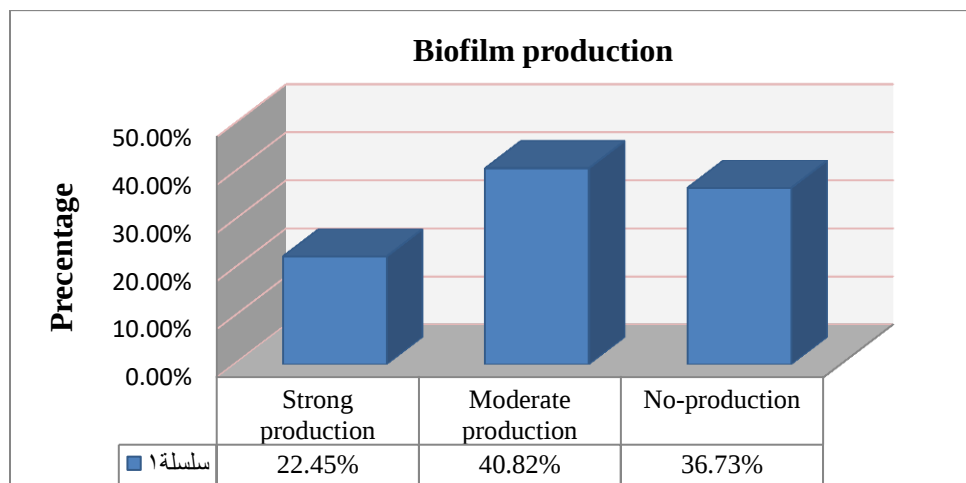




Figure (10): Percentages of biofilm formation for *P. aeruginosa* isolates by CRA method.

4.4.2 Microtiter plate Assay (MTP) method

The capacity of (49) *P. aeruginosa* isolates to produce biofilm was determined in this study using standard microtiter plates and reading in an automated ELISA reader, using a 630 nm wavelength, the absorbance of stained biofilms was measured. The optical density cut off (ODc) value was determined as mention in material and methods, and the biofilm production for each isolate was calculated by comparing the O.D of the isolates with O.D of Cut off value as listed in table (6).

Table (6): interpretation of MTP biofilm production.

ODs $\leq$ 0.05	No-biofilm production
0.05 < ODs $\leq$ 0.1	Weak
0.1 < ODs $\leq$ 0.2	Moderate
ODs > 0.2	Strong

Figures (11) and (12) present the quantitative biofilm determination using the microtiter plate assay (MTP). It was found that 44 (89.8%) of the total (49) isolates were biofilm-forming, of which 28.57% were strong biofilm producers, 48.98%, and 12.24% were moderate and weak biofilm producing isolates respectively. And only 5 isolates, i.e., (10.2%) were non-biofilm producers.

In regard to the distribution of *P. aeruginosa* isolates' capacity for production of biofilm and their sources of isolation using the Microtiter Plate (MTP) method, it was found Strong biofilm formation was recorded in sputum isolates (75%) then burn (50%), wound (22.22%), and urine (13.04%). The weak biofilm was recorded in urine and burn in percentage (21.74%) and (8.33%) respectively as illustrated in table (4.6).

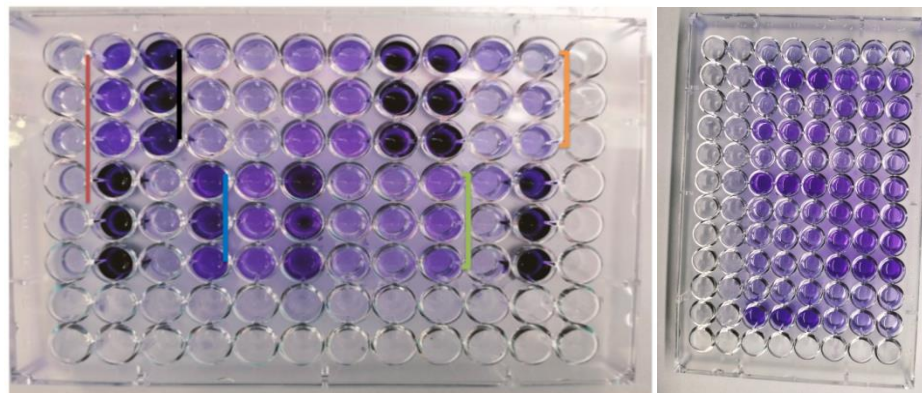


Figure (11): Microtiter plate (MTP) method results, Triplicate control are represented by the red line. A strong biofilm formed represented by the black line, a moderate biofilm formed represented by the blue line, a weak biofilm formed represented by the green line, and no biofilm formed represented by the orange line.

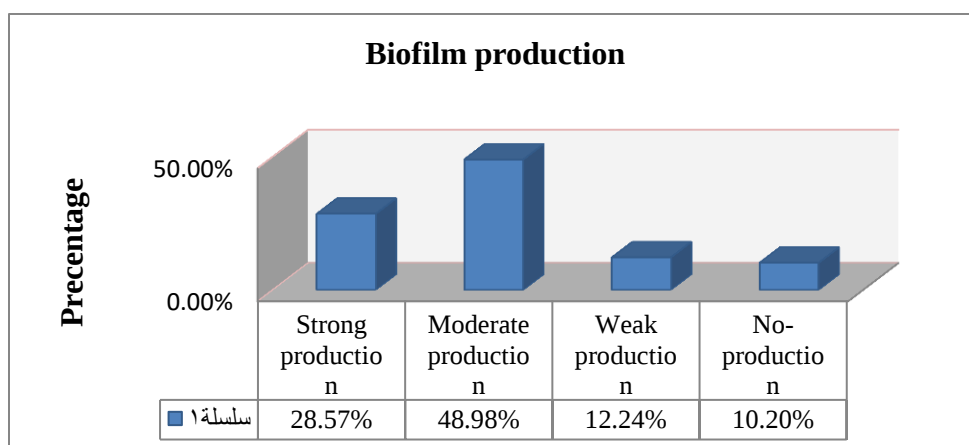


Figure (12): Percentages of biofilm formation for *P. aeruginosa* isolates by MTP method.

Table (7): The distribution of *P. aeruginosa* isolates' ability to produce biofilm and their isolation sources.



Isolation source	Isolate number	Biofilm production test								
		CRA				MTP				
		Biofilm production	Strong Biofilm production	Moderate Biofilm production	No- Biofilm production	Biofilm production	Strong Biofilm production	Moderate Biofilm production	Weak Biofilm production	No- Biofilm production
Urine	23	(14) 60.87%	(2) 14.29%	(12) 85.71%	(9) 82.61%	(19) 82.61%	(3) 13.04%	(11) 47.83%	(5) 21.74%	(4) 17.39%
Bum	12	(9) 75%	(6) 66.7%	(3) 33.3%	(3) 25%	(12) 100%	(6) 50%	(5) 41.67%	(1) 8.33%	0
Wound	10	(5) 50%	(1) 20%	(4) 80%	(5) 50%	(9) 90%	(2) 22.22%	(7) 77.78%	0	(1) 11.11%
Sputum	4	(3) 75%	(2) 66.7%	(1) 33.3%	(1) 25%	(4) 100%	(3) 75%	(1) 25%	0	0
Total	49	(31) 63.27%	(11) 22.45%	(20) 40.82%	(18) 36.73%	(44) 89.8%	(14) 28.98%	(24) 48.98%	(6) 12.24%	(5) 10.2%

The results obtained in CRA method was compared with those results of MTP method as shown in table (8). And from the result MTP methods was considered as more specific and sensitive in detection the biofilm formation than CRA method.

Table (8): Comparison of results from CRA and MTP method on *P. aeruginosa* isolated from different clinical samples.

Biofilm production test	Positive (Biofilm production)	Negative (No-biofilm production)	Total
CRA	31 (63.27%)	18 (36.73%)	49 isolate
MTP	44 (89.8%)	5 (10.2%)	

3.6 Discussion

In the present study, 49 (40.16%) of *P. aeruginosa* isolates were obtained, and the isolates were predominant in urine and burns, followed by wounds and Sputum samples. *P. aeruginosa* infection was greater in women aged 20-30 (36.74%) and 10-20 (24.50%). A study by Alsaady (2022) also shows a high prevalence of *P. aeruginosa* in urine samples (76.6%) from patients suspected of



UTIs, followed by (7.5%) wound samples and (5.4%) burns. At the same time, Hasoon (2021) reported that *P. aeruginosa* prevalence from different sources was high rate in burn samples (56.25 %), followed by wound (18.75 %), urine (5 %) and sputum (13.75). Silva *et al.* (2017) pointed out that the accurate identification of *P. aeruginosa* can be achieved by using the VITEK2 compact system. The VITEK 2 system is a fully automated tool that is easy to handle and provides a precise and rapid identification of clinically significant bacteria (Torres-Sangiao *et al.*, 2022).

CRA test results for biofilm production showed that 31 out of 49 (63.27%) isolates were biofilm producers, whereas 18\49 (36.73%) unproductive isolates. A Nader *et al.* (2017) study showed that 32/37 (86.49%) isolates with total black or light black color colonies were biofilm formation. The remaining isolates (5/36=13.51%) were red or pink, indicating non-biofilm formed. Mahdi R. J. (2020) found that 36 of 41 isolates (87.80%) were biofilm producers. Whereas 5\41 (12.19%) unproductive isolates. Also, the results mentioned by Al-kazrage (2021) on the qualitative biofilm formation number and the percentage of black color colonies were 18 (45%), moderate biofilm 16 (40%) and weak/no production 6 (15%) distributed among burning, wounds, sputum and otitis source.

Bacterial biofilm can be examined via a range of phenotypic techniques. The (CRA) test, devised by Freeman *et al.* in 1989, involves bacterial sub-culturing on brain heart infusion agar (BHIA) treated with Congo red stain and sucrose. Research findings have indicated that the accuracy of this approach is quite low. However, it has the advantages of cost-effectiveness and simplicity in execution. The assessment criteria primarily rely on visually examining the color displayed by the colonies growing on agar medium (Liberto *et al.*, 2009; Hassan *et al.*, 2011).

Additionally, The CRA technique is rapid, reproducible, and advantageous in that the colonies stay alive on the medium for subsequent analysis. The procedure is simple to perform, and the outcomes are often dependent on the developed color of the colonies that are formed, ranging from red for strains that do not produce biofilm to black for strains that do produce biofilm. The disadvantage of this approach is that it only provides qualitative information, which means it only assesses whether bacteria can form biofilms (Arciola *et al.*, 2002).

From the total of 49 clinical isolates, the MTP method detected 28.57% as strong, 48.98% as moderate, 12.24% as weak and 10.20% as non-biofilm producers. The present study's findings matched the results mentioned by the study of Al-kazrage (2021), who showed that the quantitative biofilm formation by



selected isolated using Microtiter plate assay (MTP) was 36 (90%) biofilm formations. Burns isolates formed the highest percentage of biofilm formation among the isolates, with (60%. Another study by ALrawi (2021) found that out of (100) *P. aeruginosa* isolates, (93%) were biofilm-producers, with 21 (21%) being Powerful biofilm-producers, 25 (25%) with moderate-biofilm producers, 47 (47%) were weaker-biofilm producers, and 7 (7%) were non-biofilm producers.

The MTP method is a Quantitative estimation of biofilm formation strength. The MTP method was considered superior to CRA (Hassan *et al.*, 2011). In microtiter plates, biofilm development is the most often utilized technique. Madilyn Fletcher created the MTP method in the beginning to examine bacterial attachment, and it was later shown to be compatible with the study of sessile development (Azeredo *et al.*, 2017).

The formation of biofilm by bacteria significantly contributes to their virulence and is essential in chronic and recurring infections. Biofilm formation renders bacteria more resistant to various environmental conditions, including changes in pH, exposure to antibiotics, oxygen radicals, disinfectants, and phagocytosis (Aparna & Yadav, 2008). The production of bacterial biofilms is closely related to the development of antibiotic resistance, facilitating the spread of genes for antibiotic resistance and increasing the rate of mutation within the bacterial cells (Niveditha *et al.*, 2012). The present study showed different ability to form biofilms using CRA and MTP methods. The results show that most *P. aeruginosa* isolates can produce biofilm, and MTP was more specific and sensitive than CRA in biofilm detection.

Conclusion

The current study concluded that compared to the TM and CRA methods, the TCP approach is a more quantitative and reliable technique to identify microorganisms that form biofilms and it may be used as a general screening technique to identify bacteria that produce biofilm in lab settings.

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