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Determine The Biofilm Formation and Antibiotic Resistance of Some Environmental and Clinical *Pseudomonas Aeruginosa* Isolates

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Abstract:

The purpose of the study was to investigate the biofilms formation of some environmental and clinical *pseudomonas aeruginosa* and their resistance to common antibiotics. In this study, 55 environmental water samples were collected between 13th till 30th January 2023 , from Al-Aobur water treatment station of Al-Baladiyat in middle of Baghdad, the old Rostumia project for sewage treatment of Diyala Bridge in eastern of Baghdad, and Central oil company in northern of Baghdad. Bacterial isolates were obtained and identified using standard diagnostic methods. From 700 environmental isolates were recovered, 102(14.6%) was *Pseudomonas* spp. At the same time, 130 clinical isolates of bacteria were collected in the baghdad hospitals including, Ghazi Al-Hariri Hospital, Al-Karkh General Hospital, Medical City Teaching laboratories and Al-Yarmouk teaching hospital. There were burns, wounds, blood, sputum, urine and pus as clinical sources. The clinical isolates collected between January and February 2023, were Gram-negative non-lactose fermenters, comprising 54 (41.5%) *Pseudomonas* spp. Genetic identification using 16S rRNA confirmed 52 (51%) of the environmental *Pseudomonas* spp. isolates as *P. aeruginosa*, Similarly, among the clinical isolates, 27(50%) were confirmed as *P. aeruginosa*. The environmental and clinical P. aeruginosa isolates were tested in regard to antibiotic susceptibility which showed high sensitivity in the environmental isolates. The level of resistance was greater with clinical P. aeruginosa isolates. The production of biofilm was established through Congo Red Agar (CRA) and Microtiter Plate (MTP). Among environmental isolates, 34 (65.4%) were biofilm-positive using CRA, while 18 (34.6%) were negative. For clinical isolates,12 (44.4%) positive,15 (55.6%) negative. In MTP method, strong biofilm formation was observed in 34(65.4%) environmental isolates, moderate 17(32.7%), and weak 1(1.9%). Among clinical isolates, strong biofilm formation was 4(14.8%), moderate 10(37%), and weak 13(48.2%). *Pseudomonas aeruginosa*, were constituted the largest proportion of other Pseudomonas spp isolated from environmental and clinical samples . Antibiotic susceptibility testing revealed high sensitivity among environmental isolates. Clinical isolates exhibited higher resistance levels. Environmental isolates were more productive biofilm, compare with clinical isolates.

Keywords: Antibiotic resistance, Biofilm, Congo red **,** *Pseudomonas aeruginosa*.

Introduction:

P. aeruginosa is a gram-negative, rod-shaped and most optimal at 37 o C bacterium. P. aeruginosa is known because of its fast growth rate and degrading properties (Lincopan et al., 2014). P. aeruginosa bacteria produce soluble dyes that are used in carrying of iron and mollify purulent infections. It cultivates non-lichen, non-algal, and fluorescent cultures on the media that contain the nutrients to be cultivated as well as the MacConkey agar (Jabłońska et al., 2023). It's a complex disease that causes multiple infections, the three stages are: adherence, invasion, and widespread disease (Quintieri et al., 2019). Clinical causes are composed of proteases, viral promoters of infection, Exotoxin A, lipase and phospholipase. Its colonies are characterized by the presence of pyocyanin. This chemical causes stress and promotes neutrophil death (Strateva and Mitov, 2011). Metabolic pathways and genes diversity in *P. aeruginosa* enables this bacterium to be very flexible and cause a wide range of human diseases. This is why it has been so difficult to get rid of it (Bassetti and Peghin, 2020). The bacterium *P. aeruginosa*, it is widespread in the environment and can cause severe illness in people with weakened immune systems, particularly affecting people with cystic fibrosis. Pseudomonas genus is also drug-resistant (Quintieri et al., 2019, Holger et al., 2021) The genus Pseudomonas is a wide-ranging group of bacteria with a very important ecological significance. Large numbers of members of this species are usually found in terrestrial and marine systems (Singh, 2017) Biofilm growth in environmental and pathogenic strains of Pseudomonas aeruginosa is a significant biological feature that makes them resistant to environmental and therapeutic conditions. A biofilm is a microbial community of bacterial cells attached to a surface (biological or non-biological) and surrounded by a self-produced polymeric substance known as EPS (extracellular polymeric substances) (Leid, 2009, Olsen, 2015). This structure helps bacteria: to resist antibiotic, resist the immune system and survive harsh environments. *P. aeruginosa* isolates from water and soil exhibit the ability to form biofilms, especially in humid locations These isolates typically produce a dense biofilm to aid attachment to surfaces. Clinical isolates are often isolated from patients with respiratory infections, wounds, or urinary tract infections. They show a high capacity to form biofilms, this biofilm promotes antibiotic resistance (Al-Daraghi and Al-Badrwi, 2020, Holger et al., 2021).

Environmental isolates often exhibit less resistance to antibacterial agents than clinical isolates, due to their reduced direct and repeated exposure to antibiotics (Nichols et al., 2010). However, some environmental isolates may exhibit acquired resistance due to environmental contamination resulting from the excessive use of antibiotics in agriculture or hospital wastewater (Gillings, 2017). Clinical isolates exhibit high levels of resistance, especially to common treatments, due to their constant exposure to antibiotics in hospitals. They possess multiple resistance mechanisms, including beta-lactamase production, efflux pumps, and changes in outer membrane permeability (Pachori et al., 2019).

Methods:

Environmental samples Collection:

Three stations were selected in the current study. These stations were Al-Aobur water treatment station in Al-Baladiyat in middle of Baghdad, the old Rostumia project for sewage treatment in Diyala Bridge in eastern of Baghdad, Central oil company in northern of Baghdad, the samples included water from these locations. Fifty-five water samples were collected from these different stations in the period between 13th till 30th January 2023. The samples collected by using sterile clean glass bottles, then transported from the field to the lab, where they were kept in an ice chest. Laboratory tests were done after sampling, and within 8-12 hours. Each of the samples was then diluted by decimal serial dilution between10 and 10 to the sixth part. The inoculation of aliquots (100 µL) on the nutrient agar plates was implemented in order to isolate bacteria according to the method described by (Osuoha, et al. 2019).

Clinical isolates Collection:

One hundred-thirty bacterial isolates, with a different source (burns, wounds, and biological fluids such as blood, sputum, urine, and pus based on the information that was recorded on the isolates) at Laboratories of Al-Karkh General Hospital, Ghazi Al-Hariri Hospital, the Medical City teaching Laboratories, and the Al-Yarmouk Hospital at Baghdad teaching Laboratories, were collected between January and February 2023. To isolate the bacteria, all the isolates were placed in nutrient agar plates.

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Bacterial Isolationation:

The plates of the nutrient agar were inverted and incubated at 37 0 C in 24-48 hours. The division of the types of bacterial colonies was based on color, shape, size, height, transparency, and the appearance. To obtain pure cultures, primary plates colonies were cultured in fresh nutrient agar plates. The former method was used to cultivate these subcultures in uniform conditions until pure colonies were produced. The Fifty-five water samples were collected during the current study from various locations in Baghdad distributed as: 12 samples (21.8%) from Al-Aobur water purification plant, 16 samples (29.1%) from the old Rostumia project for sewage treatment, 27 samples (49.1%) from Central Oil Company, as shown in Figure 1. On the other hand, the one hundred-thirty clinical isolates were collected according to the information recorded with the isolates, including isolates from burns (23) (17.7%), wounds (25) (19.2%), biological fluids like blood (18) (13.9%), sputum (15) (11.5%), urine (35) (26.9%), and puss (14) (10.8%) as shown in Figure 2.

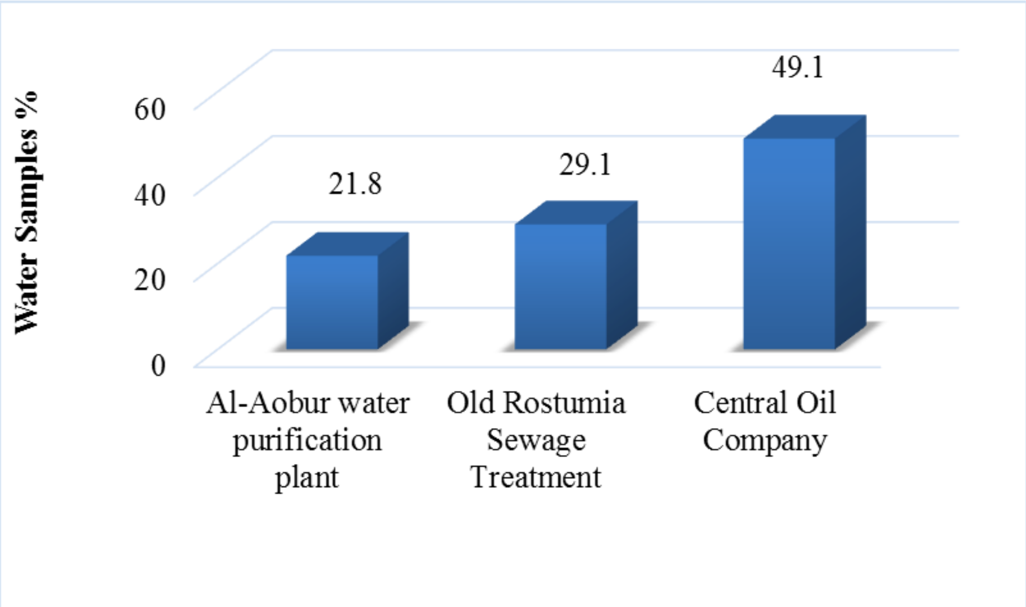

Figure 1. Distribution and percentage of the collected environmental samples

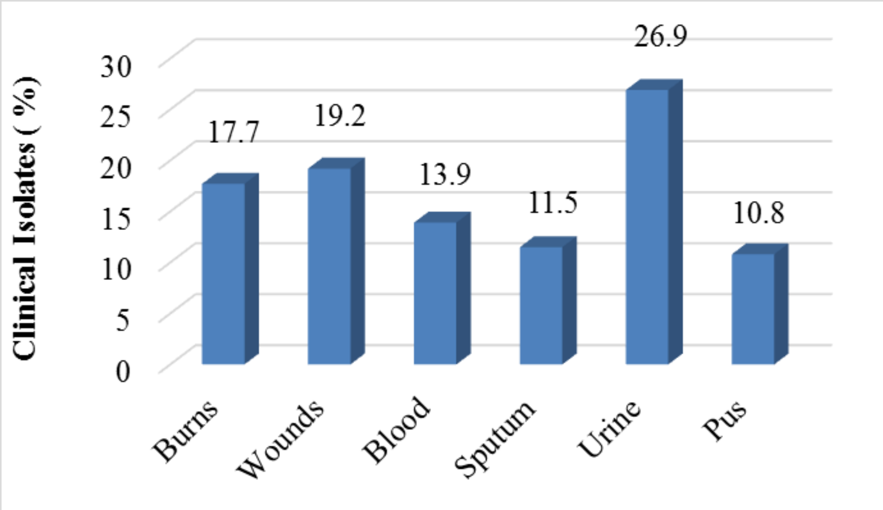

Figure 2. Distribution and percentage of clinical isolates

Bacterial Identification:

Both clinical and environmental bacterial isolates were undergoing morphological, microscopic, and biochemical tests to identification. As well, Molecular Identification was done by amplify the 16S rDNA gene (F-GGGGGATCTTCGGACCTCA and R-TCCTTAGAGTGCCCACCCG), the bacterial DNA was extracted according to (Rajabi et al., 2022). The program was including 5 min with 95° as an initial denaturation, 25 cycles each one consist of 40 sec with 95° as denaturation, 45 sec in 58° as an annealing step, and 60 sec in 72° as an extension step, and followed by 10 min in 72° as last extension step (Diggle and Whiteley, 2020). In this study, the concentration and purity of DNA in (ng/uzl) were measured on 2 ul of each sample by the thermoscientific Nanodrop spectrophotometer with a computerised interface and data storage which had, previously, been carried out on the blank sample in TE buffer (Desjardins and Conklin, 2010).

Antibiotics Susceptibility Tests:

The Kirby-Bauer method was used to check the susceptibility of various antibiotics to various antibiotics. P. aeruginosa P. aeruginosa strains Amikacin, gentamicin, Ciprofloxacin, Ceftazidime/Clavulanic acid, Piperacillin Ceftazidime/Clavulanic acid, Cotrimoxazole, levofloxacin, imipenem, ticarcillin/Clavulanic acid. There was a plate culture in the overnight culture transferred into a tube with (3 ml) of normal saline. The turbidity was set at (0.5 McFarland) tube equivalent to (1.5108 CFU/ml). In order to harvest the bacteria suspension, a sterile cotton swab was used to dip the bacterial suspension after which the excess moisture was wiped using the swab against the tube wall. The bacterial suspension was inoculated on Mueller-Hinton agar plates and the plates left to dry (10 min). Using sterilized forceps, the discs of the antibiotics were placed on the medium surface where the plates were incubated under (37 o C) within a period of 24 hrs. (Brooks et al., 2006). Millimeter (mm) measurement of the zone of inhibition around the discs was done with the help of the metric ruler after the incubation time and was compared with Clinical Laboratory Standards Institute (CLSI, 2022) and classified them as resistant(R), intermediate (I), and sensitive (S).

Observation under Congo Red Test:

Biofilm was detected using Congo Red Test.The obtained bacterial strains were placed on Congo red agar which was being prepared according to (Neopane et al., 2018) and left to incubate overnight at 37. The isolates were regarded as positive when the black colonies of a dry and crystalline nature appeared (Raksha et al., 2020).

Dioxins detected with Biofilm using Microtiter Plate Method:

Biofilm formation test was done on the microtiter plate according to (Neopane et al., 2018) by inoculating with (20 µl) of fresh bacterial suspension culture with (180 µl) of Brain Heart Infusion broth containing 2% sucrose. Two hundred thirty-three 200 2l -1. broth with 2-% broth sucrose were placed in control wells; the isolates were triplicated. After 24 h incubation of each of the wells at 37 o C, the wells were emptied and washed three times with 200 1 of distilled water (pH 7.2) after which, 200 1 of crystal violate (1%) were added into the wells and allowed to dry. Crystal violet solution was taken off and three times of the wells were washed using the distilled water to clear the unbound dye and subsequently allowed to dry at room temperature. We dissolved dye that was attached on adherent cells with the ethanol in 200 µl. The absorbance was then measured at 600 nm employing ELISA reader at the absorbance of the individual wells. The potential adherence of the test isolates of the bacteria were categorized into four groups; the mean optical density of negative control was considered the cut-off optical density (ODc) (Aziz et al., 2012).

Results:

Bacterial Identification:

All clinical and environmental isolates were subcultured on MacConkey agar, cetrimonium Pseudomonas agar as well as highly fluorescent Pseudomonas agar. Colonies are light pale, round, non-fluorescent, and saturate pyocyanin on MacConkey agar (non-fermentable lactose). Nonetheless, colonies are wrinkled and yellow-green on cetrimide agar because they produce pyocyanin. The colonies on blood agar were big, uneven, flat and brownish gray which stimulated formation of areas of beta-hemolysis. P. aeruginosa had a colorless and folded colony. Pseudomonas aeruginosa is an isolate based on the usage of HiFluoro Pseudomonas Agar Base. Bacteria use up the fluorescent compound, in the process emitting a fluorescent product. A visible fluorescent result (yellow-green) and fluorescence results in ultraviolet light were observed in this substance, as specified in the instructions of HiFluoro Pseudomonas Agar Base. Pseudomonas bacteria were identified to have a positive impact on various enzyme tests: catalase, oxidase, and positive results in growing in 42 o C, and in citrate, methyl red, and Voges Proskauer tests, whereas it was negative in Indole, methyl red, and Voges Proskauer assays, and at 4^{o}.

Out of seven hundred bacterial isolates from environmental sources, four hundred (57.2%) were recorded as Gram negative bacteria and the other three hundred isolates (42.8%) recorded as Gram positive bacteria. Out of the four hundred Gram negative bacteria, one hundred-two (14.6%) recognized as *pseudomonas spp* isolates. *Pseudomonasspp* is frequently present in wastewater from a variety of industries, including petrochemical, pharmaceutical, and food processing. These bacteria are known for their ability to degrade a wide range of organic pollutants, including hydrocarbons. They are highly versatile and often resistant to antibiotics (Iglewski, 1996). Conversely, 54 isolates (41.5) showed that they were Pseudomonas spp isolates out of one hundred and thirty clinical isolates. One such bacterium, which has a high rate of infection, a severe course, and rapidly spreads at the expense of unprotected immunology, is Pseudomonas spp(Lyu et al., 2023).

The PCR technique was used to confirm the bacterial identity. Generally, 52 (51%) isolates of environmental *pseudomonas spp* recognized as *Pseudomonas aeruginosa*, the other 40 (49%) isolates were recorded as other *Pseudomonas spp isolates*. As well as 27 (50%) of clinical *pseudomonas spp* isolates recognized as *Pseudomonas aeruginosa*.

The method of identification which is backed by the use of molecular genetic technique does not utilize the aspect of variety in phenotype and this gives a more precise identification in the species. The housekeeping

(16S rDNA gene) was identified by conventional PCR, this is suitable in the identification of P. aeruginosa. All organisms contain the 16s rRNA genes and they have species specific extensions with the help of which bacteria may be distinguished. PCR is a specific, accurate, and fast technique of identification of P. aeruginosa; most of the time, species-specific primers of

16S rRNA are used. Figure 3 showed a length of the amplified PCR products to be 956 bp.

To determine DNA Quality and Quantum of Pseudomonas aeruginosa with NanoDrop Spectrophotometer. The results justify high quality of the DNA recovered out of the RNA and proteins. The yield of a DNA genome (10200 ng/ml) with a purity of (1.8-1.9). Molecular identification on the current study depicted the value of using 16S rDNA PCR which utilized 16S rDNA to determine the diagnosis of the 102 isolates studied. It showed the size of the amplicon that was predicted (956 bp). This shows how effective the primers of the study are the exactness of the PCR machine (Desjardins and Conklin, 2010).

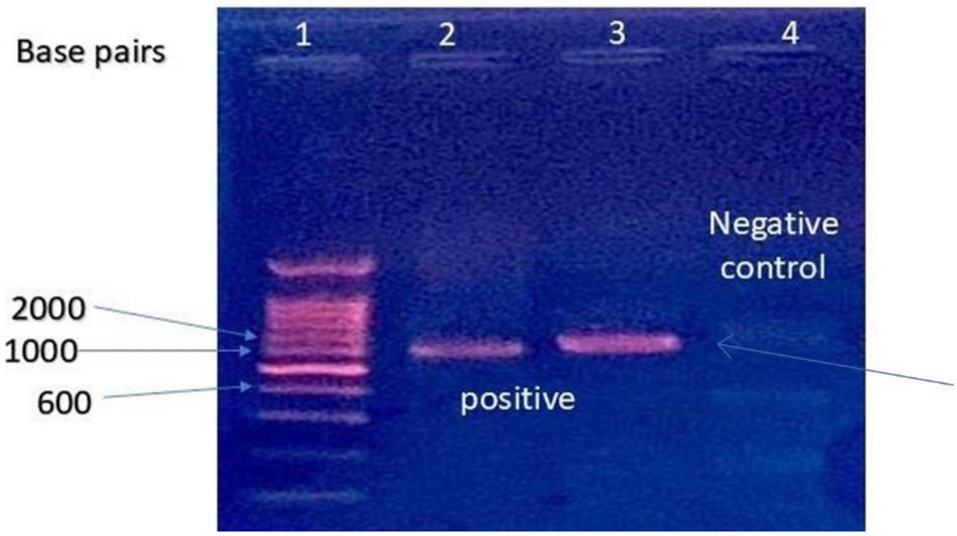

Figure 3: genotyping detection of bacterial identification

Antibiotic Susceptibility Tests:

The isolates of 52 environmental P. aeruginosa and 27 clinical P. aeruginosa were tested in terms of their susceptibility to tests traditionally implemented in disc diffusion (Kirby-Bauer) as illustrated in Figures 4 and 5. The apparent zone of inhibition size also enabled the determination of whether a bacterium was a resistant, intermediate, or sensitive bacterium to the specified recommended antibiotics. The results were taken into consideration based on the recommendations of (CLSI, 2022). The most sensitive antimicrobial disks, which belong to the different classes were observed to be very sensitive on most of the isolates of the environmental P. aeruginosa with as follows as the highest sensitivity to the lowest sensitivity: Imipenem (IPM) at (98.1%), then, Gentamycin (GEN) at (94.2%), Ciprofloxacin (CIP) at (92.3%), and Levofloxacin (LVX) at (69.2%), Piperacillin-tazobactam (TZP) at (61.2 %), Aztreonam (AZT) at (55.8 %) and Amikacin (AmK) at (50.0%) In contrast, Ceftazidime (CAZ) had the least sensitivity at (5,8%) (which showed the highest resistance rate at 92.3 %). While other antibiotics exhibited converging proportions of resistance as Ticarcillin-clavulanate (TIM) at (44.2%), and Piperacillin (PIP)at (40.4%) as in Figure 4.

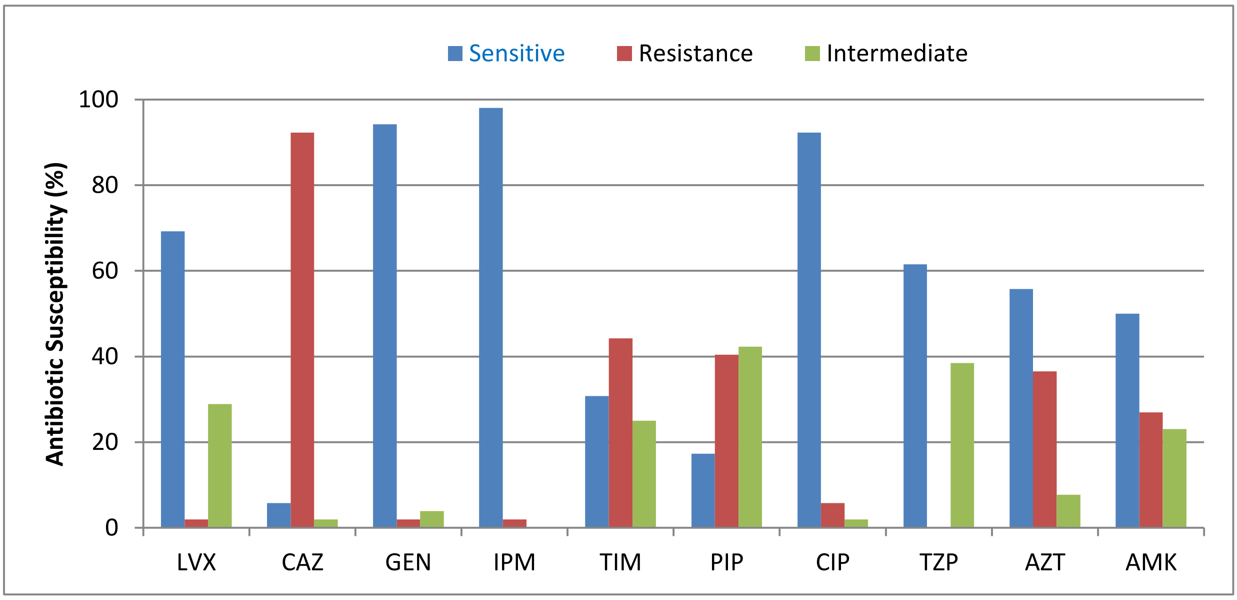

Figure 4. The percentage of antibiotic susceptibility tests of environmental *P. aeruginosa* isolates

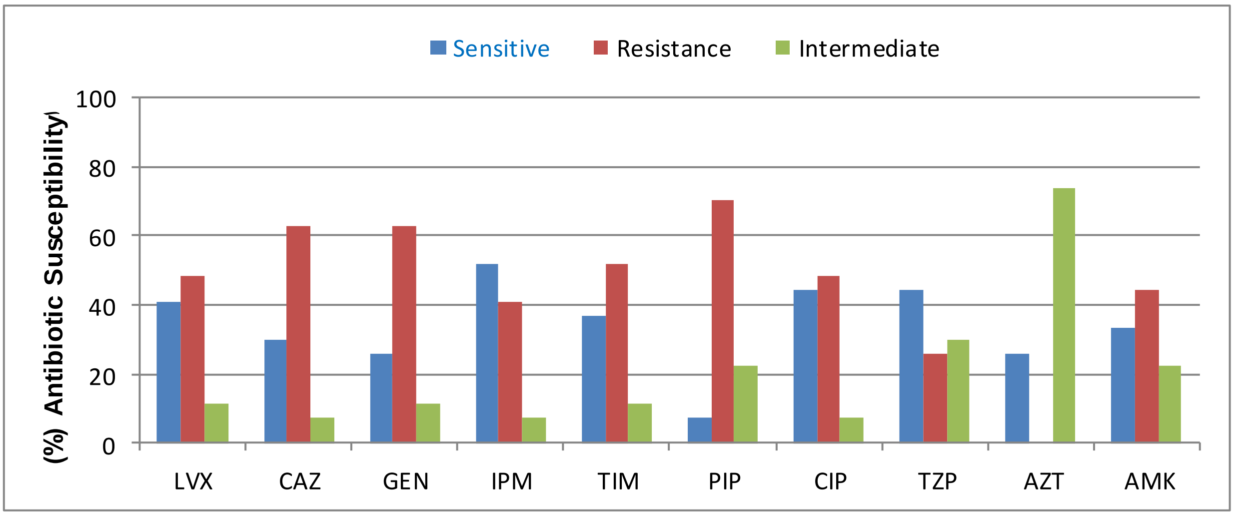

Figure 5 The proportion of all antibiotic susceptibility tests of the pathogenic isolates of *P. aeruginosa.*

Where PIP = Piperacillin, TZP = Piperacillin-tazobactam, TIM = Ticarcillin-clavulanate, CAZ = Ceftazidime, AZT = Aztreonam, IPM = Imipenem, GEN = Gentamycin, AMK = Amikacin, CIP = Ciprofloxacin, LVX = Levofloxacin.

The Clinical P. aeruginosa isolates also exhibited varying resistance to most of the antimicrobial disks, which represented the various classes. The outcome was as follows Piperacillin (PIP) was followed by Ceftazidime (CAZ) with (70.4%), then Gentamycin (GEN) followed by Ticarcillin-clavulanate (TIM) followed by Levofloxacin (LVX), Ciprofloxacin (CIP) and Amikacin (AmK). In contrast, Aztreonam (AZT) hadn't resistance (0 %). While other antibiotics exhibited different proportions of resistance as Imipenem (IPM) at (40.7 %), Piperacillin-tazobactam (TZP)at (25.9 %) as in figure 5. .Antibiotic-resistant *Pseudomonas aeruginosa* isolates can be found in various clinical samples, they are frequently identified in urine, wounds, and burns (Bakir et al., 2021).

Clinical isolates of *P. aeruginosa* often exhibit higher levels of antibiotic resistance compared to environmental isolates, primarily due to selective pressures in healthcare settings. Healthcare environments, such as hospitals and clinics, are characterized by the frequent use of antibiotics, creating a selective pressure that favors the survival and growth of antibiotic-resistant strains (Elfadadny et al., 2024). Environmental *P. aeruginosa* exhibited high sensitivity to antibiotics, the observed susceptibility levels align with previous studies such as Bhuiya *et al*, the study reported that 100% isolates showed sensitivity to levofloxacin, gentamicin, imipenem, piperacillin, amikacin, ciprofloxacin, and ceftazidime (Bhuiya et al., 2018) and Said et al reported sensitivity rates of IPM (78.9%), LVX (64.8%) CAZ (66.2) CIP (62%) GEN (52.1%) AMK (50.7%) in *P. aeruginosa* isolates (Said et al., 2023). This analysis indicated that this P. aeruginosa isolation had a similar tendency of the population having high resistance to ß-lactam. The values of the resistance levels (PIP 67.74 %, CAZ 64.51 %, IPM 35.4, TIM 45.16 %, TZP 25.8, AZT 3.22 %) seemed to be the following. It is worth noting that (Radwan et al., 2021) recorded a lower likelihood of (16%) of P. aeruginosa strains harboring a PIP resistance and (Alhayali et al., 2021) recorded a CAZ resistance probability at (35%). Additionally, (Wei et al., 2020), they documented that their strains of *P. aeruginosa* were unable to withstand any of the tested antibiotics, including Piperacillin-tazobactam, in their Chinese version. The resistance rates to aminoglycosides, including gentamicin (GEN) and amikacin (AMK), were 63.0% and 44.4%, respectively. This result was consistent with previous studies. (Attallah et al, 2024) reported a similar resistance rate to Gentamycin (GEN) and Amikacin (AK), were (61.29 %) and (45.16 %), they also reported of (51.61 %) for CIP and (48.38 %) for LVX. A local study by (Hassuna et al., 2020) also mentioned a higher resistance rate of (69.04 %) for CIP.

Biofilm Formation Detection:

Congo red agar method:

This was the potential of biofilm production by the 52 environmentally isolated P. aeruginosa strains that were cultured on CRA. The positive results were given by black and dry crystal-like colonies which showed strong biofilm formation. As demonstrated in Figure 6, 34 of the isolated (65.4 %) had the capacity to produce biofilm (positive), while 18 (34.6 %) lacked the ability to do so, as demonstrated by the color of the colonies or their lightness. Also, all 27 pathogenic *P. aeruginosa* isolates cultured on CRA, the results shown in Figure 7. 12 isolates (44.4%) positive, while 15 isolates (55.6%) negative.

Likewise, (63.27 percent) biofilm-positive and (36.73 percent) biofilm-negative isolates were found at Al-Mosul university (AL-Mojamaee and ALtaii, 2023). Nevertheless, there are also certain differences. whereas (Sultan and Nabiel, 2019) in Egypt had more non-biofilm producers (56.6 percent).

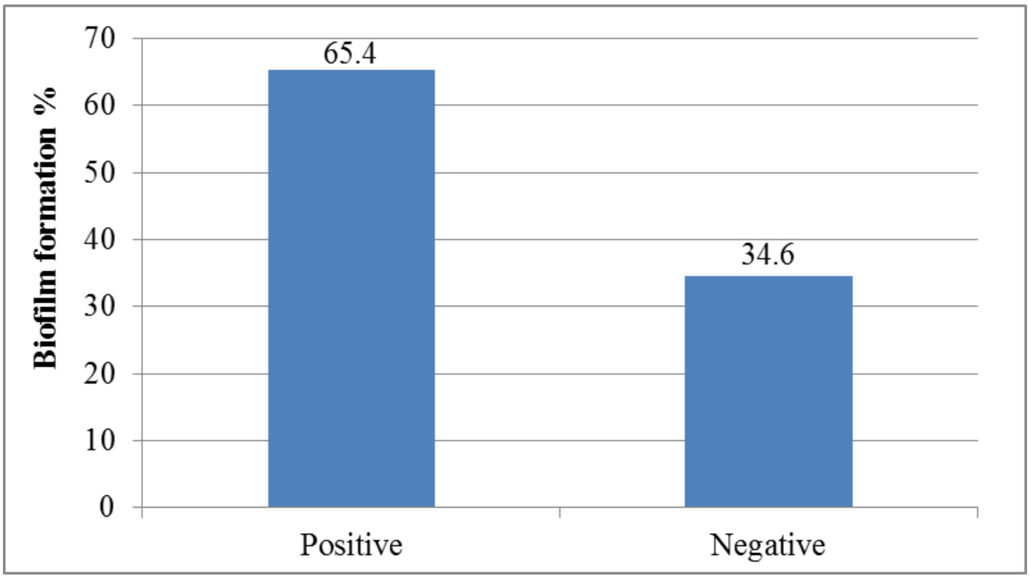

Figure 6. Biofilm formation of the environmental *P. aeruginosa* on Congo red agar

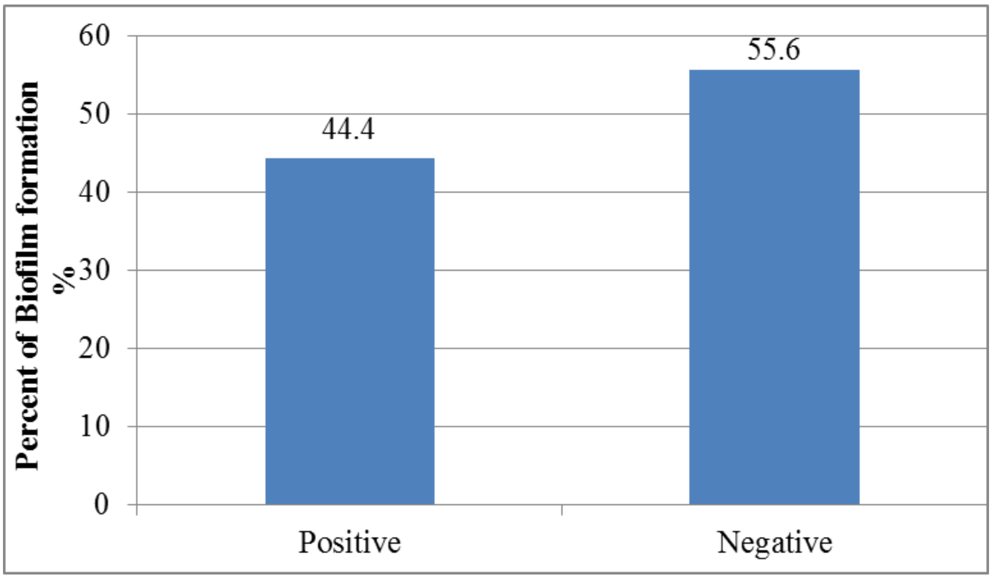

Figure 7. Biofilm formation of the clinical *P. aeruginosa* on Congo red agar

Microtiter plate method:

The MTP was used to examine the formation of biofilms by (52) environmental strains of P. aeruginosa. The analysis of the biofilm-stained attached bacteria under optical density (O.D) readings classified the biofilm formation as strong, moderate as well as weak. That is, all likelihood made biofilms were able to form and all the isolates obtained were positive. strong was the most common 34 (65.4 %), then there were Moderate 17 (32.7) and weak 1 (1.9) 19 Biofilm formation of (27) pathogenic P. aeruginosa isolates were also tested as well, and all the isolates collected in the research were positive and have the capability of biofilm formation. Figure 9 shows that the most common were Weak, 13 (48.2), Moderate, 10 (37) and Strong, 4 (14.8).

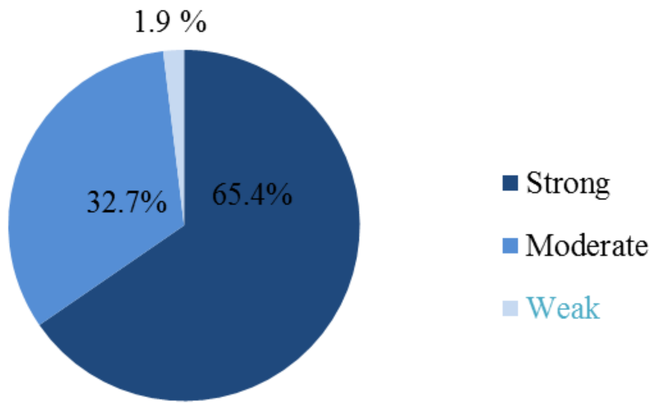

Figure 8. Biofilm formation of the environmental *P. aeruginosa* on microtiter plate method

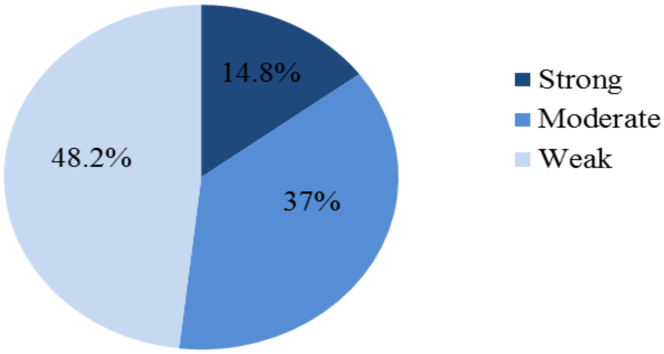

Figure 9. Biofilm formation of the clinical *P. aeruginosa* on microtiter plate method

The data regarding the production of biofilms in *P. aeruginosa* strains is in agreement with previous studies in the area. It was also reported that 19 (51 percent) were moderate producers, 12 (32 percent) weak producers, and 6 (16 percent) strong producers (Sattar and Rubaye, 2019). Another investigation demonstrated the existence of complex similarities. The researchers discovered that (93 percent) producer of biofilm was as follows (21 percent) strong biofilm producer, (25 percent) middle-range biofilm producer, (47 percent) weak biofilm producer and (7 percent) non-protein producer (Alrawi and Mahmood, 2022). The reason why environmental isolates produce biofilms at a higher rate than clinical isolates is due to several environmental, genetic, and physiological factors, including high environmental stress ,environmental isolates live in challenging environments (Nutrient deficiency , Changes in humidity and temperature ,Presence of natural or synthetic antimicrobials Competition with other microorganisms) , these conditions encourage *P. aeruginosa* to form a biofilm as a survival strategy because It protects it from harsh environmental conditions, It helps it attach to surfaces ,It facilitates cell-to-cell communication (quorum sensing) . Genomic studies show that some genes regulating biofilm formation (such as pel, psl, alg) are more active in environmental isolates compare with clinical isolates . Clinical isolates focus on other mechanisms of infection in the human body, bacteria may not always need to form a strong biofilm to survive or spread. Instead, they rely on toxins histolytic enzymes Immune system resistance . Therefore, clinical isolates may be less likely to produce biofilms because they invest their energy in other virulence factors

(Vetrivel et al ,2021)

Conclusions:

Environmental and clinical isolates of*Pseudomonas aeruginosa*, both were constituted the largest proportion of other *Pseudomonas spp*. Antibiotic susceptibility testing of environmental and clinical *P. aeruginosa* isolates revealed high sensitivity among environmental isolates. Clinical *P. aeruginosa* isolates exhibited higher resistance levels. Environmental isolates were more productive biofilm, compare with clinical isolates using CRA. In the MTP method, strong biofilm formation was observed in environmental isolates, followed by moderate biofilms and then weak biofilm. While clinical isolates, weak biofilm formation was observed in most isolates followed by moderate and a few isolates were strong biofilm formation.

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تحديد تكوين الأغشية الحيوية ومقاومة المضادات الحيوية لبعض العزلات البيئية والسريرية للزائفة الزنجارية *pseudomonas aeruginosa*

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مستخلص البحث:

الغرض من الدراسة هو التحري عن تكوين الأغشية الحيوية لبعض العزلات البيئية والسريرية للزائفة الزنجارية ومقاومتها للمضادات الحيوية الشائعة. في هذه الدراسة، تم جمع 55عينة بيئية مائية بين الثالث عشر الى الثلاثين من كانون الثاني 2023 من محطة العبور معالجة المياه قي البلديات في وسط بغداد ومشروع الرستمية القديم لمعالجة مياه الصرف الصحي في جسر ديالى في شرق بغداد وشركة نفط الوسط شمال بغداد . تم الحصول على العزلات البكتيرية وتشخيصها باستخدام طرق التشخيص القياسية. من 700عزلة بكتيرية بيئية تم جمعها، 102(14.6%) كانت لانواع بكتريا الزائفة. في جانب اخر، تم جمع 130عزلة بكتيرية سريرية من المستشفيات في بغداد، بما في ذلك مستشفى غازي الحريري، ومستشفى الكرخ العام، ومختبرات المدينة الطبية التعليمية، ومستشفى اليرموك التعليمي. وشملت المصادر السريرية الحروق والجروح والدم والبلغم والادرار والقيح. العزلات السريرية التي جمعت بين كانون الثاني وشباط 2023، كانت سالبة الجرام غير مخمرة للاكتوز، تضم54 (41.5%) من أنواع بكتريا الزائفة. التشخيص الجيني باستخدام 16S rRNA اظهر 52 (51%) من العزلات البيئية لبكتريا الزائفة كانت من نوع الزائفة الزنجارية، وبالمثل، من بين العزلات السريرية، تم تأكيد 27 (50%) على أنها زوائف زنجارية. كشف اختبار حساسية المضادات الحيوية لعزلات الزوائف الزنجارية البيئية والسريرية عن حساسية عالية بين العزلات البيئية. أظهرت العزلات السريرية مستويات مقاومة أعلى. تم تقييم إنتاج الأغشية الحيوية باستخدام طرق أجار أحمر الكونغو (CRA) وطرق صفيحة ميكروتيتر (MTP). من بين العزلات البيئية، كان 34(65.4%) إيجابيًا للغشاء الحيوي باستخدام CRA، بينما كان18 (34.6%) سلبيًا. بالنسبة للعزلات السريرية، 12(44.4%) إيجابية، 15(55.6%) سلبية. في طريقة MTP، لوحظ تكوين بيوفيلم قوي في 34(65.4%) عزلات بيئية، معتدلة 17(32.7%)، وضعيفة 1(1.9%) ومن بين العزلات السريرية، كان تكوين الأغشية الحيوية القوي 4(14.8%)، والمعتدل10 (37%)، والضعيف 13(48.2%). الزوائف الزنجارية مثلت النسبة الأكبر من أنواع الزوائف الأخرى المعزولة من العينات البيئية والسريرية , اختبار الحساسية للمضادات الحيوية اظهر حساسية عالية بين العزلات البيئية , العزلات المرضية أظهرت مستويات مقاومة عالية .العزلات البيئية كانت اكثر انتاجا للاغشية الحيوية مقارنة بالعزلات المرضية.

الكلمات المفتاحية :الزوائف الزنجارية ، مقاومة المضادات الحياتية، الغشاء الحيوي ،احمر الكونغو .