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Isolation and diagnosis of P. aeruginosa from wounds infection and antibiotics sensitivity and biofilms.

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Abstract

The present study aimed to isolate and diagnose *P.aerogenosa* bacteria from patients with wounds / males and females from different areas of Baghdad city (Mahmoudiya Hospital, Al-Kindi Teaching Hospital, General Medicine City Hospital Laboratory) by 200 swabs we got only 68 isolates of bacteria from P.aerogenosa the rest of the isolates were for various bacteria and were neglected. The isolates were diagnosed by biochemical tests, and Antibiotic Susceptibility test showed a marked variation in the pattern of susceptibility to different antibiotics. P. aeruginosa isolates displayed mixed levels of antibiotic resistance: piperacillin tazobactam 7/68 (10)% cefoperazone 13/68 (19)% amikacin 31/68 (45% cefipim 56/68 (82)% cefotaxime 57/68 (83)% ceftazidem 63/68 (92%) ciprofloxacin 27/68 (93% gentamicin 22/68 (32%) imipenem 58/68 (85%) Levofloxacin 38/68 (55%) Tegicycline 37/68 (54%) Amoxicillin 64/68 (94%) , only 52.94% of P.aerogenosa isolates were strong biofilm producers; while 19.12% and 27.94% of isolates were moderately productive and weak, respectively. Last biofilm data display that all isolate is produced in varied quantities.

Key word : Bacteria , Biofilms , Antibiotic , Antibiotic Susceptibility. *P.aerogenosa*

Introduction

According to the CDC's report from 2019, there is an increasing risk to modern medicine from infections caused by bacteria that are resistant to multiple drugs (CDC, 2019).Currently,over 700,000 individuals die each year from multidrug-resistant organisms, with a projected increase to 10 million deaths by 2050. This would surpass the number of deaths caused by cancer. In addition to improved management of antibiotics, addressing the issue of multidrug-resistant and Gram-negative bacteria through the development of new antimicrobial classes is vital in combatting this problem (O'Neill ,2016).Multidrug-resistant

Pseudomonas aeruginosa, which is a type of Gram-negative microorganism, is a significant issue when it comes to chronic and acute wound infections such as burns, particularly in healthcare settings like hospitals (Klockgether and Tümmler ,2016; Serra *et al* .,2015; Obritsch *et al*.,2005; Bodey et al.,1983). P. Aeruginosa is a bacteria that has a rod-like shape with a slightly or straight curved appearance. It is gram-negative and requires oxygen to survive. The bacteria are evenly pigmented and have a measurement of 0.5 to 1.0 micrometers in width and 1.5 to 5 micrometers in length. They are able to move using one polar flagellum and do not produce gas. Moreover, they cannot break down carbohydrates or use them as an energy source. (Uğur *et al*.,2012; Ibrahim *et al.*,2016).It can grow at a temperature of 40-4°C but not in 4°C some species which belong to pseudomonas can grow at temperature 45°C and grows well in neutral or slightly alkaline conditions pH (7.0–8.5),catalase, and oxidase-positive (Venkatakrishnan,2016).P. aeruginosa produces two types of soluble pigments pyoverdine and pyocyanin. The bacteria produce a lot of this blue pigment in low-iron conditions, and it helps with iron metabolism (Chua *et al*.2015). Another type from urine and respiratory secretions which have a mucoid look due to the presence of alginate, an exo-polysaccharide made up of guluronic and mannuronic acids. Flat and mucous colonies are thought to be important in pathogenicity and colonization (Lopez ,2017) One of the greatest opportunistic pathogens in humans is P. aeruginosa, which has numerous names during its history depending on its characteristic coloration in culture (Prabhurajeshwar, 2019). P. aeruginosa has become naturally or acquired resistant to many antimicrobial agents (Shams Eldeen *et al* .,2021).Biofilm formation is a virulence factor in P. aeruginosa that is produced by the accumulation of free-living cells and represents microcolonies bordered by P. aeruginosa exo-polysaccharides. In addition, P. aeruginosa causes both acute and chronic infections. It also has a natural resistance to several drugs, as well as the capacity to build a biofilm, a complex biological structure that renders immune defense systems and anti-biotherapy useless (Flemming *et al*.,2016) . When bacteria appear as a biofilm, they are far more resistant to

antibiotics than when they appear as planktonic cells. Bacteria that live as biofilms are far more resistant to biocides and antibiotics, and they process defense from environmental factors such as the host immune response attachment (Arciola *et al* , 2018).

MATERIAL AND METHODS CHEMICALS

1.SAMPLE COLLECTION

68 Various wound *P. aeruginosa* isolates from 200 wound swabs were obtained from males and female patients of different hospitals in Baghdad city including: Al-Mahmoudia general Hospital, AL-Kindi General Teaching Hospital and Teaching Laboratories in Medical City. The microscopically testing of *P. aeruginosa* isolates was done by using Gram stain examination under compound light microscope at 40X and 100X, Gram stain showed very small rods, single bacteria or in pairs and non-spore forming bacteria.

2. CULTURE MEDIA

Brain- Heart Infusion Broth, Cetrimide agar, MacConkey agar Man Rogosa Sharpe (MRS) agar , Mueller-Hinton agar , Nutrient agar & Nutrient broth, Pseudomonas agar, Transport medium and all other reagent grand chemicals were purchase from Hi media and sigma Aldrich , India.

RESULTS

1.Isolation and identification of p.aerogenosa

Cultural characterization all isolates of *P. aeruginosa* gave large flat colonies that produced zones of beta-hemolysis with a grape like odor on the blood agar, While on MacConkey agar pale colonies (lactose nonfermenting) small, round, convex, rough colony with irregular edges, whitish or creamy in color and has fruity odor. This bacterium was able to grow on Pseudomonas Cetrimide agar as a selective medium for *Pseudomonas* genus and also some isolates were able to produce pigments as pyocyanin and the fluorescent pigment "pyoverdine.

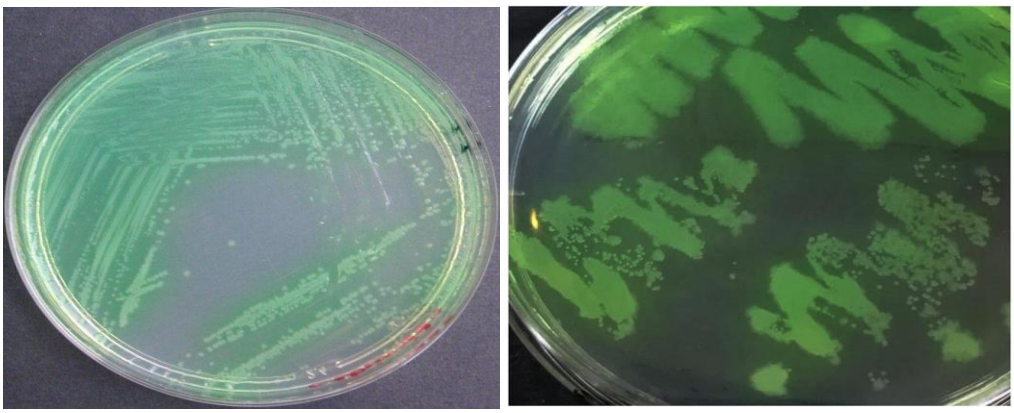

Figure (1): General characteristic of *P. aeruginosa* on, pseudo-cetrimide agar Identification of *P. aeruginosa* by VITEK 2 compact System.

2.Antibiotic susceptibility test for P. aeruginosa isolates

Antibiotic susceptibility test was conducted to the isolates of *P. aeruginosa* by using disc diffusion method to 12 antibiotics from different classes. Present data showed a marked variation in the susceptibility pattern toward different antibiotics. *P. aeruginosa* isolates showed a mixed levels of resistances to antibiotics:

Pipracillin tazobactam 7/68(10)% Cefoperazon 13/68(19)% Amikacin 31/68(45% Cefepime 56/68(82)% Cefotaxime 57/68(83)% Ceftazidime 63/68(92%) Ciprofloxacin 27/68(93% Gentamicin 22/68(32%) Imipenem 58/68(85%) Levofloxacin 38/68(55%) Tigecycline 37/68(54%) Amoxicillin 64/68(94%).

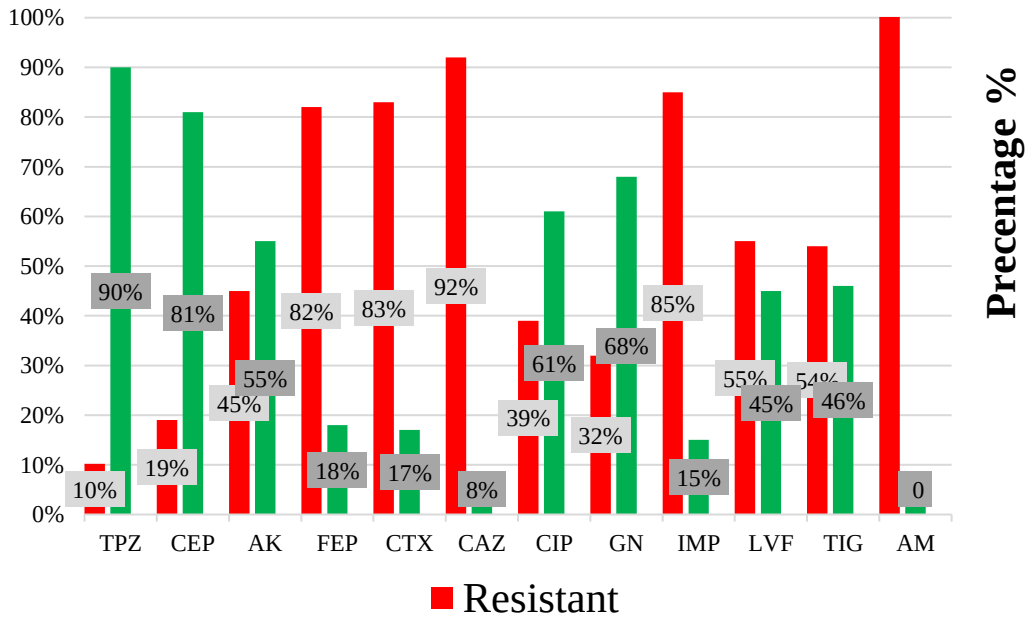

Figure (2): *P. aeruginosa* isolates isolated from wound swab susceptibility towards antibiotic

3.Determination of Biofilm formation of *P. aeruginosa* isolates

In Table 1 revealed that only 52.94% of *P. aeruginosa* isolates were strong biofilm producers; while 19.12% and 27.94% of the isolates were moderate and weak producers, respectively.

Table (1): Biofilm forming capacity of *P. aeruginosa* isolates

Production of *P. aeruginosa*	Percentage %	Number of isolates
Strong	52.94%	36
Moderate	19.12%	13
Weakly	27.94%	19
non-biofilm producer	0 %	0
Total	100%	68

DISCUSSION

P.aerogenosa can live different surfaces, and medical devices the use of its important binding factors, which includes pili flagella, and biofilms. and abundant in natural and artificial environments, with ponds, hospitals, and domestic sink drains (Remold *et al* .,2011) . Pseudomonas aeruginosa is an opportunistic pathogen that caused some infections in people, it has developed to be an crucial purpose of nosocomial infections and antibiotic resistance (Hendrie,1989;Shariati *et al.*,2018).

Pseudomonas aeruginosa may be targeted as one of the opportunistic bacteria related with healthcare infections, inclusive of ventilator-related pneumonia, in depth care unit infections, main line- related blood movement infections, urinary tract infections, operating point infections, crack infections, otitis media, and keratitis (Ito *et al* .,2021; Kalluf *et al*.,2017; Ramos *et al*.,2013; Tuon *et al* ., 2012) . From the above results it clouded nearby that pipracillin tazobactam was the most active drug followed by cefoperazon and Gentamicin as compared with other antibiotics. In difference with this result. Results would support some data found in local study done by Mahdi *et al.,* (2021) revealed that *P. aeruginosa* was the highest resistance percentages were found to Ampicillin (81.1%), Ceftriaxone and Amoxicillin-Clavulanic acid were (78.4%), Ampicillin-Sulbactam (75.6%), Cefepime (72.9%) Cefazoline (62.2%) then reasonable resistance to Ciprofloxacin (56.7%) and the lowest level of antibiotics was Amikacin (40.5%) which agreed with the results of the present study .

On the other hand, tobramycin and Amikacin were the highest active antibiotics since they noted a little resistance ratio, 33.3% and 38.8%

individually (Mahdi *et al* .,2019). Also, a study reported by Mahdi *et al.,* 2023) who found that *P. aeruginosa* resist Gentamicin (71.42%) that disagree with present study, but the resistant level of Cefepime (84.41%) agree with this study. *Pseudomonas aeruginosa* has showing various forms of resistance to a variety of antibiotics, mostly those that belong to the Multidrug-resistant (Mahdi *et al* .,2024). The very last biofilm truths show that every isolate is effective in a spread of quantities, Biofilm formation is taken into consideration as a developing procedure that consists of attaching and shifting on the floor, formation of micro colonies, maturation, and eventually spreading (Chua *et al*.,2015). Biofilm formation is idea to be a virulence indicator, and plenty of techniques were created or modified for biofilm research, resultant in a higher know-how of biofilm body structure, shape, and composition (Azeredo *et al*.,2017).

The biofilm matrix of P. aeruginosa is composed of DNA, proteins, lipids, alginate, and essential Exo-polysaccharides consisting a vital function in adherence of mobile surfaces (Ma *et al*.,2006).

Biofilm manufacturing via P. aeruginosa helps its endurance in the environment and might lead to risky bacterial infections, particularly in hospitalized patients (Abednezhad *et al.*,2023), in vitro biofilm manufacturing among scientific lines varies, and P. aeruginosa may be considered as strong, mild, weak, or no biofilm producers (Head and Yu,2004; Saxena *et al* .,2014). Biofilm formation, as an important virulence component, performs a critical function inside the pathogenesis of P. aeruginosa. It is able to protection microorganism from immune system clearance and from antibiotics (Tuon *et al*.,2022) .

CONCLUSION

P. aeruginosa isolates showed different levels of drug resistance to antibiotics and that some P.aerogenosa isolates were strong biofilm producers. The piperacillin tazobactam is very effective for P. aeruginosa .

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عزل وتشخيص P. aeruginosa من عدوى الجروح وتحديد حساسية المضادات الحيوية وتكوين الأغشية الحيوية.

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المستخلص

هدفت الدراسة الحالية الى عزل وتشخيص بكتريا P.aerogenosa من المرضى المصابين بالجروح / ذكوراً واناثاً من مناطق مختلفة من مدينة بغداد (مستشفى المحمودية ، مستشفى الكندي التعليمية ، مختبر مستشفى مدينة الطب العام) بواقع 200 مسحة حصلنا على 68 عزلة فقط من بكتريا من P.aerogenosa اما بقية العزلات فكانت لبكتريا متنوعة وقد تم هملها . وقد شخصت العزلات بواسطة الاختبارات الكيميائية الحيوية ، واظهر اختبار الحساسية تباينا ملحوظا في نمط القابلية تجاه المضادات الحيوية المختلفة. أظهرت عزلات P. aeruginosa مستويات متباينة من المقاومة للمضادات الحيوية: بيبراسيلين تازوباكتام 68/7 (10)٪ سيفوبيرازون 68/13 (19)٪ أميكاسين 68/31 (45٪ سيفيبيم 68/56 (82)٪ سيفوتاكسيم 68/57 (83)٪ سيفتازيديم 68/63 (92٪) سيبروفلوكساسين 68/27 (93٪ جنتاميسين 68/22 (32٪)إيميبينيم 68/58 (85٪) ليفوفلوكساسين 68/38 (55٪) تيجيسيكلين 68/37 (54٪) أموكسيسيلين 68/64 (94٪) و أن 52.94٪ فقط من عزلات P.aerogenosa كانت منتجة قوية للأغشية الحيوية؛ في حين أن 19.12٪ و 27.94٪ من العزلات كانت منتجة معتدلة وضعيفة على التوالي. وتظهر بيانات الأغشية الحيوية أن كل عزلة منتجة بكميات متنوعة.