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Molecular study among burn patients infected with bacterial *pseudomonas* species

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Abstract---The Invade of the Burns infaction by potentially pathogenic bacteria constitutes threatens its transmission to different human tissues and organs. A total number of 120 swabs in patient about 96/120 (80%) show positive culture of bacterial growth versus. 24/120 (20 %) show negative results for (No growth of bacteria) .A total of 96 sample were divided on the basis of(61/96)sample were Gram-negative bacteria and (35/96)sample were Gram-positive bacteria. this study provides isolation and identification of 37 isolates bacteria *pseudomonas* species for the 120 specimen(20/37)*pseudomonas.aeruginosa.10/37Pseudomonas.fluorescens.7/37Pseuomonas.putadia.*recovered from burn infections. In 120 patients according to Sex the specimens were collected from patients he suffer burns, to sex: female 64/120(53.34% samples) and male 56/120 (46.66 % samples) with age groups from 10-60 years. In this study, some of virulence factors were detected by traditional phenotypic methods. The results Bacterial Adhesion: showed *P. aeruginosa* 17/20 (85%). *P fluorescens 7/10(70%). P putadia* 4/7(57.14). most of bacteria isolate demonstrated Bacterial Adhesion:- The results showed that most isolates were able to adhere to surface epithelial cells. The results in of biofilm formation were indicated according to *Pseudomonas spp* to A total isolate *P. aeruginosa 20/20. P. fluorescence 9/10. P.putadia* 6/7. total 35/37(94.59%) strong respectively and 2/37 weak strong,form biofilms more readily in the burn wound environment. In addition, biofilm-associated cells are often more tolerant to antimicrobials than planktonic cells (Caraher *et al.,* 2006; Van *et al.*,2013)[1].[2], The result proteinase *P.aeruginosa*

20/ 20(100%) *Pfluorescens* *9/ 10(90%) P.putadia.3/ 7(42.85).P.aeruginosa* proteases can cause tissue damage during *P. aeruginosa* infections (Schmidtchen *et al.*, 2001)[3]

***Keywords*---**wound burns disease, Biofilm, Adherence activity, proteinase and Gene *Apsl* .

1. Introduction

Burns are one of the most common household injuries, especially among children. The term burn" means more than the burning sensation associated with this injury. Burns are characterized by severe skin damage that causes the affected skin cells to die (Herndon *et al.*,2018)[4]. The skin is the first immune system and large organ of the body.One of the main functions of the skin is protection as it protects the body from external factors such as microbes, chemicals and temperature (Church *et al.,* 2019)[5]The risk of burn injury are influenced by the types of burn, age of the patient, burn size, burn degree, and other chronic diseases (Borse *et al.*, 2018)[6]The skin contains gram positive bacteria located in hair follicles and sweat glands as normal flora, the intestine have gram negative bacteria that are normal flora no threat, however in immunosuppression burn patients they spread throughout the body by way of blood stream they can cause infection (Murray and Hospenthal, 2009)[7]

Pseudomonas species is a non-spore forming, non-fermentative Gram negative bacilli belong to the pseudomonadaceae family. and most of the cells possess a single polar flagellum(Kiska and Gilligan,1999)[8].The substrates that are known to support its growth ,type IV pili and flagella also have some function in biofilm formationas Type IV pili and flagella negative mutants have different biofilm architecture compared to wild type bacteria (Vasil and Iglewesk, 2008)[9] The alginate in biofilm formation protects from the act of phagocytosis by macrophages, but was not directly involved in attachment and formation of biofilm. Furthermore. (Tissot *et al*,2016)[10].Is a common Gram-negative, rod-shaped bacterium. It belongs to the *Pseudomonas* genus; 16S rRNA analysis has placed *P. fluorescens* in the *P. fluorescens* group within the genus.The alginate in biofilm formation protects *P. fluorescens* from the act of phagocytosis by macrophages, but was not directly involved in attachment and formation of biofilm. Furthermore.is a Gram-negative, rod-shaped, saprotrophic soil bacterium. Based on 16S rRNA analysis, *P. putida* was taxonomically confirmed to be a *Pseudomonas* species (*sensu stricto*) and placed, along with several other species, in the *P. putida* group, to which it lends its name. However, a recent phylogenomic analysis (Nikolaidis *et al.,*2020)[11]

2. Methods

The study was conducted at Bacteriology and Molecular Laboratories in Biology Department, Sciences Faculty, Kufa University, Iraq.

2.1 Patients and Clinical Specimens

A total of 120 burn samples were collected from patients burn in burn center in Najaf. Sader City ,AL-Zahraa Huspital,AL-Hakim, AL-Manathira and private clinics , raq during the period from September 2021 to February 2022. The patients included both sexes (female and male) and the age group ranged from 10 to 70 years.

2.2 Bacterial Isolates

The collected specimens were inoculated on three types of culture media which included blood agar and MacConkey agar, and spread on each plates with sterile loop. Plates were incubated at 37˚C for 24 hours. The plates were examined thereafter for bacterial growth and plates were then a single pure isolated colony was transferred to brain heart infusion agar for the maintenance and to submitted the morphological valuation by gram staining, carry out other biochemical tests and vitek -2 compact system that confirmed the identification of isolates.

2.3 Identification of Bacteria

The identification of *pseudomonas species* were carried out according to cellular morphology, culture characters and biochemical reactions that discribed in Collee, [12].

2.4 Vitek–2 for Confirme Identification

Gram Negative identification card was used for identification of enterobacteriaceae [13].

2.5 Phenotype Detection of Some Virulence Factors Capsule Production Detection

It was performed with used India ink stain to discover the capsule production of pseudomonas species, a single colony of bacterial growth is suspended in a drop of india ink stain and well mixed then distribution on glass slide [14].

2.6 Detection of biofilm formation activity

Semi-quantitative measurements of biofilm formation were determined using tissue culture-treated, 96- well polystyrene plates (microtiter plates MTP), based on the methods of Lizcano et al., 2010 [15].

2.7 Detection of **proteinase**

Skim milk agar plates (that was prepared as in step 3.2.5.3) were inoculated by streaking and incubated at 37ºC for 48 hours. The clear zone exists adjacent the positive result. That indicated the production of protease enzyme (Tille and Forbes, 2014)[16]

2.8 Detection of Adherence activity

Adherence activity was carried out according to (Johansson and Dahlén.,2018) [17].

2.9 *Detection of*Polymerase Chain Reaction(PCR) Assay :

The PCR assay was performed to detect the (PslA) genes for confirmation the identification of *Pseudomonas spp*,and to detect the virulence factors encoded genes

3. Results and Discussion

3.1 Virulence Factors of pseudomonas species

It is well known that the pathogenicity of *P. aeruginosa. P. fluorescens. ,P.putadia* is associated with many virulence factors. In this study, some of them were detected by traditional phenotypic methods.

3.1.Detection of proteinase

The results of the study demonstrated that the highly production of protease was release from *P.aeruginosa 20/20* (100%) *Pfluorescens 9/10(90%)P.putadia.3/7 (42.85)*

This result was consistent with a study by.(Khalil *et al.*,2015)[18] that recorded 85% protease activity among *Pseudomonas spp* isolates tested from different body sites with the highest percentage of protease activity (95%) reported in burn isolates. A study by(Shaaban *et al.*,2019)[19] investigating the prevalence of *lasB* virulence gene that code for protease enzyme in *P. aeruginosa* burn wound isolates found that 78.8% contain the gene

3.2.Detection of Biofilm Formation

The ability of all isolates of *P. aeruginosa 20/20. P. fluorescence 9/10. P.putadia* 6/7. Total 35/37(94.59%) strong respectively and 2/37 weak strong to form biofilm was detected by using microtiter plates (MTP). Biofilms were mesaered by quantify the absorbance of stained biofilms at 630 nm with a microtiter plate reader. The results in this study were indicated according to [20] The results revealed quantification of biofilm formation by microtiter plate method. A total isolate *P. aeruginosa. P. fluorescens. P.putadia.* total 18/18(100%) strong respectively isolates appeared high biofilm formation (strong positive adherence). (Table 2).

Table (2) Distribution of *P. aeruginosa. P. fluorescens. P.putadia.* Isolates According to the Types of Biofilm

Standar rang of OD (Salwa *et al.*, 2011)	Biofilm	Adherence	*P. aeruginosa* No. (%)	*P. fluorescence* No. (%)	*P.putadia* **NO.(%)**
<0.12	Non	Non	0 (0)	0 (0)	0(0)
0.12-0.24	Moderate	Moderately	3(30)	1 (25)	1(25)
>0.24	High	High	7 (70)	3 (75)	3(75)

Also the results indicated that *Pseudomonas species* isolates were the best form biofilms among other isolates its absorbance value was (100%), as shown in figure (2).

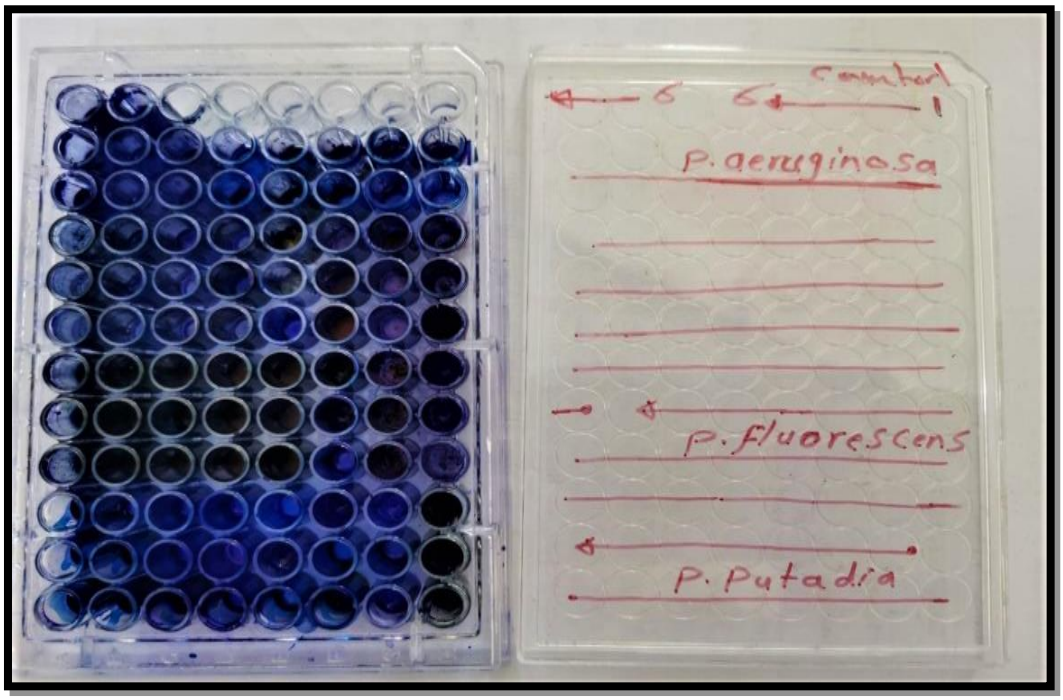

Figure (4-13): Biofilm formation of control (1-6). *P.aeruginosa(*6-53). *P fluorescens(54-74). ,P.putadia*(75-90)

3.3 Adherence to Epithelial Cells

The results showed that most *P. aeruginosa* 17/20 (85%). *P fluorescens 7/10(70%). P putadia* 4/7(57.14)isolates were able to adhere to surface epithelial cells. These observations seemed to agree with the findings of (;Das *et al.,*2020)[21], who reported the high ability of *Pseudomonas spp.* isolates to adherence to epithelial cells of skin.. Fimbriae or curli plays a vital role in attachment of the bacteria to the surface and gives a signal for initiation of microcolony formation(Ganesh *et al.,* 2019)[22].

3.4 Detection of pelA Gene

The results of PCR analysis to detected the presence gene of *PelA* gene (118bp) among of *P. aeruginosa* 9/10(90%) *P.fluorescens 3/4(75%) . P.putadia 3/4(75%)*,isolates revealed that 15/18 (83.33%) of isolates were contain *pelA* gene (figure 4-17). *Pseudomonas spp* can use either *psl* or *pel* as the primary biofilm matrix polysaccharide (Overhage *et al.,* 2005).[23]

Figure (4-17): Ethidium bromide-stained agarose gel electrophoresis of PCR products of *P. aeruginosa(1-11) P.fluorescens(12-18). P.putadia(19-23)* .using primer *pelA* gene (118bp). The electrophoresis was performed at 70 volt for 1.5-2hr. lane (L), DNA molecular size marker (100-bp ladder). Lanes (1 to 23 expet 11,12,14,19) show positive results with gene *pelA*.

4. Conclusion

1) The high frequency of specimens collection from patients infected with burns G-ve isolated are higher than G +ve .
2) Female is more frequent than Male and according to the age distribution of the specimens it appears that the highest frequency was 25.83% in age(10-20 years) (20-30years).
3) Released of many virulence factors such as biofilm protease, ,Adhesion, the phenotypic virulence of bacteria *pseudomonas spp*
4) Phenotypic and genotypic evaluation of remarks *P*seudomonas species the presence of *(,Pel-A,* The gene *Pel-A,* found of all pseudomonas spp

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