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Immunity 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, this study provides isolation and identification of 37 isolates bacteria *pseudomonas* species for the 120 specimen (20 pseudomonas.*aeruginosa.10 Pseudomonas. fluorescens.7 Pseuomonas.putadia*) recovered from burn infections. In this study, some of virulence factors were detected by traditional phenotypic methods. The results Bacterial gelatinase . The results of the study demonstrated that the high production of gelatinase was released from *P.aeruginos*a 20/20(100%, *P.fluorescens10/10(100%).p putedia 5/7(71.42%)* . the total *pseudomonas spp 35/37(94.59%)* isolates . The results haemolysin production In the blood agar plate method, 30/37 (80.08%) of *20/20(100%) P. aeruginosa*.9/10(90%) *p. fluorescens. and 1/7(14.28%) p.putedia* of all isolates demonstrate beta-hemolytic activity. Total and Deferential Count of White Blood Cells the results of WBC and differential cells. The results of WBC and differential count of neutrophils and monocytes in survivors burn patients have shown the significant increase ($P<0.05$) compared to control group. But the lymphocytes,eosinophils and basophils showed non-significant ($P>0.05$). Cytokines Detections Interleukin-6 the concentration of IL-6 in burn patients were of significant elevation ($P<0.01$) in compared to control group, yet the concentration of IL-6 showed a highly elevation burn patients, The coefficient for IL-6 was positive, indicating that the risk of mortality decreased.

***Keywords*---**wound burns disease, gelatinase, haemolysin, white blood cells count, cytokines.

Introduction

The skin is contain three barriers ,one of them a mechanical barrier (crinum layer) that enhance a dry environment not suitable for microbes growth and a chemical barrier which is provided a sweat and sebaceous glands that secretes salt or acidic material in addition to lysozyme that analyse cell membrane of microbes. The third is a biological barrier like microbes which live as normal flora on the skin that secrete fatty acids which inhabit growth of other microbes (1) A burn injury usually results from an energy transfer to the body. There are many types of burns caused by thermal, radiation, chemicals, or electrical contact (2)(3) Invasion of microorganisms into the tissue layers below the dermis lead to bacteremia or fungemia, sepsis and multiple organ dysfunctions (4) Bacteremia is the presence of bacteria in blood while septicemia is replicating bacteria to cause an infection. this leads to septic and causes a systemic inflammatory response syndrome bacteremia to progress to septicemia, especially if an individual has a weakened immune system (5)(6)The skin contains gram-positive bacteria that are normal flora and are found in hair follicles and sweat glands; the intestine contains gram-negative bacteria that are normal flora and pose no threat. However, in immunocompromised burn patients, they can spread throughout the body via the bloodstream and cause infection. (7)(8). Pseudomonas species is a non-spore-forming, non-fermentative Gram-negative bacillus of the pseudomonadaceae family. and the majority of the cells have a single polar flagellum (9)

The substrates that are known to support its growth ,type IV pili and flagella also have some function in biofilm formation. (10) as Type IV pili and flagella negative mutants have different biofilm architecture compared to wild type bacteria .(11)(12) 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 (13)(14) 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(14)

Methods

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

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 , Iraq 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.

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.

Identification of Bacteria

The identification of *pseudomonas species* were carried out according to cellular morphology, culture characters and biochemical reactions that discribed in Collee, [15].

Vitek–2 for Confirme Identification

Gram Negative identification card was used for identification of enterobacteriaceae [16].

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 [17].

Gelatinase Production:

Gelatinase activity was assessed using samples from single colonies inoculated onto agar containing 5% gelatin and incubated at 37°C for 24-48h. Gelatinase activity was evident as aclear halo around the colonies(18).

Haemolysin Production

The blood agar plates were inoculated with bacterial isolate, and then incubated at 37°C for 24-48 hr. Appearance of clear zone around the bacterial colony referred to β **–**hemolytic or green zone referred to α- hemolytic (19).

Complete Blood Count

The haematology parameter were performed on EDTA blood using (Roby-U.S.A.) in haematology laboratory . Ruby is a fully automated haematology analyser performing (CBC) on EDTA anti-coagulated blood. This instrument was used widely in human medicine. For counting the cellular blood component, the Roby uses the multi-angle polarized scatter separation (MAPSS) technique only. A measurement is made by laser light flow depolarization cytometer (20). The procedure carried out as follows:-

- One ml of blood was taken from patients and put into a test tube containing an anticoagulant (EDTA tube) to stop it from clotting.
- The blood is well mixed to mix the blood with anti-agglutination substance (though not shaken).
- A tube placed on a rack in the (Roby-U.S.A.) automated blood count.
- Automated blood count has many different components to analyze different elements in the blood; this instrument will pull the blood and then give result in one minute.
- The results will be printed out and stored in the resident memory.

Determine Levels of Cytokines

These tests were intended for quantification of serum levels of certain (IL-4 and IL-6) through the immuno enzymatic technique Enzyme-linked Immunosorbant Assay (ELISA) using Elisa washer and reader (Biotck-Japan) in immunology laboratory

Results and Discussion

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.

Gelatinase production

The results of the study demonstrated that the high production of gelatinase was released from *P.aeruginos*a 20/20(100%, *P.fluorescens10/10(100%).p putedia 5/7(71.42%)* . the total *pseudomonas spp 35/37(94.59).*This test was used to detect a microorganism's to generate gelatinase (a proteolytic enzyme) that liquefies gelatin, partial or complete inoculated liquefaction (21) as than figer3.1

Haemolysin production

In the blood agar plate method, 30/37 (81.08%) of 20/20 (100%) *P. aeruginosa*, 9/10 (90%) *p. fluorescens.* and 1/7(14.28) *p. putedia* of all isolates demonstrate beta-hemolytic activity as shown in figure (4-9), which is in agreement with (22) who stated that hemolysin is produced by 66.66% *P. aeruginosa* isolated from burns infections.

Total and Deferential Count of White Blood Cells

As shown in Fig. (4.7) and Table (4.11) the results of WBC and differential cells. The results of WBC and differential count of neutrophils and monocytes in survivors burn patients have shown the significant increase ($P<0.05$) compared to control group. But the lymphocytes,eosinophils and basophils showed non-significant ($P>0.05$) change compared to control group. This study was similar to that study of (23) who found an increasing number of neutrophils in the burn patients after injury. Further, it is correlated with the study of they obtained an

increasing count of monocytes after bacterial infection in patients who survivors. It agrees with. (24) who found an increasing number of neutrophils after burn wound infection.The results of WBC and differential count of neutrophils and lymphocytes showed the significant decrease (P<0.05).

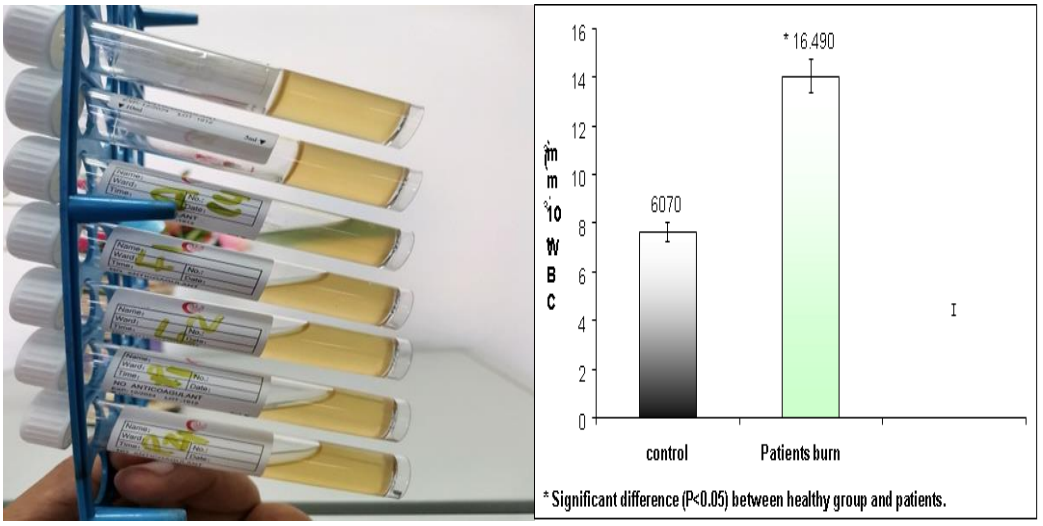

Figure (3.2): Total of Leukocyte Count in Healthy Control Group and Burn Patients

Table (3.1): Deferential Leukocyte in Healthy Control Group and Burn Patients. Significant difference(P<0.05) between healthy group and patients

Parameters mm^3)/($\times 10^3$	Control Patients (n = 10)	Patients burn (n = 110)	Normal volu
Neutrophils	4650	13100	2000-7000
Lymphocytes	2300	1590	800-4000
Monocytes	753	1370	120-1200
Eosinophils	95	70	20-500
Basophils	20	10	0-100

Interleukin-6

The current study revealed that the concentration of IL-6 in burn patients were of significant elevation (P<0.01) in compared to control group, yet the concentration of IL-6 showed a highly elevation burn patients, The coefficient for IL-6 was positive, indicating that the risk of mortality decreased The plasma levels of this cytokine are increased . Higher levels of IL-6 in patients with burn were observed compared tocontrol subjects, suggesting that this cytokine may possess a role in the pathogenesis of sepsis in burned patients. Same results was obtained from the results corresponds to results of (25) in U.S.A. that provided a significant increase in the concentration of IL-6 in the serum of burn patients after six days of burn injury and it reach to high concentration in patients. IL-6 plays an

important role in the normal development and maintenance of the human immune system. The activation of IL6 signalling pathway results in survival, proliferation, differentiation and maturation of haematopoietic cells. Human IL-6 is a major modulator of T-cell and B-cell homeostasis (26). Interleukin-6 has been shown to increase the proliferation of CD4+ and CD8+ T cells, as well as to reduce the spontaneous apoptosis of CD4+ and CD8+ T cells (28.29).

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 gelatinase, haemolysin, the phenotypic virulence of bacteria *pseudomonas spp*
4. Released White blood cells count,cytokines IL4,IL6 of all pseudomonas spp

References

1. Al-Dahash SY, Fathalla MA (2013). Veterinary Obstetrics. *2nd Edn., Mosul Iraq:* Book House for Printing and Publishing
2. Al-Mashhadani KA (2004). A Study on Diagnosis and Pathogenesis of *Pseudomonas aeruginosa* Isolated from Different Sources In Mosul City. Ph. D. Thesis. Mosul UniversityCollege of Science
3. Al-Rawi, M.A.A.; Mansel, R.E. and Jiang, W.G. (2003). Interleukin-7(IL-7) and IL-6 receptor (IL-7R) signalling complex in human solid tumors. Histol Histopathol., 18(3): 911-923.-74-
4. Anzai; Kim, H; Park, JY; Wakabayashi, H; Oyaizu, H; et al. (Jul 2000). "Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence". Int J Syst Evol Microbiol. 50 (4): 1563–89.
5. Arena, G.; Cordova, S.; Gavin, A.; Palamara, P. and Rimajova, M. (2014). Injury in Western Australia: A review of best practice, stakeholder activity, legislation, and recommendations. Perth: The University of Western Australia.
6. Bohannon, J., W. Cui, R. Cox, R. Przkora, E. Sherwood, and T. Toliver-Kinsky. 2010. Prophylactic treatment with fms-like tyrosine kinase-3 ligand after burn injury enhances global immune responses to infection. J. Immunol. 180: 3038– 3048
7. Bohannon, J., W. Cui, R. Cox, R. Przkora, E. Sherwood, and T. Toliver-Kinsky. 2010. Prophylactic treatment with fms-like tyrosine kinase-3 ligand after burn injury enhances global immune responses to infection. J. Immunol. 180: 3038– 3048
8. Brooks, C., Carroll, A., Gillies, R., and Hattie, J. (2019). A matrix of feedback for learning. Austr. J. Teach. Educ. 44, 14–32. doi: 10.14221/ajte.2018v44n4.2
9. Church, D.; Elsayed, S.; Reid, O.; Winston, B. and Lindsay, R. (2019). Burn Wound Infections. Clinical Microbiology Reviews, 19(2): 403-434
10. Collee, J.G.; Fraser, A.G.; Marmiom, B.P. and Simmon, A. (1996). Mackie and McCarteny Practical Medical Microbiology. 4th ed Churchill Livingstone Inc., USA Corvec.

11. Coque, TM.; Baquero, F. and Canton R .(2008). Increasing Prevalence of ESBL Producing *Enterobacteriaceae* in Europe. Euro Surveill. 13(47):1-11.
12. Doan, T.; Melvold, R. Viselli, S. and Waltenbaugh, C. (2008). Text book of Immunology. Wolters Kluwer Health, Philadephia: 25-38.
13. Faure, K.; Shimabukuro, D.; Ajayi, T.; Allmond, L. R.; Sawa, T and Wiener-Kronish, J. P. (2003). O-antigen serotypes and Type III secretory toxins in clinical isolates of *Pseudomonas aeruginosa*. *Journal of Clinical Microbiology, 41*(5), 2158-2160.
14. Forner, L.; Larsen, T.; Kilian, M. and Holmstrup, P. (2019). Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. J. Clin. Periodontol, 33(6): 401-407
15. Guido, F. and Pascale, F. (2005). Performance of the New VITEK 2 GP Card for Identification of Medically Relevant Gram-Negative ci in a Routine Clinical Laboratory. J Clin Microbiol, 43(1): 84-88.
16. Kao, C.C. and Garner, W.L. (2014). Acute burns. Plast Reconstr. Surg., 105(7): 2482-2492.
17. Kiska DL & Gilligan PH (1999). Pseudomonas and Bulkholderia. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC & Yolken RH (Editors), Manual of Clinical Microbiology. 7th edn. American Society for Microbiology, Washington, DC, USA.
18. Komolafe, O.O.; James, T. and Kalongolera, L. (2019). Bacteriology of burns at The Queen Elizabeth Central Hospital, Blantyre, Malaw: Burn, 29(3): 235-238.
19. Lundstrom, W.; Fewkes, N.M. and Mackall, C.L. (2012). IL-6 in human health and disease. Elsivier J. Seminars in Immunology, 24(3): 218-224
20. Mathin, I.; Lsabel, G.; Fadila, S.; Colin, S. and Rick, G. (2012). Abbott apromise for life. com. Casebook:1-91.
21. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., (2003), Manual of Clinical Microbiology, ASM, Washington, D.C.
22. Nikolaidis**,** Marios; Mossialos, Dimitris; Oliver, Stephen G.; Amoutzias, Grigorios D. (2020-07-24). "Comparative Analysis of the Core Proteomes among the Pseudomonas Major Evolutionary Groups Reveals Species-Specific Adaptations for Pseudomonas aeruginosa and Pseudomonas chlororaphis". Diversity. 12 (8): 289.
23. O'Connor, A.M.; Crawley, A.M. and Angel, J.B. (2010). Interleukin-7enhances memory CD8+ T-cell recall responses in health but its activity is impaired in human immunodeficiency virus infection.Immunology, 131(4): 525-536
24. Parales, R. E.; A. Ferrandez, A and Harwood, C. S. (Eds.). (2004). *Chemotaxisin Pseudomonas* (Vol. 1). New York: Kluwer Academic/Plenum Publishers
25. Pattanayak, M. K., Tomar, S., Gaur, A., & Goel, S. (2022). Phenotypic detection of multidrug-resistance pseudomonas aeruginosa isolated from various clinical samples. *International Journal of Health Sciences*, *6*(S6), 883–893. https://doi.org/10.53730/ijhs.v6nS6.9709
26. Ratnawati, I. G. A. A., Suandayani, N. K. T., & Sutapa, G. N. (2019). The linearity of x-ray devices radiation output and its relationship with patient thickness. *International Journal of Physical Sciences and Engineering*, *3*(3), 1–6. https://doi.org/10.29332/ijpse.v3n3.351

27. Siegel JD, Rhinehart E, Jackson M, Chiarello L. Management of multidrug-resistant organisms in health care settings, 2006. *Am J Infect Control* 2007; 35(10 suppl 2): S165–93
28. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). Health and treatment of diabetes mellitus. *International Journal of Health Sciences*, *5*(1), i-v. https://doi.org/10.53730/ijhs.v5n1.2864
29. Venet, F. (2007). Decreased monocyte human leukocyte antigen-DRexpression after severe burn injury: Correlation with severity and secondary septic shock. Critical Care Medicine, 35(8): 1910-1917
30. Yurt, R.W. and Pruitt, B.A. (1985). Decreased wound neutrophils and indiscrete margination in the pathogenesis of wound infection. Surgery, 98(2): 191-197