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Effect of some factors on extracellular hemolysin filtrate from bacterial *Pseudomonas aeruginosa* isolated from burn infection in Hilla city

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A total of (50) samples were collected from burn unit of Hilla Teaching Hospital, isolated from patient who suffering from burn cases only (40) (80%) samples were positive culture while other was negative culture (10) (20%), so, a total of (40) samples were isolated; these samples were subjected on different types of media to isolated *Pseudomonas aeruginosa*. Out of (40) samples, only (15) (37.5%) isolated were belonged to *Pseudomonas aeruginosa*. the ability of *Pseudomonas aeruginosa* isolated from patients with burn infection to produced capsule was studied; it was fond that all these isolated (100%) have capsules surrounding bacterial cell, and all isolated have not ability to produce sidrophores enzymes. Also it was studied the group of enzymes produced from bacteria, it was found that all these isolated have ability to produce lipase, protease, urease and bacitracin enzymes. On the other hand, the hemolysin production by *Pseudomonas aeruginosa* was studied and it was found that all these isolates of these bacteria were able to produce hemolysin on blood agar. Also, extracellular hemolysin filtrates of *P. aeruginosa* was detected on blood agar, it was found that (10) (66.6%) isolated of theses bacteria have extracellular hemolysin filtrates, it was appearance as transparent area around the well filled with hemolysin filtrates after incubation for 24 hours. The effect of H_2O_2 on extracellular hemolysin filtrate of *P. aeruginosa* was studied, and it was found that the H_2O_2 was decrease the activity of extracellular hemolysin filtrate on blood agar. So, the effect of mannitol and glycerol on hemolysin filtrate produce *by P. aeruginosa* was studied, and it was found that all isolates of hemolysin filtrate will increased activity when added mannitol and glycerol to it. Finally, the effect of phospholipase C on hemolysin filtrate produce by *P. aeruginosa* was studied, and it was found that all isolates of hemolysin filtrate will increase activity when added phospholipase C to it.

Key words : *Pseudomonas aeruginosa*, hemolysin, H_2O_2, glycerol, phospholipase C

Introduction:

Pseudomonas aeruginosa a gram-negative non-fermenting bacillus, and is an opportunistic human pathogen (Anzai*et al*., 2000). The organism is common in the environment, especially in water, even contaminating distilled water (Lin *et al*., 2006). *Pseudomonas aeruginosa* has been a growing prevalence of Gram-negative organisms as causes of serious infections seen in many hospitals. *P. aeruginosa* is among the most prevalent gram-negative organisms isolated from

diabetic foot wounds (Lipsky *et al*., 2004). Infection is a major complication of burn injury and is responsible for 50-60% of deaths in burn patients. It is promoted by loss of epithelial barrier of skin, malnutrition induced by the hypermetabolic response to burn injury and by a generalized post-burn immune suppression (Abston *et al*., 2000). Infection risk is directly correlated with the area of burn injury, the larger the injured area, the higher the risk of infection (Wibbenmeyer *et al*., 2006). Other contributors to infection include comorbid conditions, the use of invasive devices such as catheters and poor hand hygiene among health care staff (Hodle *et al*., 2006). Numerous *P. aeruginosa* virulence factors contribute to the pathogenesis of burn wound infections. Pilli and flagella are essential for their ability to persist in the burn wound and cause disseminated infections (Gillespie and Bamford, 2003). Protease promotes infection and dissemination, elastase degrades collagen and non-collagen host proteins and therefore elastase disrupts the host physical barriers which inhibit the spread of infections (Lyczak *et al*., 2000). *P. aeruginosa* liberates some toxic products including catalase, lipase, lecithinase, elastases, proteases and two hemolysins; a heat-labile phospholipase C and a heat-stable glycolipid, all of these are toxic and enable the organism to invasive and destroy the tissues (Kolmos *et al*., 1993). Protease, lipase, urease and asparaginase were important enzymes produced by *P. aeruginosa* as well as many other products like alginate which used in many biotechnological applications. There were an increasing interest in *P. aeruginosa* lipase and protease (Schäfer *et al*., 2005; Karadzic *et al*., 2006). Some strains produce bacteriocin with abroad spectrum activity, including important pathogens such as *Listeria monocytogenes* and *Pseudomonas species* (Cherif *et al*., 2001). Hemolysin of *P. aeruginosa* has a cytopathic action on blood and tissue culture cells (Dykes, 1995). Lysis and disintegration of the architecture of the cell involving membrane and cytoplasm were demonstrated by morphological changes (Delden and Iglewski, 1998). The hemolytic activity of hemolysin is inhibited by normal sera and by albumin; the hemolytic and cytopathic actions are explained by assuming that they alter the molecular architecture of the membranes, *P. aeruginosa*, hemolysin is responsible for the colonization of lung and other tissues (Cox *et al*., 2000).

Isolation *pseudomonas aeruginosa* from samples of patients with burn infection, detection of some virulence factors, so detection of extracellular hemolysin produced by them. Study the effect of some factors on hemolysin filtrates.

Materials and methods:

Patients: 50 samples of burn cases was collected from patients attended to Hilla Teaching Hospital through a period of three Months from November 2012 to January 2013).

- **Capsule production**: It was done according to Balowes *et al*. (1991).
- **Protease production**: It was done according to Piret *et al*. (1983).
- **Lipaese production**: It was done according to Collee *et al*. (1996).
- **Sidrophore production**: It was done according to Nassif and Sansonetti (1986).
- **Urease production**: It was done according to Collee *et al*. (1996).
- **Bacitracin production**: It was done according to Al-Qassab and Al-Khafaji (1992)
- **Hemolysin production on blood agar**: It was done according to Baron *et al*. (1994).

- **Extracellular hemolysin filtrate**: It was done according to Wyndham *et al*. 2003).
- **Effect of H_2O_2on hemolysin filtrate:** It was done according to Rennie and Arbuthnot (1971).
- **Effect of mannitol and glyserolon hemolysin filtrate:** It was done according to Johnson and Boese (1980).
- **Effect of phospholipase Con hemolysin filtrate:** It was done according to Susuma and Pinghui (1967).

Results and Discussion

Isolation of *Pseudomonas aeruginosa*: A total of (50) samples were collected from burn unit of HillaTeaching Hospital, isolated from patient who suffering from burn cases only (40)(80%) samples were positive culture while other was negative culture (10) (20%), as showed in Table (1).

Table (1) samples Isolates from Burn Patients

Total samples	Positive culture	Negative culture
50 (100%)	40 (80%)	10 (20%)

These results were similar to results obtained by De Macedo *et al*., (2003) who showed that (75%) of burn patients was contamination with different types of bacteria, these finding reflected the high percentage of bacterial contamination of burn unit with explain high percentage of positive burn culture. These results which included the high percentage of positive culture may be due to a number of specific factors having been identified in relation to infection rates in burn unit.

A total of (40) samples were isolated; these samples were subjected on different types of media to isolated *Pseudomonas aeruginosa.* Out of (40) samples, only (15) (37%) isolated were belonged to *Pseudomonas aeruginosa* as showed in Table (2).

Table (2) bacterial *pseudomonas aeruginosa* isolated from patients suffering from burn infection

Bacterial sample	*Pseudomonas aeruginosa*	%	Other bacteria	%
40(100%)	15	37	25	63

These results were agreement with results obtained by Nademm*et al*., (2008) who found that among of 2800 isolates of *Pseudomonas aeruginosa* only (230) (11.43%) isolated from burn infection. *P. aeruginosa* outbreaks in burn units are still associated with high (60%) death rates, bacteremia is associated with a 50% increase in mortality, and patients are characteristically susceptible to chronic infection by *P. aeruginosa* (Herfindal and Gourley, 2000).

Types of virulence factors detected in *Pseudomonas aeruginosa*: The ability of *Pseudomonas aeruginosa* isolated from patients with burn infection was studied; it was fond that all these isolated (100%) have capsules surrounding bacterial cell, and all isolated have notability to produce sidrophores enzymes. Also it was studied the group of enzymes produced from

bacteria, it was found that all these isolated have ability to produce lipase, protease, urease and bacitracin enzymes as showed in Table (3).

Table (3) Types of virulence factors detected in of *Pseudomonas aeruginosa*

Bacterial isolates	Capsule production	Sidrophores	Lipase	Protease	Urease	Bacitracin
15 isolates	+	-	+	+	+	+

Lassiter *et al*., (1992) showed that the capsule consist polysaccharide prevents activation of the alternative complement system protecting the bacteria from opsonization, phagocytosis and bacteriolysis. The role of siderophores is to scavenge iron from the environment and to make the mineral, which is almost always essential, available to the microbial cell. The isolates of *P. aeruginosa* appear hemolysis on the blood agar did not have siderophores; these results resemble the result obtained by Al-Mamori, (2011). The bacteria were able to produce siderophores have no ability to produce hemolysin, so bacteria need only one mechanism for obtaining iron. Iron can increase disease risk by functioning as a readily a viable essential nutrient for invading microbial and neoplastic cell, to survive and replicate in host, microbial pathogens must acquire host iron, this identical with that results obtained by (Goel and Kapil, 2001). Two distinct lipase enzymes were produced by *P. aeruginosa*, PLC-N (non-hemolytic) and PLC-H (hemolytic), both enzymes are phosphate regulated. The two enzymes could work sequentially and synergistically to lyse host cells (Van Dyke, 1991). *P. aeruginosa* could produce of large number of extracellular proteases such as alkalin protease, elastases, and exotoxin A which can cleave IgA which then lead to inhibit the function of the cells of the immune system, thus *P. aeruginosa* is resistant for phagocytosis and opsoization, this agree with that result mentioned by Stenfors and Raisanan, (1993). The result of bacitracin is identical with the results obtained by Cherif *et al*., (2001) who pointed that (90%) of *P. aeruginosa* can produced bacitracin.

Hemolysin production from *Pseudomonas aeruginosa*: the hemolysin production by *pseudomonas aeruginosa* was studied and it was found that all these isolates of these bacteria were able to produce hemolysin on blood agar as showed in table (4).

Table (4) Hemolysin production from pseudomonas aeruginosa

Total samples	*Hemolysin production*
15 (100%)	+

These results were not agreement with Van Delden and Iglewski, (1998) who demonstrated that (76%) of *P. aeruginosa* isolates that isolated from burn infection exhibit hemolysin on blood agar plates. *P. aeruginosa* has two pathways to take iron, one of these pathway is hemolysin, and these bacteria produce two hemolysin, it appear to be cytotoxic for most eukaryotic cells, so the hemolysin contribute to invasion through their cytotoxic effects on eukaryotic cells (Gadeberg *et al*., 1983).

Detection of extracellular hemolysin filtrates on blood agar: extracellular hemolysin filtrates of *P. aeruginosa* was detected on blood agar, it was found that 10 isolates of these bacteria have

extracellular hemolysin filtrates, it was appearance as transparent area around the well filled with hemolysin filtrates after incubation for 24 hours. This result with agreement with Scott, (2007) found that many type of bacteria have able to produce β-hemolysin when filtrate on blood agar.

Effect of H_2O_2 on extracellular hemolysin filtrate of *P. aeruginosa*: the effect of H_2O_2 on extracellular hemolysin filtrate of *P. aeruginosa* was studied, and it was found that the H_2O_2 was decrease the activity of extracellular hemolysin filtrate on blood agar. This result was agreement with the results obtained by Barnard and Stinson, (1996) how found that the extracellular hemolysin filtrate was inhibition and decrease activity when added to H_2O_2 on blood agar. The hemolysin was identical to hydrogen peroxide with respect to its effects on erythrocyte hemoglobin. Oxygen depended synthesis by *P. aeruginosa* to protease, in activation by catalase, differential solubility, frailer to absorb to ion-exchange chromatography resins, the amount of hydrogen peroxide present in culture media was sufficient to account for hemolytic activity.

Effect of mannitol and glycerol on extracellular hemolysin filtrate of *P. aeruginosa*: the effect of mannitol and glycerol on hemolysin filtrate produce by *Ps. aeruginosa* was studied, and it was found that all isolates of hemolysin filtrate will increased activity when added mannitol and glycerol to it. These results with agreement with results obtained by Johnson and Boese, (1980), how found that a total of 12 strain of *P. aeruginosa* this strain produced extracellular hemolysin when added glycerol and mannitol, and it was increased activity of hemolysin on blood agar. Purified hemolysin preparation contained hemolytic glycolipid, and the kinetics of hemolysin at level of purified lysine and effects of variation in lysine and erythrocyte concentration are described.

Effect of phospholipase C on extracellular hemolysin filtrate of *P. aeruginosa*: the effect of phospholipase C on hemolysin filtrate produce by *P. aeruginosa* was studied, and it was found that all isolates of hemolysin filtrate will increase activity when added phospholipase C to it. These results with agreement with results obtained by Susuma and Pinghui, (1967), how found that the bacteria *P. aeruginosa* produced extracellular hemolysin, and itwas increased activity of this hemolysin when added phospholipase C on blood agar. The hemolysin of *P. aeruginosa* was found to function as detergent in solubilizing various phospholipid in corporation of hemolysin into reaction mixtures containing phospholipids and phospholipase C, significantly increased the rate of enzyme activity.

References:

Abston, S.; Blakeney, P. and Desai, M. (2000). Post-burn infection and sepsis. Resident Orientation Manual. Galveston Shriners Burn Hospital and University of Texas Medical Branch Blocker Burn Unit . Total Burn care website.

Al-Mamori, H.H.S. (2011). Characterization of *Pseudomonas aeruginosa* Isolated from Patients and Hospital Environment in Hilla City. M.Sc. Thesis. College of medicine. University of Babylon. Iraq .

AL-Qassab, A. O. and AL-khafaji Z. M. (1992). Effect of different conditions on inhibition activity of enteric Lactobacilli against diarrhea-causing enteric bacteria. J. Agric. Sci. 3(1): 18-26.

Anzai, K., H., Park, J. Y., Wakabayashi, H. and Oyaizu, H. (2000). Phylogenetic affiliation of the *Pseudomonas* based on 16S rRNA sequence. Int. J. Syst. Evol. Microbiol. 50(4): 1563–89 .

Balowes, A.; Hausler, W. J.; Herrman, J.R.; Isenberg, K. L. and Shadomy, H. J. (1991). Manual of clinical microbiology. 5th ed., American Society for Microbiology. Washington D. C. PP. 1303-1370 .

Barnard, J.P and Stinson, M.W. (1996). The alpha-hemolysin of *Streptococcus gordonii* is H_2O_2. Infect. Immune. 64(9): 3853-3857.

Baron, E. J.; Peterson, L. R. and Finegold, S. M. (1994). Bailey and Scott's Diagnostic Microbiology. 9th ed., the C.V. Mosby Company, U.S.A.

Cherif, A.; Ouzari, H.; Daffonchio, D.; Cherif, H.; Ben Slama, K.; Hassen, A.; Jaoua, S. and Boudabous, A. (2001). Thuricin 7: A novel bacteriocin produced by *Bacillus thuringiensis* BMG1.7, a new strain isolated from soil. Lett. Appl. Microbiol. 32: 2432-2447.

Cherif, A.; Ouzari, H.; Daffonchio, D.; Cherif, H.; Ben Slama, K.; Hassen, A.; Jaoua, S. and Boudabous,A. (2001). Thuricin 7: A novel bacteriocin produced by *Bacillus thuringiensis* BMG1.7, a new strain isolated from soil. Lett. Appl. Microbiol. 32: 2432-2447.

Collee, J.G.; Fraser, A. G.; Marmion, B. P. and Simmons, A. (1996). Mackie and Mecartney. Practical Medical Microbiology. 14th ed. Churchill Living stone, USA.PP:413 – 424.

De Macedo, J.L.S.; Rosa, S.C. and Castro, C. (2003). Sepsis in burned patients.Revista da SociedadeBrasileria de Medicina Tropical. 36(6):647-52 .

Dykes, G. (1995). Bacteriocins: ecological and evolutionary significance. Trends Ecol. Evol. 10: 186-189.

Gadeberg, O.V.; Orskov, I. and Rhodes, J.M. (1983). Cytotoxic effect of an alpha-hemolytic Escherichia coli strain on human blood monocytes and granulocytes in vitro. Infect. Immune. PP: 337-341.

Gillespie, S. and Bamford, K. (2003). Medical microbiology and infection at a glance. Blackwel Publishing . Pp (90)

Goel, U. K. and Kapil, A. (2001).Monoclonal antibodies against the iron regulated outer membrane proteins of *Acinetobacter baumannii* are bacteriocidal B.M.C. Microbial.1: 16-20.

Herfindal, E. T. and Gourley, D. R. (2000). Textbook of Therapeutics, Drug and Disease Management 7th ed. Lippincott Williams & Wilkins, a Wolters Kluwer Co., USA. PP: 1049-1054.

Hodle, A.E.; Richter, K.P. And Thompson, R.M.(2006). Infection control practices in U.S. burn units. J. burn care Res. 27(2):142-51.

Johanson, M.K and Boese, M.D. (1980). Production and preparation of heat- stable extracellular hemolysin from *P. aeruginosa*. Infect. Immune. 29(3):1028-33.

Karadzic, I.; Masui A. L.; Zivkovic, I. and Fujiwara N. (2006). Purification and characterization of an alkaline lipase from *Pseudomonas aeruginosa* isolated from putrid mineral cutting oil as component of metalworking fluid. J. Biosci. Bioeng. 102: 82-89.

Kolmos, H. J.; Thuesen, B.; Nielsen, S. V.; Lohmann, M.; Kristoffersen, K. and Rosdahi, V. T. (1993). Outbreak of Infection in Burns Unit due to *P. aeruginosa*. J. Hosp. Infect 40:32-39.

Lassiter, H. A.; Watson, S. W.; Seifring, M. L. and Tanner J. E. (1992). Complement factor 9 deficiency in serum of human neonates. J. Infect. Dis. 166: 53-57 .

Lin, Y. C., Chen, T. L., Chen, H. S., Wang, F. D. and Liu, C. Y. (2006). Clinical characteristics and risk factors for attributable mortality in *Enterobacter cloacae* bacteremia. 39(1):67-72.

Lipsky, B. A., Berendt, A. R. and Deery, H.G. (2004). Infectious Diseases Society of America. Diagnosis and treatment of diabetic foot infections. Clin. Infect. Dis. 39: 885–910 .

Lyczak, J.B.; Cannon, C.L.; and Peir, G.B. (2000). Establishment of *Pseudomonas aeruginosa* infection lessons from a versatile opportunist. Microbes Infect. 2:1051-60.

Nadeem, S. G.; Qasmi, S. A.; Afaque, F.; Saleem, M. and Hakim , S. T. (2008). Comparison of the in vitro susceptibility of clinical isolates of Pseudomonas aeruginosa in a local hospital setting in Karachi, Pakistan. BJM. 2(4) 35-39.

Nassif, X. and Sansonetti, P. J. (1986). Correlation of the virulence of *Klebsiella pneumoniae* K1 and K2 with the presence of a plamid encoding aerobactin. Infect. Immune. 54(3): 603-608.

Piret, J.; Millet, J. and Demain, A. (1983). Production of intracellular protease during sporulation of Bacillus brrevis. Eur. J. Appl. Microbiol. Biotechnol. 17: 227-230.

Rennie, R.P. and Arbuthnot, J.P. (1971). Effect of carbohydrates on hemolysin of E.coli. Infect. Immun. 3(6):849-850 .

Schäfer, T.; Kirk, O.; Borchert, T. V.; Fuglsang, C. C.; Pedersen S.; Salmon, S.; Olsen, H. S.; Deinhammer, R. and Lund, H. (2005). Enzymes for Tech. Appl. Wiley .13: 377-437.

Stenfors, L. E. and Raisanen, S. (1993). Opsonization and phagocytosis of bacteria during various middle ear infections. Int J Pediatr Otorhinolaryngol. 27(2):137-45.

Susuma, K and Pinghui, V. (1967). Effect of hemolysin of *P. aeruginosa* on phospholipases and phospholipase C. J. Bacteriol. 93(2):670-674.

Van Delden, C. V. and Iglewski, B. H. (1998). Cell – to – cell signaling and *P. aeruginosa* infections. Emerg. Infect. Dis. 4. (1): 77-88 .

Van Dyke, M. I.; Lee, H. and Trevors, J. T. (1991). Applications of microbial surfactants. Biotechnol. Adv. 9: 241-252.

Wibbenmeyer, L.; Danks, R. and faucher, L. (2006). Prospective analysis of nosocomial infection rates, antibiotic use, and patterns of resistance in a burn population. Burn care Res. 27(2):152-160.

Wyndham, W.L., Lassa, B., Barah, E.W., Nicole, T.P. and Rodney, A.W. (2003). Acquisition of stcE and C1 esterase inhibitor metallo protease, during evolution of *E.coli* O157:H7. J. Infec. Dis. 14:187-1907.