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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\textsubscript{2}O\textsubscript{2} on extracellular hemolysin filtrate of *P. aeruginosa* was studied, and it was found that the H\textsubscript{2}O\textsubscript{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\textsubscript{2}O\textsubscript{2}, glycerol, phospholipase C

**Introduction:**

*Pseudomonas aeruginosa* a gram-negative non-fermenting bacillus, and is an opportunistic human pathogen (Anza 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. Pili 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).
- Lipase 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 H₂O₂ on 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 C on 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 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%), as showed in Table (1).

<table>
<thead>
<tr>
<th>Total samples</th>
<th>Positive culture</th>
<th>Negative culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 (100%)</td>
<td>40 (80%)</td>
<td>10 (20%)</td>
</tr>
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</table>

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>
<thead>
<tr>
<th>Bacterial sample</th>
<th>Pseudomonas aeruginosa</th>
<th>%</th>
<th>Other bacteria</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>40(100%)</td>
<td>15</td>
<td>37</td>
<td>25</td>
<td>63</td>
</tr>
</tbody>
</table>

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*

<table>
<thead>
<tr>
<th>Bacterial isolates</th>
<th>Capsule production</th>
<th>Sidrophores</th>
<th>Lipase</th>
<th>Protease</th>
<th>Urease</th>
<th>Bacitracin</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 isolates</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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 opsonization, 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

<table>
<thead>
<tr>
<th>Total samples</th>
<th>Hemolysin production</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 (100%)</td>
<td>+</td>
</tr>
</tbody>
</table>

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 H₂O₂ on extracellular hemolysin filtrate of *P. aeruginosa*:** the effect of H₂O₂ on extracellular hemolysin filtrate of *P. aeruginosa* was studied, and it was found that the H₂O₂ 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 H₂O₂ 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, trailer 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 it was 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:**


