Magazin of Al-Kufa University for Biology / VOL.6/ NO.2/ Year : 2014
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Study of some virulence factors of *Pseudomonas aeruginosa* isolated from different clinical sources.

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Summary:
A total of 25 isolates of *Pseudomonas aeruginosa* out of 80 samples isolated from various clinical sources (burns, wounds, urinary tract infections (UTIs),sputum and otitis media), From patients were attending to Hilla Teaching Hospital.
Maximum isolates were obtained fromburn samples9(36%),wound and otitis media samples5(20%), UTIsandsputum3(12%).
Some virulence factors of all isolates were studied, and the results showed that all bacterial isolates (100%) having bacteriocin, extracellular protease, lipase and urease enzyme, most the isolates (86.8%) being able to produce hemolysin, but some isolates (13.2%) producing the siderophores.
Key words: *Pseudomonas aeruginosa*, virulence factors, UTIs, burns, wounds, otitis media.

Introduction:

The genus *Pseudomonas* is Gram-negative, rod-shaped bacterium, strict aerobic with unipolar motility, The genus *Pseudomonas* contains more than 140 species, most of which are saprophytic; more than 25 species of *Pseudomonas* are associated with humans(1).Most *Pseudomonas* known to cause disease in humans are associated with opportunistic infections especially *Pseudomonas aeruginosa*(2).It causes disease in individuals; it is a major threat to hospitalized and immunocompromised patients, particularly those with serious underlying diseases such as cancer and burns (3).The high motility associated with these infections is due to a combination of weakened host defense, bacterial resistance to antibiotics, and the production of extracellular bacterial enzymes and toxins (4).*Pseudomonas aeruginosa* is a pathogen that causes nosocomial infections, accounting for 20% of pneumonia and 16% of urinary tract infections according to recent data from national nosocomial surveillance system (5). The medical problem results from this organism are its ability to resist almost all antibacterial agents, leading to predominate over it when the sensitive organisms are suppressed by these agents (6). There are limited numbers of antimicrobial agents including the anti-pseudomonal Penicillins, Cephalosporin, Carbapenems, Aminoglycosides and Fluoroquinolons with reliable activity against its (7). Also the medically importance of this organism may be lies in its ability to produce a variety of toxins, extracellular enzymes including elastases, proteases and hemolysins (8).

Aims of the study

1 - Isolation and identification of *Pseudomonas aeruginosa* from different clinical speciemens.
2-Study some factors associated with pathogenecity of *Pseudomonas aeruginosa* such as haemolysin, sidrophore, bacteriocin, extracellular protease, lipase and urease.

Material and methods

Specimen and isolates:
25 *Ps. aeruginosa* isolates out of 80 samples from different clinical sources.

1-Detection of haemolysin production was achieved according to methods of (9).

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.6/ NO.2/ Year : 2014
http://www.kufabiojournal.org

2-Detection of sidrophore production was achieved according to methods of (10).
3-Detection of bacteriocin production was achieved according to methods of (11).
4-Detection of Protease production was achieved according to methods of (12).
5-Detection of Lipase production was achieved according to methods of (13).
6-Detection of Urease production was achieved according to methods of (13).

Results and discussion

In this study a total of (80) samples were obtained from patients suffering from burns, wounds, urinary tract infections (UTIs), throat infections and otitis media, Table(1)

Table (1) Distribution of samples and number of *Ps. aeruginosa* isolates:

Sample	Total Number of sample	Number of *Ps. aeruginosa* isolates	%
Burns	22	9	36
wounds	13	5	20
Urine	9	3	12
sputum	12	3	12
Otitis media	24	5	20
Total	80	25	100

In the present study, the distribution of *Ps. aeruginosa* isolates according to the site of infection was studied; it was found that the most infections of this bacterium occur in the burns (36%). This result resemble the result obtained by (14) who found that among the (1500) isolates of *Ps. aeruginosa*300(20.0%) isolates from burn infections, but the results were not agreed with the results obtained by (15) who found that among the 65 isolates of this bacterium 5(7.7%) burns were found.
In the present study *Ps. aeruginosa* in wounds infection was studied it was found that infection with this bacterium in wounds less than the infection in burns (20%).
This result was resemble with the result reported by (16) who found that *Ps. aeruginosa* present in wound infection in rate (15.84%).
The result obtained in this study showed that five isolates (20%) of *Ps. aeruginosa* were isolated from patients with otitis media.(17) showed that the isolation percentage of *Ps. aeruginosa* was reached to (11%) if compared with other causative agents (aerobic and anaerobic bacteria) isolates from otitis media.
Also, Urinary tract infection is one of the most common disease entities deal with by urologists, general practitioners, surgeon and all other members of the medical profession. The possible factors leading to UTI include pregnancy, diabetes mellitus, nephrolithiasis urological instrumentation, urinary tract abnormalities, burns, and vesico-urethral reflux (18).

The results of the present study showed only 3 isolates (12%) of *Ps. aeruginosa* were isolated from sputum samples. This result was acceptable with the result obtained by (19) who found that 55 isolates of *Ps. aeruginosa*(26.57%) can be isolated from sputum. but did not accept the result obtained by (20) who has succeeded to isolate *Ps. aeruginosa* from sputum at rate reaching (2.76%),
In this study some virulence factors,(haemolysin, sidrophore, bacteriocin, extracellular protease, lipase and urease) were examined. Production of hemolysin by *Ps. aeruginosa* ismostly associated with pathogenic bacteria, therefore it is considered as important factor that participates in their pathogenesis (21). Hemolysin production by *Ps. aeruginosa* was

studied, it was found that (86.8%) isolates were able to produce extracellular hemolysin on blood agar,these results shown in Table (2).
These results bring into line with (22) who demonstrated that (76%) of *Ps. aeruginosa* isolates that isolated from different clinical sources exhibit hemolysin on blood agar plates, but(23) found that (10%) strains produced hemolysin.
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Ps. aeruginosa has two pathways to take iron, one of these pathway is hemolysin, and these bacteria produce two hemolysins, it appear to be cytotoxic for most eukaryotic cells, hemolysins contribute to invasion through their cytotoxic effects on eukaryotic cells. Bacteria may use hemolysins as a way to obtain nutrients from host cells. For example, iron may be a limiting factor in the growth of various pathogenic bacteria (24).

Hemolysin is lytic to erythrocytes and it is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury, and impaired host defenses. Exposure of PMNLs and release of leukotriene and ATP; cause marked morphologic alterations; and impair chemotaxis and phagocytosis. Lysis occurs at higher concentrations (25.).
In present study, *Ps. aeruginosa* isolates are also investigated for their ability to produce siderophores synthesis. The results show that (13.2%) isolates of *Ps. aeruginosa* are able to produce siderophores, these results shown in Table (2).
The role of siderophores is to scavenge iron from the environment and to make the mineral, which is almost always essential, and available to the microbial cell. Most aerobic and facultative anaerobic microorganisms synthesize at least one type of siderophores. The microbes requires iron for variety of functions including the electron transport chain, in deoxyribonucleotide synthesis, in the synthesis of heme and for incorporation in the proteins involved in nitrogen fixation (26).

The isolates of *Ps. aeruginosa* appear hemolysis on the blood agar and do not have siderophores; these results resemble the result obtained by (27).

The bacteria that are able to produce siderophores have no ability to produce hemolysin, so that bacteria need only one mechanism for obtaining iron that 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, that identical with the results obtained by (28).

All isolates (100%) in the present study produce bacteriocin when being tested with the sensitive gram negative indicator isolates like (*E. coli*, *Klebsiella pneumonia*, *Proteus mirabilis*).This result is agreed with the results obtained by (29) who pointed that (90%) of *Ps. aeruginosa* can produce bacteriocin.These results shown in Table (2).

(30) reported that (85%) of *Ps. aeruginosa* isolates were a producer of bacteriocin.

The antimicrobial protein produced by *Pseudomonas aeruginosa* that kill or inhibit the growth of other bacteria related to the same group or species (31).
Bacteriocin directly or indirectly is associated with virulence because bacteriocin binds to receptors at the bacterial cell surface. The sensitivity of isolates to bacteriocin is dependent on the formation of specific receptors found on the outer surface of the cell. The producer strains present a self-protection mechanism. This bacteriocin is resistant to a wide range of pH, high temperatures and also to several proteolytic enzymes(32).
The bacteriocin showed high activity of antibacterial action, mostof the bacteriocine exert their lethal activity by the adsorption to specific receptors in the external surface of the sensitive bacteria (31) and then they are able to interact with the cytoplasmic membrane leading to the bacterial death (33).

Protease production by *Ps. aeruginosa* isolates was studied; it was found that all these isolates(100%) have this enzyme appears as a zone around the colony when being grown on

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.6/ NO.2/ Year : 2014
http://www.kufabiojournal.org

M_9 media after adding of 3ml of 5% Trichloroacetic acid and incubation for 24 hrs. as shown in Table (2).

Ps. aeruginosa could produce of large numbers of extracellular protease such as alkaline protease, elastase, and exotoxine A which can cleave IgA which then lead to inhibit the function of the cells of the immune system, thus *Ps. aeruginosa* is resistant to phagocytosis and opsonization (34).

Proteases play a crucial role in numerous pathologic processes, arthritis, tumor invasion and metastasis. The Infection and a number of degenerative diseases have been linked with the involvement of one or more proteolytic enzymes. The proteolytic activity has a maximum activity similar to other experiment of protease from microbial origin (35).

The proteases proposed as virulence factors ina variety of diseases caused by this microorganism. The virulence of *Pseudomonas aeruginosa* is multifactorial, but it is partly determined by exo-products such as alkaline protease and elastase that are responsible for the damage of tissues by degrading elastin collagen and proteoglycans.These enzymes also shown to degrade proteinsthat function in host defense *in vivo* (36).

Ability of *Ps. aeruginosa* to produce lipase has been investigated and found that all these isolates (100%) are able to produce lipase after incubation for 48 hrs. on egg yolk agar. These results shown in Table (2).

This result was consistent with (37), who found that all isolates examined in their study gave positive result in a test for lipase production. Lipase is water-soluble enzyme that catalyzes the hydrolysis of ester chemical bonds in water insoluble lipid substrate, most lipases act as a specific position on the glycerol backbone of lipid substrate (38).

Two distinct lipase enzymes were produced by *Ps. 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 (39).

The lipase production can be arrested by various compounds such as Tetracycline which are effective against lipase production by interfering with protein synthesis by binding to bacterial ribosome (40).

Urease production by *Ps. aeruginosa* isolates was studied, and found that all these isolates (100%) were able to produce urease, these result shown in Table (2).

The result of this study came in agreement with the result obtained by (41) who found that all isolates of *Ps. aeruginosa* were able to produce this enzyme.

(42) found that ureases are enzymes widespread among organisms that hydrolyze urea into ammonia and carbon dioxide. Bacterial ureases are multimers of two or three subunit complexes.

Urease activity enables bacteria to use urea as the sole nitrogen source urease provided a suitable condition for bacterial survival in the site of infection through changing the pH and the removal of the toxic effect of urea by converting it to CO_2 and NH_3.

Print ISSN: 2073-8854 & Online ISSN: 2311-6544
Magazin of Al-Kufa University for Biology / VOL.6/ NO.2/ Year : 2014
http://www.kufabiojournal.org

Table (2) Virulence factors of *Pseudomonas aeruginosa* isolates:

Virulence factors	*Total No.(%)*
Hemolysin	86.8
Sidrophore	13.2
Bacteriocin	100
Protease	100
Lipase	100
Urease	100

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