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Virulence factors of *Pseudomonas aeruginosa* Isolates from Iraqi patients : Literature Review

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الخلاصة

تهدف هذه الدراسة الى إستعراض و تحليل للمراجع المتعلقة بعامل الضراوة لبكتريا (Pseudomonas aeruginosa) والتي يمكن عزلها من أنواع مختلفة من العدوى. تمتاز هذه البكتريا بتغيير الضراوة مما يؤدي الى زيادة الامراضية و تنوع الالتهابات المصاحبة للاصابة. عدوى ال Pseudomonas بشكل عام يتم علاجها بالمضادات الحيوية. الاستخدام المتكرر و العلاج المستمر بالمضادات الحيوية جعل علاج الاصابة ب Pseudomonas اكثر صعوبة نتيجة لزيادة عدد السلالات المقاومة للمضادات الحيوية. ان من الاسباب المهمة لهذه المقاومة هي الطفرة الوراثية في الجين gyrA نتيجة الاستخدام المتكرر للمضادات الحيوية و تحديدا علاج fluoroquinolone كما اشارت الى ذلك العديد من الدراسات و الجدير بالذكر ان هذا الجين مسؤوول عن عامل الضراوة DNA gyrase و الذي يعتبر هدفا رئيسيا لعمل المضاد الحيوي fluoroquinolone. لذا من الضروري، ولتقليل خطر ظهور سلالات مقاومة من هذه البكتريا، اتباع انظمة قائمة على اسس علمية، كاختبار الحساسية مثلا، لغرض صرف المضادات الحيوية و كذلك ارشاد المصابين بالعدوى باتباع التعليمات المثالية لاستهلاك العلاج.

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Abstract

The aim of this research is a review study of virulence factor of Pseudomonas aeruginosa pathogen which can be isolated from different infection types. Most infections accompanying this bacteria have a variable virulence factors increasing bacterial pathogenicity. The treatment of Pseudomonas infections is maintained by antibiotics though in hospitalized patients; the treatment of these infections is becoming harder as a result of the increasing amount of antibiotic-resistant strains.

Keywords: P. aeruginosa, antibiotic resistance, virulence genes.

Introduction:

P. aeruginosa is an aerobic Gram-negative bacteria, rod, facultative anaerobic, non-fermentative, nonsporulation, motile bacterium by one polar flagellum, nutritionally versatile bacteria. (Al-Marzoqi etal.,2013) It belongs to pseudomonadaceae family .
(Pathmanathan etal.,2009) In fact, P. aeruginosa can colonizes various eukaryotic hosts comprising humans, plants, animals and worms .(Ngai ,2008).

The spread of P. aeruginosa in health organizations is highly dangerous, with infiltrates, by any deficiency at the main defense line in the human host particularly in the intensive care units (ICUs) causing nosocomial infections. (Lyczak etal., 2000 ;Farahi etal.,2018) and (Al-mayahi and salman ,2020) It causes nosocomial infections, particularly among patients in the ICU. Various infections are caused by P. aeruginosa, such as nosocomial pneumonias, severe burns, skin and soft tissue infections, urinary tract infections (UTIs), and infections in immunocompromised individuals (Babay,2007).

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In most of the laboratories, the detection of P.aeruginosa is still achieved by microbial culture and biochemical tests. Consequently, primary detection and suitable treatments are the best approaches against these infections. Though a

study has revealed that these approaches contain dependable detection results, they need many days to be accomplished and time-consuming. Many studies have revealed that unsuitable primary antimicrobial treatments were related to adverse consequences for infection therapy. Contrariwise, incorrect detection can cause the management of ineffective antimicrobial treatments through the first (48-72) hrs(Riou ,2010).

Additionally, in cases of low bacterial count, particularly in patients treated with antibiotic, false negative results can be accomplished in usual laboratory tests. Therefore specific and rapid approaches with a high sensitivity rate is of great significance. Recently, the identification and detection of P.aeruginosain in clinical samples by the polymerase chain reaction (PCR) technique has been improved considerably. (Chuang *etal.*, 2013).Since 1992, when the first PCR detection of this bacterium, several genes have been stated as PCR marks for P.aeruginosa detection. Later, many studies have revealed that these diagnostic genes do not have thorough specificity or sensitivity for detection and accordingly have false positive and false negative results. The genome of P.aeruginosa has a highly polymorphic nature, so it can affect the PCR specificity and consistency (Vitkauskiene *etal*.,2010).

Pathogenicity:

P. aeruginosa is a pathogenic bacterium responsible for severe opportunistic infections, particularly in the immunocompromised patients . As a nosocomial bacterium, P. aeruginosa has a capacity to colonize wounds and catheters, therefore it can be promoted among various hospital sectors such as urology unites, burn unit and ICUs .(Rello etal.,2014) (Al-Saeedi and

Raheema.,2019). Urinary tract infections, respiratory tract infection, burn and wound with blue green pus, eye infection and ear infection, soft tissue infections, dermatitis, joint infections and bacteremia, were communal infections resulted by this bacterium .(Al-Dahmoshi ,2017). During the hospitalization, patient exposed to bacteria from many sources such as other patients and healthcare staff, environment as well as hospital waste .(Naher etal.,2014)

As a nosocomial pathogen, P. aeruginosa has established most consideration , it is infrequently infects healthy tissues, nevertheless, in compromised defenses, it can infect almost all tissues and it is easily able to adapt to environmental change, require minimal growth nutritional requirement, rapidly improve antibiotics resistance and produces virulence arsenal . (Meenakumari etal.,2011)

P. aeruginosa is a human opportunistic microbe and has consequence in morbidity and health care costs both in the community and hospitals including economic burden and prolonged stay Infections resulted by this bacterium is commonly life threatening and hard to be treated because it shows essentially elevated resistance to several antimicrobial, mainly multidrug resistance in the health care institutes ,the study revealed that the maximum ratio of P. aeruginosa infections was in wound infection about (33.8%) (Naher etal.,2014), while other study found that the ratio was (17.5%) (Al-mayali and salman ,2020), this pathogen is considered as the main factor of wound nosocomial infections.

Followed by the ear infection about (26.8%) . (Naher etal.,2014), UTI 12 was (16.9%) , whereas in throat infection (7%) , then burn infection (5.6%) , while other study found that the ratio was (17.5%) , sputum was (4.2%) while others found that the ratio was (0%) , stool (2.8%),while other study found that the ratio was (4%) , both C.S.F seminal and fluid was (1.4%) . %) (Al-mayali and salman ,2020) .It was

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observed that the results of this study was similar with the results found by Hassan, 2012 in Kurdistan, which revealed that the ratio of P. aeruginosa isolated from burn was (10.9%) (Bunyan etal.,2018).

Antibiotic Resistance:

In fact, the pathogenesis of P.aeruginosa is multifactorial, caused by the multiple mechanisms existence of resistance to most antibiotics, due to multidrug efflux, impermeability and the chromosomal AmpC β-lactamase, leading to the production of a various cellular structures set and extracellular molecules playing an important role in developing pathogenicity. (Babay ,2007). Its common resistance is belong to several factors . It is essentially antimicrobial resistant, resulting from the low cell wall permeability. P. aeruginosa is identified to have intrinsic multi-drug resistance abilities . (Ergin and Mutlu ,1999).

P.aeruginosa interacts with outer media through its outer membrane. The outer membrane consist of proteins, Lipo polysaccharaid (LPS) and phospholipid. The main structural proteins in the outer membrane characterized by three types: lipoproteins, porins and heat labile proteins like OmpA. LPS is a significant bacterial toxin which has been widely studied as a gram negative sepsis mediator.

P. aeruginosa has the genetic ability to show a wide resistance mechanisms repertoire (Saleh etal.,2012).

Generally, the antibiotics are natural products which inhibit the bacteria in either bacteriostatic or bactericidal mode, fluoroquinolones (FQs) are artificial antibiotics with a bactericidal action against various Gram -ve bacterium species. (Nejma etal., 2018). They are used principally in the infections treatment which produced by P. aeruginosa, nevertheless, an elevated resistance to fluoroquinolones was showed between clinical samples at high rate, resulting in the failure of the P. aeruginosa treatment (Babay ,2007). The main mechanism of the fluoroquinolones resistant in P. aeruginosa is the mutations. Also study revealed that the mutations occur in the gyrA gene in P. aeruginosa isolates (25% resistant, 7.5% sensitive isolates and 67.5%

intermediate). It can gain extra resistance genes from other bacteria by plasmids, bacteriophages and transposons. Mutations occur in chromosomal genes regulating the resistance genes, the gene gyrA which encode the subunit A in DNA gyrase, consequently resulting in the transcription inhibition and blocking of DNA replication .(Fridkin and gayness ,1999).

Moreover, an increased incidence of the multidrug resistance (MDR) in P. aeruginosa has been remarked, relating to an increased mortality and morbidity (Farahi etal.,2018) Regional differences in antibiotic resistance found for various organisms, comprising P. aeruginosa and this may be associated with the variation in the prescribing habits of antibiotic. It has been reported that a remarkable rise in antibiotic resistance in gram negative pathogens improved from the hospitalized patients, particularly for critically sick patients, infections which is resulted from multidrug resistant (MDR) G-ve bacteria, particularly multidrug-resistant P. aeruginosa (MDRPA), are related to increased mortality, morbidity and costs. (Vrnaz etal.,2011). The extensive usage of antibacterial factors has led to elevated levels of antibiotic resistance (Speert 1993), consequently, it is necessary to choose suitable antibacterial factors, to evade the elevated patient mortality and the economic

problems affecting patients and society. Nevertheless, In USA, illogical usage of antibacterial factors has been detected, which leading to adverse concerns, comprising increased mortality and high medical costs . (Franco etal.,2009).

Phagocytic cells (including neutrophils and mononuclear phagocytes (tissue macrophages and circulating monocytes) are the body's initial defense against P. aeruginosa. The phagocytosis is stimulated in the existence of serum factors containing complement and IgG. The host defenses against various invading gram-ve bacteria based on native immune response of endotoxin(LPS). Anti- Pseudomonal vaccines which LPS based have been revealed to offer limited serotype specific defense. Moreover, the LPS endotoxic characteristics have claimed against the usage of such vaccines in lung infection in cystic fibrosis patients and burn patients . (Al-khafaji

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etal.,2020).Consequently, OMP has been used as main defensive antigens in vaccines development against Pseudomonal infection. This Antigen was revealed to be extremely immunogenic. OMP as antigen has some benefits, the most significant are their preservation among the 17 P. aeruginosa serotypes as well as no cross reaction with the other gram-ve bacilli .(Fraylick etal.,2001).

P. aeruginosa is the most predominant bacterium and it is principally hard to be treated. Actually, P. aeruginosa possesses various virulence characters contributing to the bacterial toxicity and invasion, comprising exotoxin A, elastase, phospholipase and homoserine lactone. Furthermore, this bacterium harbours various mechanisms of drug resistance in suppression or activation the activity of efflux pump system, formation of biofilm and loss or reduced expression of the outer membrane proteins. Consequently, MDR and extensively drug resistant (XDR) strains are wide-spread .(Franco etal.,2009).

P. aeruginosa has virulence traits that increase its potential for disease containing fluorescein, pyocyanin, pigments, pyoverdin and pyorubin, lytic enzymes and toxins, thus it may be isolated from environmental, clinical and hydrocarbon contaminated pools. The infection settlement and the immune system avoidance can be due to set of virulence factors . (Fraylick etal.,2001).

Virulence Factors:

P. aeruginosa has a many virulence factors, like las B elastase,which is encoded by las B gene6. nan1 gene which encodes neuraminidase helping bacteria in adherence to the epithelial cells. In addition, There are two outer membrane genes helping the rapid detection of P. aeruginosa oprL and oprI gene . (Naher etal.,2014)

The secretion system that has been identified in gram-negative bacteria is a third(type III) which inject Pseudomonal toxins directly into the adjacent host cells by T3SS to begin the infection. This system secrets four identified effector proteins (also termed Exoenzymes): ExoA, ExoT, ExoS, ExoY and

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ExoU .Exotoxin A (ExoA) is encoded via the toxA gene, obstruct synthesis of protein in the host cells9. As well as, produces phenazines which is redox-active and are toxic to the human cell . (Naher etal.,2014)

Exotoxin T (ExoT) and Exotoxin S (ExoS) share (75%) amino acid identity, they are bifunctional exotoxins having C-terminal ADP ribosylation actions, nevertheless ExoT has a lower catalytic action, with (0.2 %) of the ADP ribosyl transferase action of ExoS .(Meenakumari etal.,2011) . ExoT is considered as a GTPase trigger protein (GAP) to Rho family. This protein was proven to be in charge for prohibiting the wound repair in vitro.

ExoS is GTPase triggering protein domain, in addition to ADP ribosyl transferase (ADPRT) action. It can stimulate a cytotoxic activity and is associated
with the ability to produce lung impairment and depraved result from infection with this bacterium. The domain ADP ribosyl transferase (ADPRT) in ExoS responsible for the ability to obstruct the synthesis of DNA in cultured cells .(Fraylick etal.,2001 ; Fadhil etal.,2016) .ExoS is encoded via the exo help P.aeruginosa to keep away from phagocytosis leading to the host cell death .(Naher *etal*.,2014)

Exotoxin U (ExoU) toxin is cytolytic to cell types in many mammalian comprising neutrophils, macrophages, epithelial cells and fibroblasts. Acute pneumonia in animal models, the exoU gene disorder caused reduced virulence, while conversion with an exo U- expressing plasmid raised the strains virulence that did not normally secrete ExoU. (Meenakumari etal.,2011). ExoU is associated with carrying phospholipase action playing a role in the hydrolyzing of the ester bond in cell membrane phospholipids resulting in membrane disturbance, elaboration of fatty acid. (Fraylick etal.,2001).

Exotoxin Y (ExoY) is extracellular adenylate cyclase, when it introduced to mammalian cells resulting in increasing of the intracellular 3',5' cyclic adenosine monophosphate (cAMP) concentration and finally disruption of the cytoskeleton activity, improved endothelial penetrability and interruption of

bacterial uptake by the host immune cells.(Fraylick etal.,2001). Exo Y displays a definite homology to extracellular adenylate cyclases of Bacillus pertussis and Bacillus anthracis. ExoY rises cytosolic cAMP, improved by a eukaryotic cofactor, this raised cytosolic cAMP produce an increased pulmonary micro vascular intercellular gap as well as raised lung permeability. (Meenakumari etal.,2011). In fact, the environmental bacteria are less pathogenic, consequently their management can be less risky in comparison to the clinical isolates .

A recent study showed that the incidence of the virulence factors genes in P. aeruginosa isolates was as the following: las B (10.6%), opr I (6.4%), oprL (7.5%), exo S (9.4%) (Fadhil *etal*.,2016).

Another modern study showed that (73.7%) and (42.1%) of the isolates harbour (exo S) gene in the clinical and the environmental isolates correspondingly , other study showed that exo S gene established in (50%) of isolates while another study showed that exo S gene was in (50%) of isolates .(Saleh etal.,2012). Exo U was

(4.5%) (Fadhil etal.,2016) while other study showed that exo U gene is found in all (100%) isolates, whereas it found in (94.7%) of the environmental isolates while it was found in (25%) of isolates (Saleh etal.,2012). About (89.5%) of clinical isolates and (89.5%) of environmental isolates have exo T gene ,while it was detected in (85%) . Only (63.2%) and (42.1%) of the clinical and environmental isolates correspondingly have exoY (Fraylick etal.,2001), another study revealed that exoY gene was (60%) of isolates. (Saleh *etal*.,2012).

Conclusions

In conclusion, the current study is in agreement with any previous studies, indicated mutations in gyrA gene resulting from the excessive antibiotics usage which is one of the mechanisms leading to the resistance of fluoroquinolone in P. aeruginosa isolates, it was showed that DNA gyrase which encode gyrA gene is the main target for fluoroquinolon.

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