STUDY THE EFFECT OF SOME INHIBITOR FACTORS ON PRODUCTION OF SOME VIRULENCE FACTORS OF *PSEUDOMONAS AERUGINOSA* AND THEIR ABILITY FOR ADHESION TO CONTACT LENSES

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Abstract

Two isolates of *Pseudomonas aeruginosa* were obtained from contact lenses weares with eye infection. *P. aeruginosa* isolates were able to produce gelatinase, elastase and protease. *The P. aeruginosa* isolates serotypes were A:p9 and F:P12 . The sensitivity of the isolates to the antibiotics was tested, the results showed that both isolates were resistant of the used antibiotics except Chloramphenicol and Ciprofloxacin. The ability of *P. aeruginosa* isolates to adhere to soft contact lenses was tested. The effect of the antibiotics (Chloramphenicol and Ciprofloxacin), soft contact lenses care solution, normal saline and sterilization solutions (drops) like Methadin and Nazordin and enzymes (Papain,Neuraminidase) on production of protease and elastase. The results showed that Nazordin and Methadin were reduced the production of protease and elastase (residual activity of protease 47%&42% respectively), and (the residual activity of elastase 42&%49% respectively).The effect of antibiotics (Chloramphenicol,Ciprofloxacin),lens contact care solution , normal salin, Methadin Nazordin and enzymes (papain ,Neuraminidase) on *P. aeruginosa* adhesion to contact lenses was tested. The results showed that sterilization drop (Nazordin) was more effective ratio on the bacterial adherence, the inhibitory ratio of Nazordin was 90.68%, and the inhibitory ratio of Methadin was 72.06 % of cells from adhesion to contact lenses. While the enzymes (Papain, Neuraminidase) did not reduce adhesion.

دراسة تاثير بعض العوامل المثبطة في انتاج بعض عوامل الفوعة لبكتريا *Pseudomonas aeruginosa* و قابليتها على الالتصاق على العدسات اللاصقة

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الخلاصة

تم الحصول على عزلتين من بكتريا السيدوموناس الهوائية من العدسات اللاصقة من اصابات العين.كانت عزلات السيدوموناس الهوائية قادرة على انتاج الجيلاتينيز و ايلاستيز و بروتييز الانماط المصلية للعزلتين كانت A:p9 و F:P12 . تم التحري عن حساسية العزلتين للمضادات الحياتية ،فاظهرت النتائج ان العزلتين مقاومة للمضادات الحياتية المستخدمة، فيما عدا الكلورامفينيكول والسيبروفلاكسين. و اختبر تاثير كل من المضادات الحياتية (الكلورامفينيكول والسيبروفلاكسين)ومحاليل حفظ العدسات اللاصقة الناعمة والسلاين والمحاليل المعقمة)مثل الميثادين والنازوردين،و انزيمات البابائين و نيوروامينيديز في انتاج البروتييز وايلاستيز

فاظهرت النتائج ان النازوردين والميثادين لها القدرة على تقليل انتاج البروتييز والايلاستييز (الفعالية المتبقية للبروتييز هي٤٧% و٤٢% على التوالي) في حين ان (الفعالية المتبقية للايلاستييز ٤٢% و٤٩% على التوالي) .تم اختبار تاثير المضادات الحياتية (الكلورامفينيكول و السيبروفلاكسين)، و سائل حفظ العدسات اللاصقة، والمحلول الملحي، و الميثادين والنازوردين، وانزيمات(البابائين و النيوروامينيديز)على قدرة التصاق السيدوموناس الهوائية على العدسات اللاصقة،حيث اظهرت النتائج ان قطرات التعقيم (النازوردين) اكثر تاثيرا على التصاق البكتيريا على العدسات اللاصقة ، ان نسبة التثبيط للنازوردين هي 90.68% في حين ان نسبة التثبيط للميثادين كانت 72.06%،و بينما الانزيمات(البابائين والنيروامينيدز) لم تؤثر على الالتصاق.

Introduction

Pseudomonas aeruginosa is a prevalent opportunistic pathogenic in human , causing chronic lung infections in cystic fibrosis patients , burn victims and other immunocompromised people [1]. *Pseudomonas aeruginosa* is the most frequently found pathogen in cornal ulcers of patients who wear soft contact lenses[2]. The development of cornal ulcers in individuals who wear soft contact lenses depended on a variety of contributing factors including compromised ocular surface (eg:truma from contact lenses wear, hypoxia , dry eye, exposure to pathogens(eg: poor hygiene,contact lens colonization of bacteria from extended wear) and virulence of organism [3]. A multitude of virulence factors and mechenisms allow *P. aeruginosa* to adhere ,survive ,and replicate in corneal tissue, lipopolysaccharide and pili may mediate initial adhesion to contact lenses or the cornea [4]. *Pseudomonas aeruginosa* produces many extra celluler virulence factors ,which are associated with extensive tissue damage, invasiveness , colonazition, and are able to promote the destruction of the cornea[5]. *P.aeruginosa* is a common cause of contact-lens-related microbial keratitis. This bacterium is becoming increasingly resistant to antibiotics, damage to the cornea resulting from the combined effect of bacteria and host factors can lead to loss of vision [6]. *P.aeruginosa* is known for its intrinsic and acquired resistance against a wide range of antimicrobial drugs which leads to difficulty in treatment [7]. The study aims to determine the virulence characteristic,serotypes, antibiotic resistance of *P. aeruginosa* isolated from contact lenses wears with eye infections and the effect of different inhibitors ,and antibiotics on *P. aeruginosa* isolate adherence to soft contact lenses.

Material and methods:

Isolation:

A collection of fifteen *Pseudomonas* isolates were obtained from contact lenses wears with eye infection from Ibn Al-Haitham hospital for eye infections.The bacteria were identified by biochemical tests [8], and all isolates maintained on nutrient broth containing 20% glycerol in deep freez. The identification was confirmed by API 20 E system (BioMeriex).

The ability of the isolates to produce gelatenase ,elastase, and protease was tested[9]. The isolates were subjected to O-antigen serotyping by slid agglutination test using Sanofi Diagnostic Pasteur anti– O antisera for the grouping of *P. aeruginosa*.

Antibiotic sensitivity test:

The sensitivity of bacteria to antibiotics (Chloramphenicol, Cephalothin, Ofloxacin, Ciprofloxacin, Piperacillin, Moxifloxacin) were tested and sub minimal inhibitory concentrations (sub MICs) of Chloramphenicol ,Ciprofloxacin were determined [10].

Bacterial adherence to soft contact lenses:

The isolates were cultured on MacConkey agar and then sub cultured on tryptic soy broth(TSB) to encourage its growth.It was incubated overnight and then it was sub cultured in TSB (1:100) with contact lenses. The bacteria were incubated for (1 hour) at room temperature (two types of contact lenses: medical and cosmetics were used) ,then rinsed with sterile Phosphate Buffer Saline, and assayed by homogenizing each one in 3ml PBS, then the number of adherence cells to the contact lenses was determined [11].The sterial contact lenses used as control (the contact lenses with PBS without bacteria).

The effect of antibiotics and different inhibitors on production of several virulence factor of *P. aeroginosa* :

The effect of antibiotics (sub MICs of Chloramphenicol and Ciprofloxacin),and different materials include soft contact lens care

solution, normal salin Methadin, Nazordin,and enzymes (Papain0.1mg/ml, Neuraminidase 0.1mg/ml) on production of
elastase ,and protease of isolates were tested.After growth in the prescence or absence of antibiotics, different materials and enzymes, and then incubation at 37˚C for 24 hrs.The activity of elastase and protease was exsamined [12,13].

The effect of antibiotics and different inhibitors on *P. aeroginosa* adherence to soft contact lenses:

The effect of antibiotics (sub MICs of Chloramphenicol and Ciprofloxacin),and different materials include soft contact lens care solution, normal salin Methadin, Nazordin,and enzymes (Papain0.1mg/ml, Neuraminidase 0.1mg/ml) on *P. aeruginosa* adherence to soft contact lens was tested. The contact lenses were incubated with bacteria in presence of tested materials for (1hrs) at room tempreture and the number of adherence cells to contact lenses was determined[11].

Results and discussion:

Isolation:

From 15 isolates of *Pseudomonas*,two isolates were identified as *P. aeruginosa* .
P. aeruginosa isolates were able to produce gelatinase , elastease ,and protease.Determination of O-antigen serogroup by means of agglutinating sera demonstrated that the *P. aeroginosa* 1 belong to O-antigen group F:P12 and *P. aeroginosa* 2 belong to O-antigen group A:P9 . Choy *et al*[14] found that the most frequent serotypes of *P. aeroginosa* isolates from eye infections were G,A,C,E,Iand B. The ability to produce alkaline protease and gelatenase and invade the corneal epithelium may play amajor role in the pathogensis of contac lens-related *P. aeruginosa* keratitis[15].

Antibiotics sensitivity test:

The results showed that the two isolates of *P aeroginosa* were resistant to Cephalothin, Ofloxacin, Piperacillin, Moxifloxacin , but sensitive to Chloramphenicol and Ciprofloxacin, table(1).

Table 1:bacterial sensitivity to different antibiotics

Isolation no.	P	CE	MO	OF	C	CI
P.aeroginosa 1	R	R	R	R	S	S
P.aeroginosa 2	R	R	R	R	S	S

P: Piperacillin,
CE: Cephalothin,
MO: molaxacillin,
OF: Oflaxicillin,
C: Chloramphenicol,
CI: Ciprofloxacin.

The sub MIC were determined for chloramphenicol and Ciprofloxacin , it was 512 µg/ml for both of them , table(2).

Table 2 : sub minimal inhibitory concentrations with antibiotics:

Antibiotics	sub MICs
Chloramphenicol	512 µg/ml
Ciprofloxacin	512 µg/ml

The resistant of *p. aeroginosa* to penicillines as a result of production of penecillinase like(PSE-1,OXA2 and TEM-2),extended spectrum beta -lactamase, and chromosomal Cephalo-sporinase [16]. *P. aeroginosa* resists to fluroquinolones due to formation of biofilms ,and type II toxin-encoding genes [17], so that there was a major concern when antibiotics such as fluroquinolones are used as amonotherapeutic agent [14].

The effect of antibiotics and different inhibitors on production of several virulence factor of *P. aeroginosa* :

The results showed that in cultures with antibiotics and different inhibitors the ability of *P. aeruginosa* to produce elastase and protease was reduced ,residual activity of protease and elastase rang (47% -92%) ,and (42% -90%) respectively, table(3).

Table 3:The effect of antibiotics and different inhibitors on production of several virulence factors of *p. aeroginosa*

Inhibitors	Resedual activity %	
	Protease	Elastase
Chloramphenicol	84	80
Ciprofloxacin	87	88
Lens care solution	78	76
Methadin	52	49
Nazordin	47	42
Papain	92	90
Neuraminidase	89	86

The subMICs of Chloramphenicol and Ciprofloxacin had slight effect on production of protease and elastase while Nazordin and Methadin were reduced the production of protease and elastase (residual activity of protease 47%&42% respectively), and (the residual activity of elastase 42&%49%) respectively. The results also demonstrated that enzymes(Papain0.1mg/ml, Neuraminidase 0.1mg/ml) had a significant impact on the production of protease and elastase. The inhibitory antibiotics play important role in regulating bacterial genes including virulence factor genes, also antibiotics at low concentrations can regulate virulence factors ,and therefore influence bacterial pathogenesis [18].

The effect of antibiotics and different inhibitors on *P. aeroginosa* adherance to soft contact lenses:

The results showed that sub MICs of Chloramphenicol and Ciprofloxacin inhibit adhesion of *p. aeroginosa* to contact lens (62.5% and 2.26% inhibition) respectively. Nazordin inhibit 90.68% of bacterial adherence while Methadin inhibit 72.06%of cell adhesion to contact lens.The contact lens observed(care) solution also prevented adhesion of *p. aeroginosa* to contact lens (42.25% inhibition).While the enzymes (Papain ,Neuroaminidase) not reduced adhesion of *p. aeroginosa* ,(figure 1).

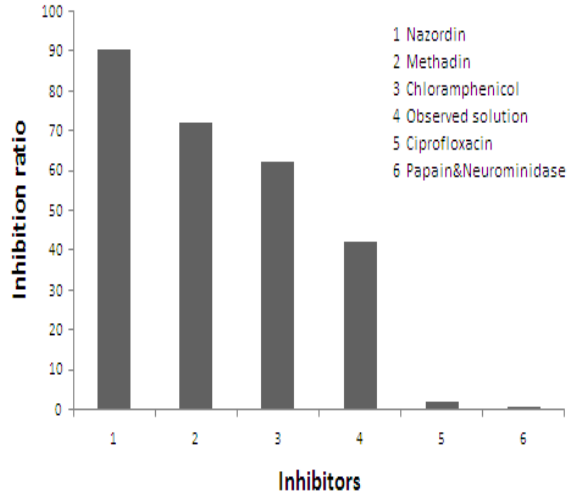

Figure 1:Inhibition ratio with antibiotics and different inhibitors.

P. aeruginosa adhesion to contact lenses depends on number of factors ,including hydrophobic interaction between the bacterial particles and contact lens polymer ,and a vailable space between polymers for binding (there is more space in polymers with higher water contact) [19]. One strategy to minimize contact lens induced infection is the development of an antimicrobial or antiadhesive contact lens[20].

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