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Study the effect of Antibiotics on pyocyanin production from *Pseudomonas aeruginosa* and pyocyanin as Antibiotic against different pathogenic bacteria"

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ABSTRACT

P. aeruginosa was cultured on nutrient agar containing different antibiotics, the growth and pigment of bacterial colonies were treated. Azithromycin killed the bacteria on low concentration, colonies of *P. aeruginosa* treated with Ampicillin, Amoxycillin, and Cefixime appeared light green .Cephalexin and Azithromycin led to formation of light green-yellow colonies, while Doxycillin ,and Clindamycin appeared green ,but there was slight change in colonies color.

The pigment (pyocyanin) was extracted from the untreated cells of bacteria and its effect as antibiotic used against many different pathogenic bacteria have been studied, the pigment inhibited *E. coli*, *Acinetobacter*, *Staphylococcus auraus*, and *Streptococcus pneumoniae but not Klebsiella pneumoniae* ,and *Proteus vulgaris* .

Introduction:

Pseudomonas aeruginosa synthesized a characteristic blue-green, chloroform-soluble compound called pyocyanin (1-hydroxy-s-methel-phenazine)(1). Pathophysiollogial effects of *P. aeruginosa* are often associated with a number of virulence factors secreted by this bacterium. Among these virulence factors secreted by *P. aeruginosa* is Pyocyanin ,a low molecular weight phenazine redox pigment(2).

Numerous studies have been made to determine the specific stimuli for pigment production in *P. aeruginosa* and related species, but the process still appears to be incompletely understood (3).

Pyocyanin has antibiotic activity against wide variety of micro-organisms ,which may benefit *P. aeruginosa* by elimination of competing micro-organisms ;pyocyanin used as antimicrobial agent ,selectively inhibitors for gram-positive and gram negative bacteria other than *Pseudomonas* spp.(4).

Pyocyanin is a virulence factor can cause death, a research indicates that salicylic acid can inhibit pyocyanin production (5).

The phenotypic change was the increased production of the phenazine redox –active molecule pyocyanin in the OxyR mutant. The OxyR mutant could not grow in LB medium,and restoration of normal pyocyanin levels, was only possible when the oxyR was present in a multicopy vector (6). This study aimed to determine the effect of different antibiotics on pyocyanin production from *P. aeruginosa* and used it as antibiotic against pathogenic bacteria.

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Materials and methods:

P. aeruginosa isolates :

P. aeruginosa isolates were obtained from clinical specimens urine form Baghdad educational hospital, and then identified by biochemical tests (7).It was maintained on nutrient agar slant as the culture of these bacteria were presented in nutrient with 20% glycerin in a deep freeze.

Antibiotics preparation:

Ampicillin, Amoxycillin, Azithromycin, Clindamycin, Cephalexin, Cefixime ,and Doxycillin. These antibiotics were prepared as serial double concentrations (2-256µg/ml) in nutrient broth.

Treatment of bacteria with antibiotics:

P. aeruginosa (0.1ml of 18 hrs aged culture) was inoculated in 5 ml of nutrient broth containing antibiotics ,and incubated at 37˚C for 24 hrs.

The control was prepared without antibiotic. (0.1ml) of the culture was streaked on nutrient agar

surface, and incubated at 37˚C for 18 hrs.The change in colonies color were observed, the colonies resulted from this treatment then cultured on nutrient agar containing the same antibiotics.

Pyocyanin extraction and it´s activity as antibiotic:

Pyocaynin was prepared by growing P. aeruginosa in LB media ,and then sub cultured on tryptic soy broth, and incubated at 37˚C for 18 hrs, centrifuged (3000 rpm for 30min), and supernant was taken. An aliquot (5ml) from culture then extracted into (1ml) of chloroform, and then reextracted into (1ml) of 0.2N HCl. The absorbance of this solution was measured at 520 nm (8).

The different pathogenic bacteria were cultured on brain-heart broth (24 hrs.),and the bacteria was cultured on a nutrient agar by spreader and left to dry for 15min after that wells made by cork borer ,and filled with (0.1µl) of pyocyanin extraction incubated for 24 hrs ,and the results were observed .

Discussion and Results:

Effect of antibiotics on bacterial growth and pyocyanin production:

P. aeruginosa was cultured in media containing different antibiotics, P. aeruginosa grew at different concentrations in media containing the other antibiotics ,most of them had an effect on color of pigment .The colonies of bacteria with Ampicillin, Amoxycillin, Cefixime appeared light green at low concentration of these antibiotics . The colonies of P. aeruginosa treated with Ampicillin, Amoxycillin ,and Cefixime appeared light green. Cephalexin , and Azithromycin led to formation of light green-yellow colonies, while Doxycillin ,and Clindamycin appeared green ,but there was slight change in colonies color at (64µg/ml) to Doxycillin and (256 µg/ml) to clindamycin(table 1).

The ability of three macrolide antibiotic (Erythromycin, Clathromycin ,and Azithromycin) to inhibit the expression of several pathogenicity traits of P. aeruginosa at sub inhibitory concentration that do not affect the rate of growth of this Micro-organism was investigated. Only Azithromycin suppressed synthesis of pyocyanin in all isolates. These results indicate that newer macrolides ,and especially Azithromycin are endowed with a remarkable ability to inhibit in vitro the expression of a number of physiological processes that are considered more essential than replication in the pathogenesis of P. aeruginosa (9).

Many studies have shown that sub inhibitory antibiotics play important roles in regulating bacterial genes including virulence factor genes. Secreted virulence related gene clusters of P. aeruginosa, and important opportunistic pathogen was examined in the presence of sub inhibitory concentration of Amipicillin and Azithromycin. Activation of gene expression was observed with phzA1 ,rh1AB ,phzA2 ,IasB ,exoy, and exoS. These antibiotics at low concentration can up-regulate virulence factors and ,therefore, influence bacterial pathogenesis (10).

Pyocyanin production was relatively low and inducible on antibiotic challenge; Pyocyanin production in the ampR mutant was significantly higher in the absence and presence of the β-lactam agent (11).

In the course of determining chromogenic variations of Pseudomonas species, some differences of pigmentation were observed. Some pigmentation differences are inherent to antibiotic sensitivity and chromogenic variations have been observed in the presence of several antibiotics (12).

The activity and pyocyanin used as antibiotic:

Pyocyanin activity (measured at 520nm) decreased in all cultures treated with antibiotics comparing with untreated one. Azithromycin gave the lowest activity (O.D= 0.12) which was 10.33% of the origin culture ,Ampicillin, and Cephalexin reduced the pigment to 25.15%-38.7 % ,while the pigment obtained from Amoxycillin, and Cefixime from 46.52%-50.40% ,Clindamycin ,and Doxycillin ranged from 70.5%-77.89 % (table 2).

When pyocyanin extracted from culture of *P. aeruginosa* without treated with antibiotics against pathogenic bacteria (gram negative and gram positive), the extracted pyocyanin inhibited *E.coli*, but it´s effect was slight and appear small inhibition zone its diameter (10mm) , and pyocyanin inhibited *Acinetobacter* , the inhibition zone was (12mm).While there was not inhibition zone observed around the wells of extracted pyocyanin aganist *K . pneumoniae* ,and *Proteus vulgaris* ,but *S. auraus*, and *Streptococcus pneumoniae*

inhibited by pyocyanin and their inhibition zones were (16mm) and (18mm) on subsequently (table3). Hassan and Fridovich demonstrated that *E. coli,* and *S. aureus* were susceptible to pyocyanin (4).

Pyocyanin categorized as bacteriostatic agents ,these investigatiors used term bacteriostatic to indicate inhibition of bacterial growth on an agar plate and ,therefore, in context, bacteriostatic agent could be bacteriocidal in action. The It was demonstrated that organisms growing aerobically with nitrate as a terminal electron accepter were as sensitive as, or more sensitive to the action of pyocyanin than organisms grown under aerobic condition ,and it was conclude that the mechanism of action is the result of pyocyanin interacting with the cell membrane respiratory chain in such a way to render the cell unable to perform energy-requiring, membrane-bound metabolic process such as active transport (13). Gram positive bacteria were much more susceptible to pyocyanin than were gram negative bacteria (6).

Pyocyanin has antibiotic activity against a wide variety of micro-organisms which may benefit *P. aeruginosa* by climination of competing micro-organisms and also has been shown to inhibit mammalian cell respiration, and inhibit both epidermal cell growth and lymphocyte proliferation, Oxygen-dependent toxicity of pyocyanin due to auto oxidation by reduced toxicity, pyocyanin leading to O^{-}_{2} and /or H_2O_2 generation, and causing cell death (14).

The conclusion from these results that pyocyanin that extracted *Pseudomonas aeruginosa* from effected by different antibiotics were used in this research and pyocyanin has antibacterial activity, and can use as antibiotic against pathginic bacteria.

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Table (1): Color of *P. aeruginosa* colony after growing in nutrient broth containing antibiotics

Antibiotics	Concentration (µg/ml)	Color
Ampicillin	16	Light green
Amoxycillin	32	Light green
Cephalexin	128	Light green-yellow
Cefixime	64	Light green
Azithromycin	8	Light green-yellow
Doxycillin	256	Green
Clindamycin	64	Green

Table(2):The percentage of pyocyanin activity after treated with different antibiotics

Antibiotics	O.D (520nm)	Pigment%
Ampicillin	0.292	25.15%
Amoxycillin	0.54	46.52%
Cephalexin	0.45	38.7%
Cefixime	0.585	50.40%
Azithromycin	0.12	10.33%
Doxycillin	0.904	77.89%
Clindamycin	0.78	70.5%

Table (3):The average of inhibition zone diameter of untreated pyocyanin extracted without treated with antibiotics from culture of *P. aeruginosa* .

Pathogenic bacteria	Diameter of inhibition zone of untreated pyocyanin with antibiotics in (mm)
E.coli	10mm
Acinetobacter	12mm
K. pneumonia	Omm
Proteus vulgaris	Omm
S. auraus	16mm
Streptococcus pneumonia	18mm

دراسة تأثير المضادات الحياتية على البايوسيانين المنتج من بكتريا *Pseudomonas aeruginosa* واستخدام البايوسيانين كمضاد حياتي ضد انواع بكتيرية ممرضة مختلفة

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الخلاصة

زرعت بكتريا P.aeruginosa في اوساط تحتوي مضادات حياتية مختلفة ،و تم متابعة النمو والصبغة لمستعمرات البكتريا الناتجة .لوحظ ان الازيثرومايسين قتل البكتريا بتراكيز قليلة في حين ظهرت المستعمرات النامية من المزارع المعاملة بالامبسيلين والاموكسيلين والسيفكسيم بلون اخضر فاتح، بينما المعاملة الازيثرومايسين والسيفالكسين ظهرت بلون اخضر–مصفر فاتح اما البكتريا المعاملة بالدوكسي سايكلين والكلندامايسين فتاثير المضادات عليها كان قليل جدا فظهرت بلون افتح من العزلة المعاملة.استخلصت صبغة البايوسيانين من الخلايا الغيرالمعاملة ودرست فعالية البايوسيانين كمضاد حياتي ضد العديد من العزلات البكتيرية الممرضة. البايوسيانين اعطى مناطق تثبيط واضحة ضد *E.coli* و *Acinetobacter* و *auraus* و *Staphylococcus* و *Streptococcus pneumoniae*. *Klebsiella* neumoniae ولا بكتريا Proteus vulgaris الا انها لم تثبط بكتريا.