THE EFFECT OF SUB-MINIMAL INHIBITORY CONCENTRATION OF AMIKACIN ,CEFTAZIDIM AND CIPROFLOXACIN ON THE PRODUCTION OF PYOCYANIN BY *Pseudomonas aeruginosa*[+]

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Abstract

The effect of sub-minimal inhibitory concentrations (sub-MICs) of three antibiotics (amikacin, ceftazidim and ciprofloxacin) on production ability of pyocyanin by *Pseudomonas aeruginosa* was studied .It was found that ciprofloxacin and ceftazidim were the most effective antibiotics followed by amikacin .The treatment with ciprofloxacin rendered the production of pyocyanin restricted to control growth. Ciftazidim completely suppressed the production on 0.5 and 0.25 of the MIC . On the other hand 91.66% of the isolates were suppressed on 0.125 of the MIC. Amikacin completely inhibited the production on 0.5 of the MIC and 75% of the isolates were suppressed on 0.125 of the MIC,but only 8.33% were suppressed on 0.125 of the MIC.

المستخلص

درس تاثير التراكيز تحت المثبطة الدنيا للمضادات الحياتية ،الاميكاسين والسفتازديم والسبوفلوكساسين على انتاج صبغة البايوسيانين فى *pseudomonas aeroginosa*

لقد وجد ان المضاد الحياتى السبروفلوكساسين والسفتازديم هما الاكثر فعالية يليهما المضاد الحيـــاتى الاميكاسين .اظهرت المعاملة بالمضاد الحياتى السبروفلوكساسبن ان الانتاجية قد االقتـــصرت فقـــط علـــى السيطرة ،امـــا الـــسفتازديم فقـــد كـــبح تمامـــا الانتاجيـــة فـــى ٠,٥و ٠,٢٥ مـــن التراكيـــز المثبطـــة الدنيا،بينما٩١,٦٦%من العزلات قد كبح انتاجها فى ٠,١٢٥ من التراكيز المثبطة الدنيا . اما الاميكاسين فقد ثبط الانتاجية تماما فى ٠،٥من التراكيز المثبطة الدنيا و٧٥% من العزلات كبح فى ٠,٢٥من التراكيز المثبطة الدنيا ولكن فقط ٨,٣٣% من العزلات قد كبح فى ٠,١٢٥ من التراكيز المثبطة الدنيا .

Introduction

In parallel with the rapid development of a wide antimicrobial agents since the 1940s bacteria have proved extremely adaptable to developing resistance to each new agent that comes along ,and this has led to the ineffectiveness of many promising agents [1].

There are several factors concerning the widespread of resistance throughout bacterial population. These factors, including self prescription, random uses and

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continuous exposure to different antibiotics in the form of underdosage, tend to accumulate the antibiotics at sub-minimal inhibitory concentration in sub-MIC in the body[2] .

The ability of antibiotics to suppress many virulence factors has been reported by many. studies In *P. aeruginosa* , many studies investigated the effect of sub-therapeutic levels of several antibiotics on a number of virulence factors which were related to invasive disease including alginate, protease, toxin and cell-associated surface structure [3,4,5] .

In this study, we evaluat the influence of sub-MIC (0.5MIC, 0.25MICand 0.125MIC) of three antibiotics (amikacin, ceftazideme and ciprofloxacin)on one of virulence factors ,the pyocyanin a blue phenazine pigment secreted in patients infected by *P.aeroginosa* isolated .

Materials and Methods

Bacterial isolates

Twelve isolates were selected to be used in this study which was made to produce pyocyanin from previous work [6].

Determination of minimal inhibitory concentrations (MIC)

The MICs of amikacin, ceftazideme (Eron S.A.,Coba) and ciprofloxacin were determined by using difold dilution method on Muller-Hinton broth as recommended by [7] .In this study , the point of minimum inhibitory concentrations (MIC) and the point of pyocyanin inhibition concentrations were determined. The MICs were defined as the lowest concentrations of antibiotics inhibited the visible growth of bacteria after 18hr of incubation at 37C[8] .

The production of pyocyanin tested on the tubes represents the 0.5 ,0.25 and 0.125 of the MIC by visible color .

The Procedure can be summerized as shown below

1. 1 ml of serial difold dilution of each antibiotic was prepared in addition to positive and negative controls.
2. 1 ml of bacterial suspention (1000000 CFU /ml)was added to each test tube except negative control tube.
3. All the test tubes were incubated at 37 C for 24 h.
4. The results of MIC and pyocyanin inhibition concentrations were read as the example below Ex.isolate number 1 , antibiotic ciprofloxacin

Antibiotic concentration	8	4	2	1	0.5	0.25	0.125	Positive control	Negative control
isible growth	-	-	-	+	+	+	+	+	-
Visible pyocyanin	-	-	-	-	+	+	+	+	-

This table reveals the MIC =4µg/ml , 0.5MIC = 2µg/ml,0.25MIC=1µg/ml, 0.125MIC= 0.5µg/ml that for(isolate number 1) ,Morever this table shows that the 0.5MIC , 0.25MIC (which have visible growth)inhibited pyocyanin production .

Results and Discussion

The effect of amikacin on pigmentation ability is shown in figure (1) The obtained results reveal that the isolates are completely suppressed to produce pyocyanin on 0.5 of the MIC ,whereas (75%) of the isolates are suppressed on 0.25 of the MIC . While in 0.125 of the MIC ,only (8.33%) of tested isolates comissioned their ability to produce the pyocyanin .

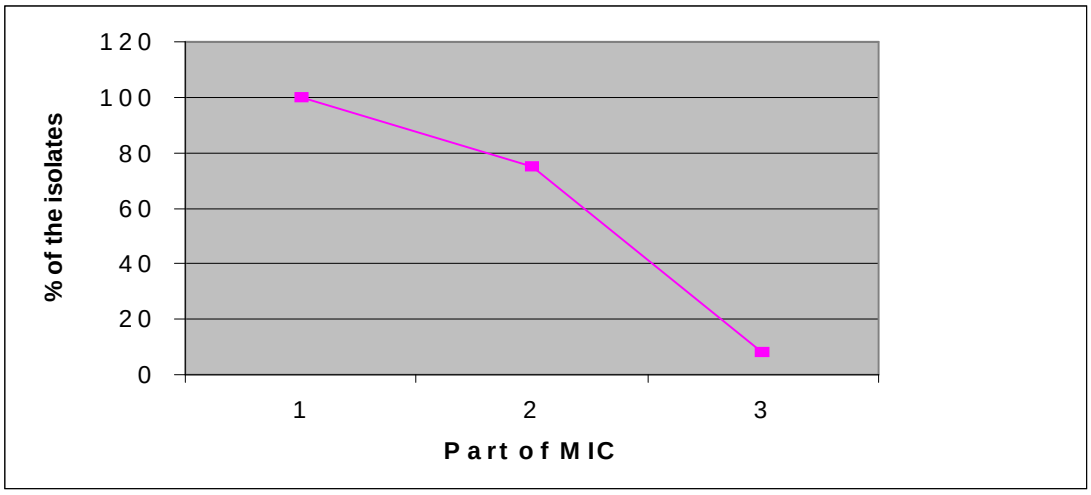

Fig. (1): The effect of amikacin on production of pyocyanin

When the isolates were treated with ceftazideme ,the established results show a complete suppression on the ability to produce the pigment on 0.5 ,0.25 of the MIC and
(91.66%) of the isolates were suppressed on 0.125 of the MIC (figure 2).

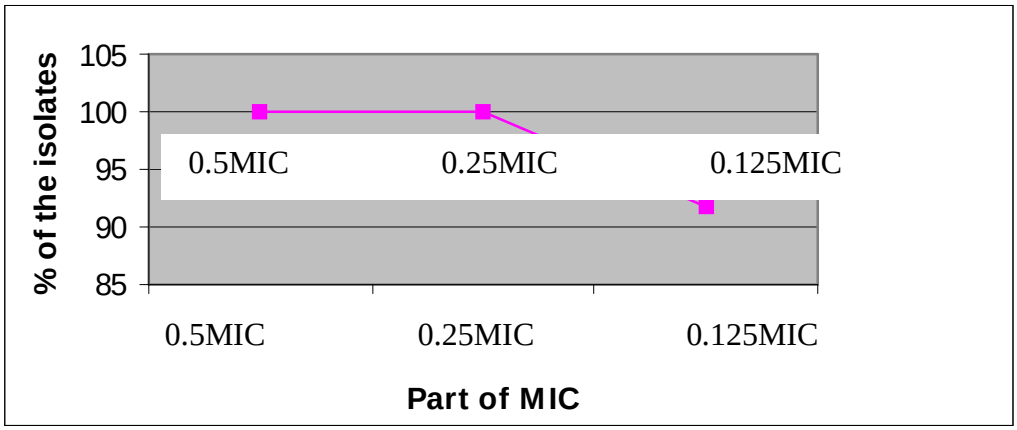

Fig. (2): The effect of ceftazideme on production of pyocyanin .

However, the treatment with ciprifloxacin exhibits that the production of pigment was restricted to the control growth (no antibiotic) except, in one isolate which was inhibited on 0.5 of the MIC (figure 3) .

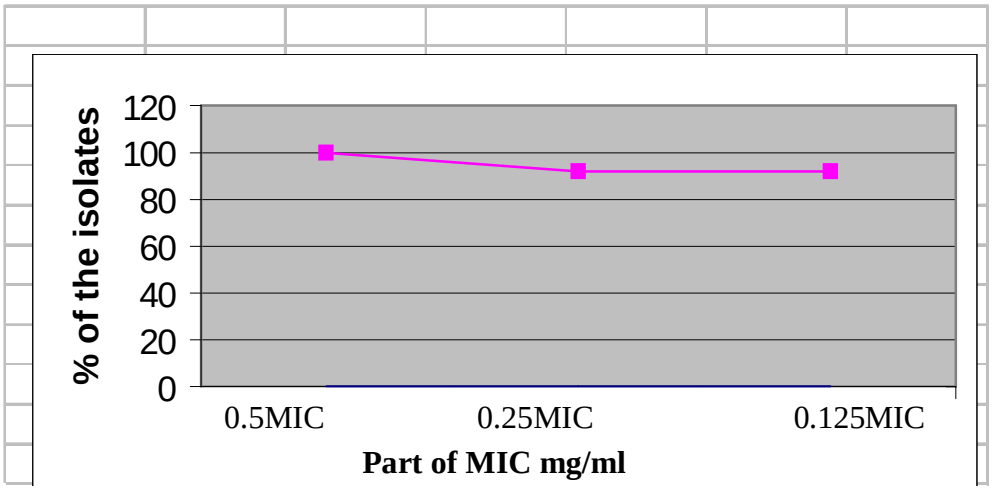

Fig. (3) The effect of ciprofloxacin on production of pyocyanin .

The treatments with ciprofloxacin and ceftazideme were found to be the most effective ,even when it was used in very low dosage (0.125 of the MIC) and followed by amikacin . These results may be explained according to mechanism role of antimicrobial agents which are adopted in this study . Ciprofloxacin acted by inhibiting the activity of DNA gyrase and the therapy prevented supercoiling of bacterial chromosome ,mining the activity on the DNA replication stage [9] therefore, the antibiotic may interfere with the expression of pyocyanin production . Ceftazideme, which is considered as a member of β-lactam antibiotic, acts by inhibiting cell wall synthesis by binding to enzymes which is known a penicillin binding protein (PBPs) which is responsible for final stage of cross linkage of bacterial cell wall [9] .so the exposure to antibiotic may alter the binding site of the pigment with the cell wall especially early known[10] which bond the pyocyanin with the cell wall of the bacteria .

Amikacin inhibits protein synthesis by blocking the binding site on ribosome, mining the actual role on the translation stage [9] ,so the treatment may interfere with one or more enzymes which are essential in the pigment formation ,therefore; we observed the influence has gradually decrease with treatment dose .

Furthermore, the exception which is noted with ciproflxacin may refer to individual variation or the specificity which distinguishes each isolatese, especially the pyocyanin production reported previously as one of secondary metabolic substances which is influenced by environmental culturing and ionic strength medium [11, 12].

Our results come in agreement with several other studies using other antibiotics [3,4] . On the other hand many authors emphasize the importance of suppression of the pyocyanin production by *P .aeroginosa* as one of virulence factors which impairs the normal function of human nasal cilia [13], disturbs respiratory epithelium[13], exerts pro inflammatory effect on human phagocytes and is possibly responsible for neutrophil- mediated tissue damage [14] , and acts synergistically with siderophore to generate hydroxyl radical capable of producing endothelial cell damage [15].

In addition to that different mutations may occur as a result of continuous exposure to antibacterial agents. These mutations may alter many factors like sigma factor (Rpos). The rops mutation leads to increase the production of the phenazine antibiotic pyocyanin and these appear to enhance chronic lung infection in rat [16].

Finally, more investigations are needed to study the precise action of antibiotics of sub-MICs on expression of pyocyanin production by *P. aeruginosa*.

References

1. Koch A.L.,"Evolution of antibiotic resistance gene function,". *Microbiol.Rev.*Vol.45 (2). pp. 355-378, (1981).
2. Murray D.M.and Barbara E ., "Problems and dilemmas of antimicrobial rsistance," *Pharmacotherapy* ,Vol. 12(6pt2).pp. 865-935,1992.
3. Sato K.K.,Linuma Y.,Hasegaswa T.,Morii T., Yammashino T., and Ohta M., "Effect of sub - inhibitory concentration of macrolides on expression of fiagellin in *P.aeruginosa* and *Proteus mirambilis,*" Antimicrob. *Agent Chemother*. Vol.44(10).pp. 2869-2872, 2000.
4. Tanaka G., Shigeta M, Komatsuzawa H ., "Suginnka H., and Usu i,T.Effect of clarithrommycin on *P . aeruginosa* biofelms," *Chemotherapy,* Vol.46(1).pp. 36-42.2000.
5. Mizukane R.,Hirikata Y., Kaku M., Ischii Y., Furuya N., Ishda K., Koga H., Kohno S., and Yamaguchi.K. "Comparative in vitro exoenzyme-suppressing activity of azithromycin and other macrolides antibiotic against *P.aeruginosa* ," *Antimicrob.Agent chemother*.Vol.38 (3).pp. 528-533, 1994.
6. AL-Shalchi S.A., "Influence of sub-lethal concentration of ceftazidime on *P.aeruginosa* exoenzymes production," *J. Basic. Med. Sci* .(In publication). 2003.
7. Miles R.S.,and Amyes C.G., "Laboratory Control of antimicrobial Therapy," In : Makie and MaCareney *Practical Medical Microbiology* Eds by Collee,J.G.; Fraser A.G.; Marmion,B.and Simmon.A. 14(ed.), Churchill Livingstone, New York. pp. 413-418,1996.
8. *National Committee for Clinical Laboratory Standards*.Approved Standard M2-A6.Villanova.Pa.1997.
9. Laurence D.R., Bennet P.N., and Brown M.S., *Clinical Pharmacology* .8(ed) Churchill Livingstone.London 1997
10. Frank L H., and Demoss R. D. , "On the biosynthesis of pyocyanin," *J. Bacteriol*,Vol. 77,pp. 776-782, 1959.
11. Kanner D.;Gerber,N. and Bartha,R. "Pattern of phenazine pigment production by a strain of *P .aeruginosa" .J. Bacteriol* . 134(2).pp. 690-692,1978.
12. Palumbo S.A., "Role of iron and sulfur in pigment and slime formation by *P.aeruginsa,"* J.*Bacteriol*.Vol. 111. pp. 430-436, 1972.
13. Kanthakumer K.; Taylor, G. and Tsang, K.W. "Mechanisms of action of *P.aerugiosa* pyocyanin on human ciliary beat in vitro" . *Infect.Immun*. Vol.61:pp. 2848-2853,1993.
14. Ras G.J., Anderson,R .and Tayler,G.W. "Proinflammatory interactions of pyocyanin and 1- hydroxyphenazine with human neutrophils in vitro," *J.Infect*.Dis.Vol. 162:pp 178-185. 1990.

15. Britigan B.E., Roedel,T.L. and Rasmussen,G.T. "Interaction of the *P.aeruginosa* secretary product pyocyanin and pyochelin generates hydroxyl radical and cause synergistic damage to endothelial cells. Implication for *Pseudomonas* associated tissue injury" .*J.Clin. Invest*.Vol.90,pp. 2187-2196, 1992.
16. Suh S.J.,Silo S.J., and Woods D .E. , Effect of rops mutation on the stress response and expression of virulence factors in *P. aeruginosa* .J.Bacteriol.Vol.181(13):pp. 3890-7, 1999.