

EFFECT OF SUB-MICS OF SOME ANTIMICROBIAL AGENTS ON SWARMING OF *PROTEUS* SPP. ⁺

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Abstract

19 swarming isolates of genus *Proteus* were isolated from urinary tract infection; these isolates were cultured on different concentrations of cetrime, methanol, tetracycline swarming. The and gentamycin to study the effect of these materials on *Proteus* spp. results show that all these materials have abolition effect on swarming. The sub-MICs of cetrime had a very good effect on *Proteus* spp. swarming and at concentrations of 0.04%, 0.03%, 0.02% and 0.01% and the percentages of isolates that didn't show swarming under each of the concentrations reached to 100%, 100%, 94.73% and 26.31% respectively while lower concentrations didn't show any effect on swarming. From treatment with methanol the results reveal that the sub-MICs of methanol also had abolition effect on *Proteus* spp. swarming at the following concentrations: 5%, 2.5% and 1.25% and the percentages of isolates that didn't show swarming were 94.73%, 41.6% and 8.3% respectively while lower concentrations didn't show any effect. From treatment with tetracycline the results showed that the sub-MICs of tetracycline show a remarkable suppression effect on *Proteus* spp. swarming at the concentrations: 64, 32 and 16 µg/ml and the percentages of isolates that didn't show swarming was 100%, 63.5% and 8.3% while lower concentrations didn't show any effect on *Proteus* spp. swarming.

Finally the effective sub-MICs of gentamycin were variable and as follows: 32, 16, 8, 4, 2 and 1 µg/ml; the percentages of isolates that didn't show swarming were: 100%, 63.5%, 63.5%, 63.5%, 45.45% and 16.6%.

المستخلص

تم عزل ١٩ عزلة من جنس الـ *Proteus spp.* من أصابات المجاري البولية وتم زرعها على تراكيز مختلفة من كل من السترامايد، الميثانول، الجينتاميسين و النيتراسايكلين لدراسة تأثير هذه المواد في الحركة الموجية لبكتريا الـ *Proteus spp.* . لقد أظهرت النتائج أن كل هذه المواد لديها القدرة على إلغاء الحركة الموجية. لقد كان للتراكيز المثبطة تحت الدنيا للسترامايد تأثير جيد جداً في الحركة الموجية لبكتريا الـ *Proteus spp.* وذلك في التراكيز ٠.٠٤% و ٠.٠٣% و ٠.٠٢% و ٠.٠١% وكانت نسبة العزلات التي لم تظهر الحركة الموجية تحت كل من هذه التراكيز كما يلي: ١٠٠% و ١٠٠% و ٩٤.٧٣% و ٢٦.٣١% على الترتيب ، بينما لم تظهر التراكيز

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الأقل أي تأثير على الحركة الموجية. ومن المعاملة بالميثانول أظهرت النتائج أن التراكيز المثبطة تحت الدنيا للميثانول أيضا" كان لها قدرة من ألغاء الحركة الموجية لهذه البكتريا وذلك ضمن التراكيز التالية : ٥% و ٢,٥% و ١,٢٥% وكانت نسبة العزلات التي لم تظهر الحركة الموجية تحت تأثير هذه التراكيز كالتالي : ٩٤,٧٣% و ٤١,٦% و ٨,٣% على الترتيب ، بينما لم تظهر التراكيز الأقل أي تأثير على الحركة الموجية . أما من المعاملة بالتتراسايكلين فقد أظهرت النتائج أن التراكيز المثبطة تحت الدنيا للتتراسايكلين كان لها قدرة واضحة على ألغاء الحركة الموجية لبكتريا الـ *Proteus spp* . وذلك في التراكيز التالية : ١٦,٣٢,٦٤ مايكروغرام / مل وكانت نسب العزلات التي لم تظهر حركة موجية كالتالي : ١٠٠% و ٦٣,٥% و ٨,٣% على الترتيب بينما لم تظهر التراكيز الأقل أي تأثير في الحركة الموجية . وأخيرا" كانت التراكيز المثبطة تحت الدنيا المؤثرة للجينتاميسين متغايرة وكما يلي: ١٦,٣٢,٦٤, ٨,٣, ٢,٥ مايكرو غرام / مل . وكانت نسبة العزلات التي لم تظهر حركة موجية كالتالي : ١٠٠% و ٦٣,٥% و ٦٣,٥% و ٤٥,٤٥% و ١٦,٦% .

Introduction

Proteus spp .are well known to be frequently involved in urinary tract infection pathologies and are also responsible for various systemic and localized infections [1].

Swarming behavior is an important feature of development of *Proteus mirabilis* catheter related infection [2]. Bacteria can ascend to the bladder through the column of urine in the catheter lumen or by extra-luminal colonization and cause the infection.

Swarming is described as the formation of the concentric zones of bacterial growth, able to cover the whole surface of solid culture medium [3]. This phenomenon causes problems in microbiology investigation and enhances the difficulty of isolating other organisms from mixed cultures involving *Proteus spp*.

Numerous media have been reported to prevent this swarming, among these are the very dry plates described by Whitby [4], the MacConky medium containing bile salts [5], ferrous ions [6], *p*-nitrophenyl glycerin [7], sodium azide, barbiton, and sulfonamide also inhibit *Proteus spp* swarming [8].

Detection of antiswarm agents allows an easier isolation of gram-positive cocci and members of the families *Enterobacteriaceae* and *Pseudomonads*. Cetrimide is an antiseptic with detergent properties [9] and variable activities against different types of bacteria and some fungi so it is used as preservative in pharmaceuticals [10].

This research is aimed to study the effect of cetrimide, methanol, tetracycline and gentamycin on *Proteus spp* swarming to be using new medium that can prevent *Proteus spp* swarming and make microbiological investigation and diagnosis easier.

Material and Methods

Bacterial isolates: 19 clinical isolates of swarm *Proteus spp*.isolated from urinary tract infection were supplied kindly by Miss Yasmine AlBayati from College of Sciences, Baghdad University.

Detecting the effect of antimicrobial agents on swarming: 4 types of Antimicrobial agents (cetrimide, methanol, tetracycline, gentamycine) were used in this research to detect their ability to suppress the swarming phenomenon of *Proteus spp.*

Cells of each strain were inoculated on a series of nutrient agar containing different concentrations of cetrimide (0,0.01%, 0.02%, 0.03%, 0.04%) or methanol (0,1.25%, 2.5%, 5%) or sub-MICs of tetracycline (0,16,32,64)µg/ml; or sub-MICs of gentamycine (0,1,2,4,8,16,32) .All these were incubated over night at 37°c. Cultures were then examined visually and compared with the control to determine the effect of each antimicrobial agent on swarming and at which concentration. From the obtained results we select the lowest concentration that gave the highest suppression activity percentage against swarming to be used in a medium containing mixed growth involving *Proteus spp* and test the growth ability of other bacteria like *E.coli*, *S.aureus*, *P.aeruginosa* on this medium.

Results and Discussion

The results show that the cetrimide has a good inhibitory effect on *Proteus spp* swarming. The concentrations 0.04% and 0.03% of cetrimide inhibit swarming of 100%of isolates. The concentration 0.02% inhibits the swarming of 94.73% of isolates. The concentration 0.01% inhibits the swarming of 26.31% of isolates, while the lower concentrations (0.005%, 0.0025%, 0.0012%)didn't show any effect on swarming (Fig 1).

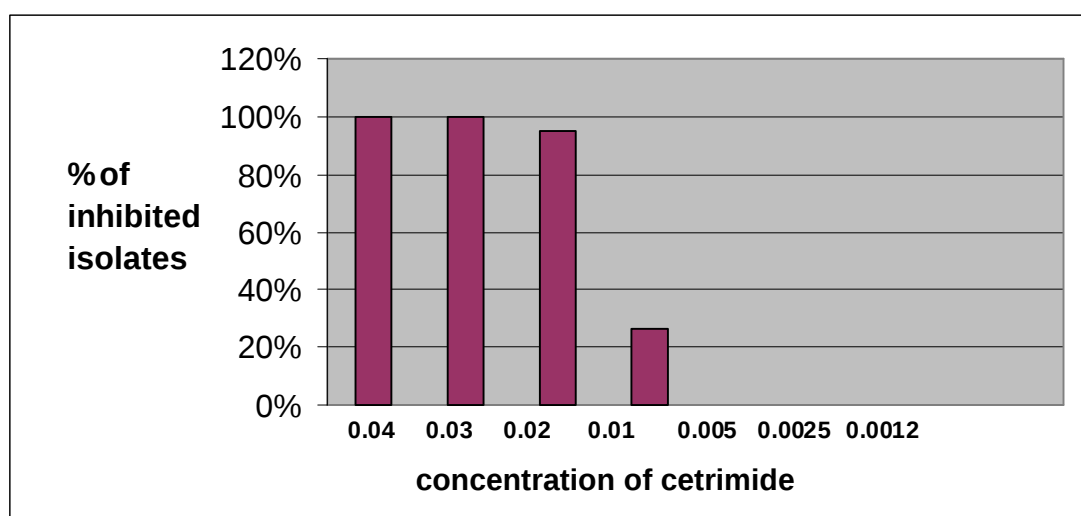


Figure (1): Effect of different concentrations of cetrimide on swarming of *Proteus spp.*isolated from urinary tract infections.

Methanol also shows a good suppression effect on swarming. The concentration of 5% inhibits swarming in 94.73%of isolates , and the concentrations 2.5% and 1.25% suppressed swarming in 41.6% and 8.3% of isolates respectively; while lower concentrations (0.6,0.3) didn't show any effect on swarming (Figure 2).

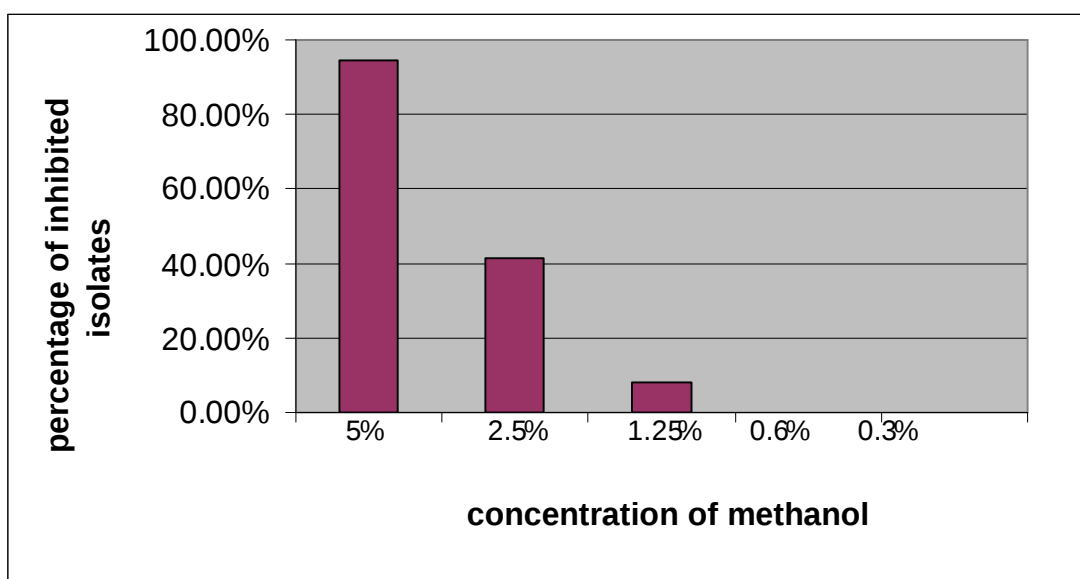


Figure (2): Effect of different concentrations of methanol on swarming of *Proteus spp.* isolated from urinary tract infections

Moreover tetracycline also shows good suppression effect on *Proteus spp.* swarming but in high concentration. 64µg/ml of tetracycline suppressed swarming in 100%of isolates and the concentration 32µg/ml of tetracycline inhibits swarming in 63.5%of isolates, the concentration 16µg/ml inhibits the swarming in 8.3%of isolates. The lower concentrations of tetracycline (8,4,2,1) µg/ml didn't show any suppression effect on swarming (figure 3).

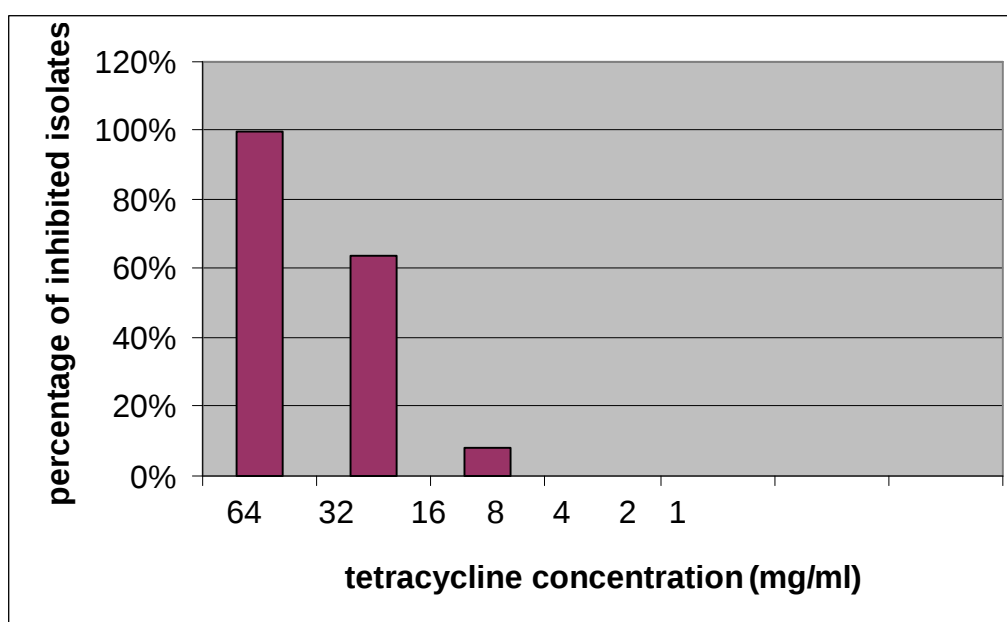


Figure (3): Effect of different concentrations of tetracycline on swarming of *Proteus spp.* isolated from urinary tract infections

The results show that gentamycin has variant inhibitory effect on *Proteus spp* swarming .32µg/ml of gentamycin inhibits swarming in all isolates and the concentrations (16,8,4) µg/ml inhibits swarming in 63.5% of isolates, the concentration 2µg/ml inhibits swarming in 45.45% of isolates while 1 µg/ml of gentamycin inhibits swarming in

16.6% of isolates. Lower concentrations (0.5, 0.25) µg/ml didn't show any effect on swarming (Figure 4).

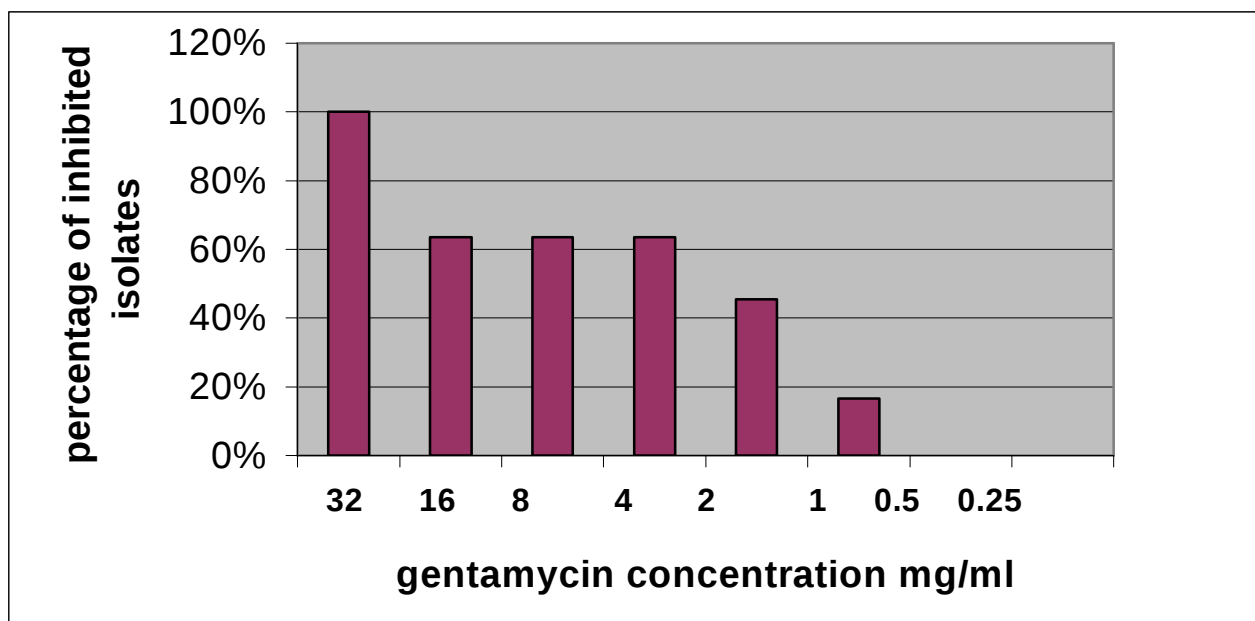


Figure (4): Effect of different concentrations of gentamycin on swarming of *Proteus spp.* isolated from urinary tract infections

There is no previous study that refers to the effect of cetrimide on swarming of *Proteus spp.*, but there are many studies that deal with suppression of *Proteus spp.* swarming by using other materials [6], [8], [7], [11].

The results prove that 0.02% of cetrimide is suitable to prepare a medium with suppression effect on swarming of *Proteus spp.* and the results show that this concentration doesn't have any inhibitory effect on the growth of *S.aureus*, *P.aeruginosa*, and *E.coli* so we may use this concentration in preparing medium to separate single colony of mixed culture of these bacteria with *Proteus spp.*

In addition there are many studies that deal with the effect of antibiotics and their modes of action on swarming but there aren't any studies that deal with effect of tetracycline and gentamycin on swarming. Molinari *et al.* (1992) show that the sub-MICs of azithromycin inhibit motility of *P.mirabilis* and this inhibition is due to the absence of flagella [12] and Sato *et al* (2000) showed that the macrolides antibiotics (erythromycin, clarithromycin, azithromycin), which have inhibitory effect on motility of *P. mirabilis* at sub-MICs, suppresses the expression of flagellin in these concentrations.

References:

- 1-Brooks, G.F.; Buter, J.S. and Morse, S.A., Enteric Gram-Negative rods (Enterobacteriaceae. In: Jawets, Melnick and Alderberg *Medical Microbiology*, 21st edition, Librairie du Liban Beirut, Lebanon, pp. 218-231, 1998.
- 2-Belas, R. & Flaherty, D., "Sequence and Genetic Analysis of Multiple Flagellin-encoding Genes From *P.mirabilis*" *Gene*, Vol.148, pp.33-41, 1994.
- 3-Crichton, P.B., Enterobacteriaceae: *Escherichia*, *Klebsiella*, *Proteus* and other genera. In: Mackie and McCartney "*Practical Medical Microbiology*" Cruickshank, by Collee, J.G.; Fraser, A.G.; Marnion, B.P. and Simmons, Churchill Livingstone, New York, pp.361-385, 1996.
- 4-Whitby, L., *Medical Microbiology*, Churchill and A Churchill, London, 1945. Cited by: Hernandez, E.; Ramisse, F.; Carallo, J., "Abolition of swarming of *Proteus*" *J.Clin.Microbiol.* Vol.37, pp. 3435-3438, 1999.
- 5-MacConkey, M., Mackie and McCartney's, *Handbook of Bacteriology*, E.Livingstone, London, UK, 1905. Cited by: Hernandez, E.; Ramisse, F.; Cavallo, J., "Abolition of swarming of *Proteus*" *J.Clin.Microbiol.*, Vol.37, pp.3435-3438, 1999.
- 6-Sokolski, W.T. and Stapert, E.M., "Medium for the Control of Bacterial Swarmer" *J.bacterial.*, Vol.85, pp.718, 1963.
- 7- Williams, F.D., "Abolition of Swarming of *Proteus* by *P*-nitrophenyl glycerin general properties" *Appl.Microbiol.*, Vol.25, pp. 745-750, 1973.
- 8-Alwen, J. & Smith, D.G., "Medium to Suppress Swarming of *Proteus* spp" *J.Appl.Bacteriol.* Vol.30, pp.389-394, 1976.
- 9-Dictionary of Medicines, Oxford University Press, Market House Books Ltd. 2000.
- 10- FeF Chemicals A/S com. (Internet).
- 11-Vanasten, F.J. & Gastra, W., "Urea Restrains Swarming of *P.mirabilis*" *J.Clin.Microbiol.* Vol.37, No.5, pp.1625, 1999.
- 12 - Molinari, G., Paglia, P. and Schito, G., "Inhibition of Motility of *P.aeruginosa* and *P.mirabilis* in sub inhibitory concentration of azithromycin" *Eur.J.Clin.Microbiol.Infec.Dis.* Vol.11, pp.469-471, 1992.
- 13-Sato, K.; Linuma, Y.; Hasegawa, T.; Yamashino, T. and Otha, M., "Effect of Subinhibitory concentrations of macrolides on expression of flagellin in *P.aeruginosa* & *P. mirabilis*" *Antimicrob.Agents.Chemother.* Vol.44, No.10, pp. 2869-2872, 2000.