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Curing of some Antibiotic Resistance in *Pseudomonas aeruginosa* Isolates by the Action of Sodium Dodecyl Sulphate and Elevated Temperature

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ABSTRACT

Twelve isolates of *Pseudomonas aeruginosa* were collected from different human infection cases, from Al-Razzi teaching hospital laboratories in Mosul. They were diagnosed using the API 20E System. The bacterial isolates were classified into four groups according to their resistance to six antibiotics (ampicillin, tetracycline, chloramphenicol, gentamycine, streptomycin and trimethoprim).

No spontaneous curing for plasmid DNA content that confer resistance to above antibiotics, was observed in the tested isolates. But, by using the sodium dodecyl sulfate at concentration of 1% (W/V) with different incubation periods as curing agent, the bacterial colonies that lost their antibiotics resistance appeared with different curing rates. The results showed 100% of curing for streptomycin resistance while for chloramphenicol and tetracycline the curing percent occurred between 17% to 83% and little effect of SDS on ampicillin and trimethoprime resistance in some isolates. In addition, curing of plasmid DNA by elevated temperature (45°C) was carried out and the results revealed high percentage of curing for all antibiotics resistance as they were compared with that induced by the action of SDS. The percentages of bacterial colonies that lost their antibiotic resistance were more than 60% and reached to 100% for curing the ampicillin resistance in the three tested isolates.

تحييد بعض المورثات المقاومة للمضادات الحيوية في عزلات جرثومة *Pseudomonas aeruginosa* بوساطة كبريتات دوديكايل الصوديوم ودرجة الحرارة المرتفعة

الملخص

تم جمع 12 عزلة من جرثومة *Pseudomonas aeruginosa* من حالات إصـابات جرثوميـة مختلفة في الإنسان من مختبرات مستشفى الرازي التعليمي بالموصل. شخصت العزلات باستخدام نظـام API ذي الحفر الدقيقة. صنفت العزلات الجرثومية الى (4) مجاميع نسبة لمقاومتها الى سـتة مـضادات حيوية (الامبسلين ، التيتراسايكلين ، الكلورامفينيكول ، الجينتامايسين، الستربتومايسين ، التراي ميثبريم).

لم يلاحظ تحييد تلقائي لمحتوى الـ DNA البلازميدي مانح المقاومة للمضادات الحيوي المستخدمة في العزلات المختبرة. ولكن عند استعمال كبريتات دوديكايـل الـصوديوم بتركيـز 1% (وزن\حجـم) وبفترات تحضين مختلفة كعامل محيد فان المستعمرات الجرثومية الفاقدة لمقاومتها للمـضادات الحيويـة ظهرت وبنسب تحييد مختلفة. النتائج أوضحت إن 100% من التحييد لمقاومة الستربتومايسين بينما نسب التحييد للمضادين الكلورامفينيكول والتيتراسايكلين كانت تتراوح ما بين 17-83% وتأثير قليل للـ SDS لتحييد مقاومة الامبسيلين والتراي ميثبريم في بعض العزلات أضف الى ذلك فقد تم تحييـد الـ DNA البلازميدي بواسطة درجة الحرارة (45°م). أظهرت النتائج نسبة عالية من تحييد المقاومـة للمـضادات الحيوية مقارنة بالنتائج التي تم الحصول عليها باستخدام الـ SDS. أن نسب المـستعمرات الجرثوميـة الفاقدة للمقاومة كانت اكثر من 60% لجميع المضادات الحيوية وقد وصلت الـى نـسبة 100% لتحييـد مقاومة الامبسيلين في ثلاثة عزلات مختبرة.

INTRODUCTION

Curing plasmids is a process by which the bacterial cells have lost their plasmids during cell division, these type of cells were said to be cured. Curing may occur naturally through cell division or by treating the cells with chemical and physical agents (Freifelder, 1983).

The possibility of losing a plasmid during cell division is increased if the plasmids form multimers during replication. The formation of multimers effectively lowers the effective copy number because each multimer will segregate into the daughter cells as a single plasmid therefore multimer greatly increase the chance of a plasmid being lost during cell division (Snyder and Champress, 1997).

There have been a number of reports demonstrating the ability of various chemical and physical agents to increase the rate of loss of plasmid DNA from bacteria, of these, (Ingram et al., 1972) found that drug resistance of *P. aeruginosa* could be eliminated from RPI containing strains by treatment with SDS, (Villar et al., 1981) show that plasmid curing carried out after treating the bacterial cells with 1% SDS in broth medium.

In addition, (Padilla et al., 1992) had observed that in *P. aeruginosa* the presence of cured plasmid was not more than 20% at optimal experimental condition. (Pattnakik et al., 1995) found that acridine orange could not effect *P. aeruginosa* due to impermeability of cell membrane while ethidium bromide and SDS affected on the curing of antibiotic resistance plasmids at concentrations of 700-3000 µg/ml and 1.2% (w/v) respectively.

MATERIALS & METHODS

Bacterial isolates collection:

Twelve isolates of *P. aeruginosa* were collected from different sources of human infections (urine, wounds, burns and vagina) from Al-Razzi hospital laboratories.

Identification of the Bacterial isolates:

They were diagnosed using API 20E microtubes system (Atlas et al., 1995).

Preparation of antibiotic stock solutions:

The method used by (Ahmed, 1989) was followed for preparation of stock solution of the antibiotics, ampicillin (Am), tetracycline (Tc), chloramphenicol (Cm), gentamycine (Gm), streptomycin (Sm) and trimethoprim (Tri). They were used at the final concentrations of 25, 10, 34, 10, 20 and 10 µg/ml, respectively and were added to the nutrient media after had been autoclaved and cooled to 50ºC.

Plasmid curing:

Spontaneous curing:

Spontaneous curing in *P. aeruginosa* isolates for their plasmid DNA content was detected according to method described by (Meyer, 1974).

Induced curing of plasmid DNA content in *P. aeruginosa* isolates by:

A. Sodium Dodecyl Sulfate (SDS):

Curing by SDS was done by the procedure mentioned by (Tomoeda et al., 1974).

B. Elevated temperature (45ºC):

This curing was carried out according to (Baldwin et al., 1969).

Selection of the cured bacterial cells:

In all treatments of curing agents, master plates were prepared containing 100 bacterial treated colonies. Replica plating technique was used in order to determine the cured cells onto the nutrient agar with antibiotics.

RESULTS & DISCUSSION

Identification of *P. aeruginosa* isolates:

A total of 12 bacterial isolates were collected from different sources of human infection. Most of the isolates produced pyocyanin (blue green pigment). The bacterial cells from smear preparation are Gram-negative, rods, arranged single or in short chain. Furthermore, biochemical tests were performed to support the above results using API 20E, which is rapid and accurate technique for identification at the family enterobacteriaceae. All the isolates are oxidase positive which is important characteristic for all the bacteria.

Antibiotic resistance of *P. aeruginosa* isolates:

The bacterial isolates were screened for their resistance to six antibiotics used. All the isolates show resistance to ampicillin while for others revealed variation in their resistance. The bacterial isolates were grouped according to their resistance to antibiotics understudy into four groups. And labeled P1, P2, P3, and P4.

Table 1: Shows the groups of *P. aeruginosa* isolates and their resistance and sensitivity to the antibiotics used.

No. of groups	Nutrient agar plates containing the final concentrations in µg/ml of					
	Am (25)	Tc (10)	Cm (34)	Sm (20)	Gm (10)	Tri (10)
P1	R	R	R	R	R	R
P2	R	R	R	S	S	R
P3	R	S	R	S	S	R
P4	R	S	R	S	S	S

R refer to resistance and S to sensitivity.

Plasmid Curing:

Spontaneous Curing:

Spontaneous curing of the plasmid DNA content for *P. aeruginosa* isolates (representing the groups understudy) were performed according to Meyer (1974). No spontaneously losses of antibiotic resistance were obtained for any of tested isolates and this may be due to the ability of the antibiotic resistance plasmid in these isolates to segregate regularly and are stable within them.

These results are disagree with that obtained when spontaneous curing carried out on, *E. coli* isolates (Mohammed, 1999) and Klebsiella pneumonia (Hasan, 2000) where there are differences in the population count of these bacteria in the absence and presence of antibiotics.

Induced Curing:

A. By Sodium Dodecyl Sulphate (SDS) action:

Concentration of SDS used were 0.1%, 0.5%, 1% and 2% (W/V) with the cultures of bacterial isolates. The results obtained, indicate that SDS at concentration 0.1%, 0.5% has no effect on plasmid curing and complete lysis of treated bacterial cells at 2% but curing of antibiotics resistance were observed at 1% and with different incubation periods.

Table 2: Show the effect of SDS at concentration 1% on curing the antibiotics resistance from *P. aeruginosa* isolates with different incubation periods.

Antibiotics	% of colonies losing their resistance to antibiotics											
	24 hours				48 hours				72 hours			
	P1	P2	P3	P4	P1	P2	P3	P4	P1	P2	P3	P4
Ampicillin	0	0	0	8	8	0	0	0	14	0	0	0
Chloramphenicol	17	0	27	0	40	55	25	0	27	0	0	0
Gentamycin	0	S	S	S	0	S	S	S	0	S	S	S
Tetracyclin	83	38	S	S	37	37	S	S	20	0	S	S
Streptomycin	100	S	S	S	100	S	S	S	10	S	S	S
Trimethoprim	0	0	0	S	0	20	0	S	0	0	0	S

It is clear from (Table 2) that the effect of SDS on antibiotic resistance plasmid DNA differed with different isolates and with the incubation time used as will. Bacterial colonies appeared 100% sensitive to streptomycin for all treated isolate and during all incubation times.

Other antibiotic resistances were affected, by SDS of all isolate with different rates including Ampicillin, chloramphenicol, and tetracycline.

The response of bacterial isolates in different rates to 1% SDS may be related to the permeability through outer membrane and the location of antibiotic resistance genes.

(Sonstein and Baldwin, 1972) elucidated that the effectiveness of SDS may be related to plasmid copy number or amount of the enzyme which inactivate antibiotics, this conclusion supported by (Ingram *et al*., 1972) who mentioned that the amount of *ß*-

lactamase produced by cured strains was only 40-60% of the β-lactamase produced by fully resistance strains.

In addition, comparing with other genus of bacteria, (Saffawi, 2001) found that the SDS affected the antibiotic resistance genes in *S. aureus* isolated from different environment and these resistance which were cured are conferred by Am, Cm, Sm genes with 100% and 65% on penicillin gene when the SDS used at concentration at 0.002% (W/V).

B. By Elevated temperature:

Elevated temperature 45˚C was used to cure plasmid DNA that confers resistance to antibiotics understudies from *P. aeruginosa* isolates.

Table 3 : Curing of plasmid DNA from *P. aeruginosa* isolates by elevated temperature.

Isolate No.	% of colonies losing their resistance to antibiotics:					
	Am	Cm	Gm	Sm	Tc	Tri
P1	63	60	80	95	80	91
P2	100	85	S	95	97	90
P3	100	85	S	S	S	87
P4	100	92	S	S	S	S

It is clear from (Table 3) that the elevated temperature has a remarkable effect on all antibiotic resistance conferred by the bacterial isolates. P1 isolate losses its resistance to Am, Cm, Gm, Sm, Tc and Tri in a range between (60-95)%, while P2, P3 and P4 loss their resistance to Am in 100% and ranging for other antibiotic resistance loss from 85% - 97%.

From above results conclusion can be made that curing by elevated temperature is an efficient curing agent among others. This may be due to that the enzymes of DNA replication become more affected by this temperature.

Our interpretation involves changing the shape (folding of the polypeptide) of the enzyme responsible for DNA replication of plasmids and may be the change make these enzyme inactive at this temperature.

Studies had been made on the effect at the elevated temperature on DNA synthesis and plasmid curing, of these, (May et al,. 1964) who obtained high frequency of loss result after growing of some bacterial strains at elevated temperature and observed that tetracycline and penicillinase positive strain of *S. aureus* grown at 43-44˚C give rise to increase proportion of tetracycline-sensitive and penicillinase negative bacteria, (Al-Amir, 1999) reported that there is a clear effect of elevated temperature on curing the plasmid DNA content from isolates of *P. aeruginosa* which agree with our results.

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