

Curing of some Antibiotic Resistance by the Action of Sodium Dodecyl Sulphate and Ethidium bromide in *Pseudomonas aeruginosa* Isolated from urine of human, cow meat and horses wound.

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SUMMARY

Thirty seven isolates of *Pseudomonas aeruginosa* Twenty-two isolates were collected from urine in Al-Diywania teaching hospital, eleven isolates from cow meat and four isolates from wound horses. They were identified using the Vitek 2, for detecting characteristics of the biochemical tests and morphological appearance. All isolates resisted to Nalidixic acid, Oxacillin, Vancomycin, Ampicillin, and Ciprofloxacin, while show sensitive to Kanamycin and Tetracycline. The minimum inhibitory concentrations (MICs) of two curing agents, sodium dodecyl sulphate (SDS) and ethidium bromide, used in this study were determined and the results indicated that the curing percentage and efficiency of each curing agent was determined. From treatment with 700 µg/ml ethidium bromide, it was observed that no cured cells were obtained for all antibiotics used and obtained for all antibiotics used by SDS at a concentration of (1, 0.9, 0.8)% (W/V) where had no effect on plasmid curing and bacterial cells complete lysis, the isolates appeared sensitive to (0.5, 0.6 and 0.7)% concentration of SDS were Nalidixic acid 10mg was (35.1, 94.5 and 100) %, Vancomycin 30mg (59.4, 86.4 and 100) %, Kanamycin 30mg was (97.2, 100, 100)% and Tetracycline 30mg was (75.6, 100 and 100)% respectively, no there sensitivity for each Oxacillin 30mg, Ciprofloxacin 5mg and Ampicillin 10mg.

Key word: *Pseudomonas aeruginosa*, SDS, curing, plasmid

تحديد بعض المورثات المقاومة للمضادات الحيوية بواسطة كبريتات دوديثيل الصوديوم و الاثيديوم
الزوائف الزنجارية المعزولة من إدرار الإنسان , لحوم الأبقار وجروح الخيول برومايد في جرثومة

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الخلاصة

تم جمع 37 عزلة من جرثومة الزوائف الزنجارية من مصادر مختلفة فكانت 22 عزلة من إدرار الإنسان من مختبرات مستشفى الديوانية التعليمي, 11 عزلة من لحوم الأبقار و4 عزلات من جروح الخيول شخّصت العزلات باستخدام جهاز الفايثك 2, الصفات المظهرية واختبارات البايوكيميائية. أظهرت جميع العزلات مقاومة للمضادات الحيوية النالديك اسد ,

الاوكتاسيلين , الفانكوميسين , الاميسيلين و السبروفلاكسيدين , اظهرت حساسية للكناميسين والتتراسيكلين . التركيز المثبط الادنى للمادتين صوديوم دودسيل سلفات والاثيديوم برومايد لتحديد كفاءة تحييد البلازميدي للعزلات , إذ لم يلاحظ تحييد لمادة الاثيديوم برومايد 700ملغ/ مل المستخدمة في العزلات المختبرة. ولكن عند استعمال كبريتات دوديسيل الصوديوم بتركيز (1, 0,9, 0,8) % وزن/حجم كعامل محيد فان المستعمرات الجرثومية تحللت , أضف الى ذلك فقد اظهرت نسب تحييد بتركيز (0,7, 0,6, 0,5) % وزن/حجم النتائج كانت النالسك اسد 10 ملغم (100 و 94,5, 35,1) % , الفانكوميسين 30ملغم (59,4 , 86,4 , 100) % , الكناميسين 30 ملغم (100, 100, 97,2) % والتتراسيكلين 30 ملغم (75,6 , 100, 100) على التوالي ولم يظهر تحييد 0,0 % لكل من الاوكساسيلين 30ملغم , الاميسيلين 10 ملغم و السبروفلاكسيدين 5 ملغم.

Introduction

Pseudomonas aeruginosa is bacteria spread rapidly and increasingly each day. It usually associated with infection of hospital-outbreaks, strains found as multiabtibiotics resistant strains. Multiantimicrobial-resistant of *Pseudomonas aeruginosa* colonizes in hematology/oncology units, a burn unit and urology wards, also in units of neonatal critical care (1, 2).

P. aeruginosa is often a part of the normal flora (normal population of bacteria that is there all the time) of the skin, mucous membranes, and intestinal contents of many healthy animals .It is also a cause of diseases in both livestock and companion animals, including otitis and urinary tract infections in dogs, mastitis in dairy cows, endometritis in horses (3).

Pseudomonas aeruginosa is very common in nosocomial infections. It is the causative agent of about 10-15% of these infections. It exhibits high resistance to different groups of antibiotics by intrinsic or acquired mechanisms. This remarkable resistance makes the treatment of *P.aeruginosa* infections very difficult (4, 5).

It is of great value to investigate new drugs to overcome such resistance either by use of these agents individually or in combination with antibiotics, antibiotic resistance is a common problem spread over the worldwide and is spreading each day due to disuse of antibiotic (6).

The resistance to antimicrobial drugs may be occurring due to reduced

permeability of the cell wall, β -lactamases production by chromosomal and plasmid and an active multidrug efflux mechanism (7).

The aim of this study to:

- 1) Isolate *Pseudomonas aeruginosa* from urine of human, wound of horses and meat of cow.
- 2) Detection the sensitivity patterns of all isolates to some selected antibiotics.
- 3) cure by use sodium dodecyl sulphate (SDS) all the isolates and Ethidium bromide.

Materials And Methods

1.Sample collection

A total of 85 different samples were collected, were Fifty samples were collected from Urine in Al-Diywania teaching hospital, twenty five sample meat of cow and ten samples horses of wound, then transferred to the lab. and reactivated by using media brain heart infusion broth . Then cultured on MacConKey agar and blood agar, Orientation chrome agar, the colonies were chosen for more purification, and all isolated submitted to oxidase test., Gram (-) where show rod in shape then subculture on nutrient media. After that incubated at (37°C) for (24) h, then freeze at (4°C), for make remain biochemical tests (8). Confirmed the isolates by Vitek 2 assay.

2.Antibiotic susceptibility test

The antibiotic discs were used included, Nalidixic acid (NA), (10mg), Oxacillin (OX)(30 mg), Vancomycin (VA) (10mg), Ampicillin (Amp) (10mg), Kanamycin (K)

(30 ug), Tetracycline , (TE) (30 mg) Ciprofloxacin (CIP) (5mg).

Antimicrobial Susceptibility tests were performed by the disc diffusion method according to the National Committee for Clinical Laboratory Standards(NCCLS) guidelines . (Oxoid, UK). Pure cultures of the bacterial isolates standardized to 0.5 McFarland turbidity standards were aseptically streaked on Mueller Hinton Agar plates using sterile cotton swab. The antibiotic disks were aseptically placed on the MH agar plates; and these were incubated at 37C⁰ for 24 hrs. The inhibition zone diameters were measured to the nearest millimeter using meter rule (9).

3- Plasmid curing

Resistance curing was conducted on multidrug resistant isolates. This was done to determine whether the gene coding for resistance is carried on the chromosomes or plasmids. Plasmid being an extra chromosomal DNA molecule is eliminated from host bacteria after exposure to sub-lethal concentrations of intercalating agents such as sodium dodecyl sulphate (SDS). The curing agent used in this work was SDS and Ethidium bromide. The experiment was done according to a previously used methodology (10).

Use of sodium dodecyl sulphate (SDS): six concentrations (1 ,0.9, 0.8, 0.7, 0.6 and 0.5)% of SDS in nutrient broth were used in this experiment. Preparation of Nutrient broth and mix with supplemented (1 , 0.9, 0.8, 0.7, 0.6 and 0.5) g of SDS in one batch of 100 ml achieve a final concentration of(1,0.9, 0.8, 0.7, 0.6 0.5) % and (w/v) SDS

respectively. It was then sterilized by autoclaving at 121°C for 15 min. Selected overnight cultures of isolates were standardized to 0.5 McFarland turbidity standards using sterile saline. From these, 0.1 ml of each culture was inoculated separately into 5 ml of SDS supplemented nutrient broth in test tubes and incubated at 37C⁰ for 24 hrs. After incubation, cultures were standardized and spread on Mueller Hinton agar and susceptibility testing was carried out on each of the cured isolates. This method was described by (11), in which the elimination of antibiotics resistance plasmid DNA from *P. aeruginosa* isolates was done by ethidium bromide, as follow: 10 ml of nutrient broth containing 700 µg/ml ethidium bromide was inoculated with 0.3 ml of overnight culture of *P. aeruginosa* isolates, and incubated at 37°C for 24, 48 and 72 h. Serial dilution was performed up to 7-10 by 0.1 ml of interval incubated samples, and 0.1 ml of the last three dilutions was placed on nutrient agar plates, then all plates were incubated at 37C⁰ for 24 hrs. Serial dilution was done by (7-10) by add (0.1) ml of distal water in the last three dilutions after that placed on plates of nutrient media, then all plates were allowed to grow for (24) at (37°C) h in incubator .

The Results & discussion:

Isolation and identification:

Thirty seven isolates of *Pseudomonas aeruginosa* Twenty-two isolates were collected from urine in Al-Diywania teaching hospital, eleven isolates from cow meat and four isolates from wound horses.table (1).

Table (1): Isolation of *P. aeruginosa* from urine of human, meat of cow and wound of horses.

<i>Pseudomonas aeruginosa</i> isolates			
Samples	Positive	Negative	Total
Urine of human	22	28	50
Cow meat	11	14	25
Horses wound	4	6	10
Total	37	48	85

Characteristics of cultural and morphological done by use nutrient agar, MacConkey agar, blood agar and orientation chrome agar. The colonies of *P. aeruginosa* isolates has smooth, large and oval shape, with flat margins. Production of pyocyanin by Thirty seven isolates (blue green stain) as figure (1), according to that mentioned by (12). The bacterial cells were gram-negative rods, short chain or arranged. All isolates produced without color colonies and did not ferment lactose sugar on MacConkey's agar. The Vitek 2 apparatus were support our results. All the isolates were positive to catalase test oxidase test. This results is in agreement with the results of (13 , 14).



Figure(1). *Pseudomonas aeruginosa* colonies produced pyocyanin (bluegreen pigment) on orientation chrome agar.



Figure (2). *Pseudomonas aeruginosa* colonies on blood agar

Antibiotic susceptibility

Antibiotic susceptibility of *P. aeruginosa* isolates included test seven antibiotics. Our study showed high percentage of multiantibiotics resistance *P. aeruginosa* isolates see (table 2). All the isolates resisted to Nalidixic acid, Oxacillin, Vancomycin, Ampicillin and Ciprofloxacin, but some isolates were sensitive (24.3%) to Kanamycin and (45.9%) to Tetracycline (figure 3).

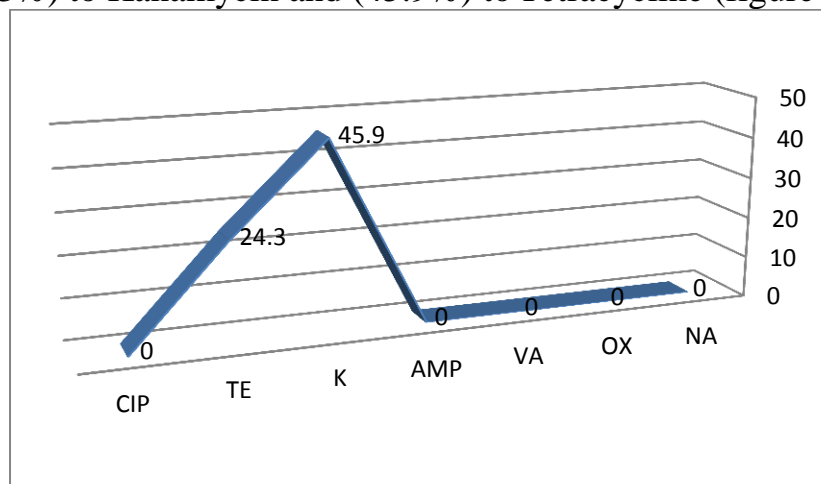


Figure (3). Percent rates of antibiotic susceptibility of the bacterial isolates.

P. aeruginosa have R-plasmid that encoding to resistance to antimicrobial (15) (16).

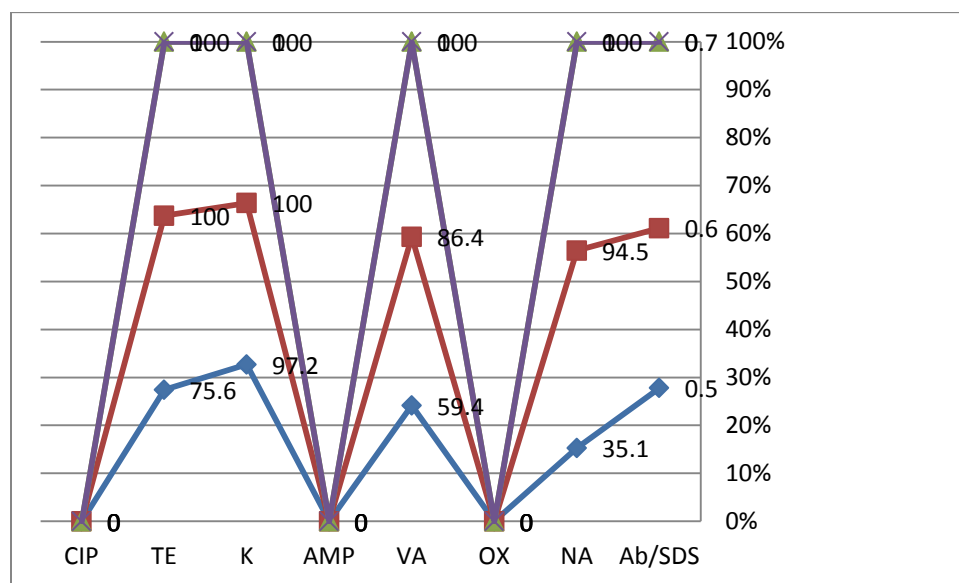
The study show has great similarity with (17) with one or more antibiotics resistance by *P. aeruginosa*.

Sodium dodecyle sulphate and plasmid curing:

Six concentrations used of SDS (1, 0.9, 0.8, 0.7, 0.6 and 0.5) % were done on the isolates to determine the changes in plasmid composition.

SDS at concentration of (1, 0.9 and 0.8) % hadn't effectiveness on curing of the plasmid and complete analysis of the bacterial cells, but curing of antibiotics resistance was observed at concentration (0.7, 0.6 and 0.5) %.

Most thirty seven multidrug resistant strains before curing, recorded the effect of (0.5, 0.6 and 0.7) % SDS as curing agent on the plasmid DNA of *P. aeruginosa* isolates. all bacterial colonies sensitive for (0.5, 0.6 and 0.7) % consternation of SDS were Nalidixic acid 10mg was (35.1, 94.5 and 100) %, Vancomycin 30mg (59.4, 86.4 and 100) %, Kanamycin 30mg was (97.2, 100, 100)% and Tetracycline 30mg was (75.6, 100 and 100)% respectively After curing, there was improvement in sensitivity due to removal of resistance plasmid DNA. There was 0.0% sensitivity each to Oxacillin 30mg , Ampicillin 10mg and Ciprofloxacin 5mg figure(4).



Figure(4). Percentages of plasmid curing by(0.5, 0.6, 0.7)% sodium dodecyl sulphate (SDS)

Current study found resistance genes, the results revealed that isolates of *P. aeruginosa* responded to varies rates to SDS, and this may be depend on permeability of the membrane, also depend on location of the gens which carried by the plasmids(18), the effectiveness of SDS may be depend on number of plasmid copies, and quantity of enzymes which inactivate antimicrobial (14).

Table (2): In vitro Antibiotic Susceptibility Patterns of *P. aeruginosa* Isolates strains before and after treatment to plasmid curing substance(SDS).

Source of isolation	No. of isolation	SDS		Antibiotic						
Urine	22			NA 10m g	OX 30m g	VA 10m g	AMP 10m g	K 30m g	TE 30m g	CIP 5mg
		Before		0s	0s	0s	0s	12s	3s	0s
		After	0.5	19s	0s	6s	0s	22s	16s	0s
			0.6	20s	0s	17s	0s	22s	22s	0s
		0.7	22s	0s	22s	0s	22s	22s	0s	
Meat	11	Before		0s	0s	0s	0s	3s	6s	0s
		After	0.5	0s	0s	11s	0s	10s	11s	0s
			0.6	11s	0s	11s	0s	11s	11s	0s
			0.7	11s	0s	11s	0s	11s	11s	0s
Wound	4	Before		0s	0s	0s	0s	2s	0s	0s
		After	0.5	4s	0s	4s	0s	4s	1s	0s
			0.6	4s	0s	4s	0s	4s	4s	0s
			0.7	4s	0s	4s	0s	4s	4s	0s
Total %	37 100%	Before		0%	0%	0%	0%	45.9 %	24.3 %	0%
		After	0.5	35.1 %	0%	59.4 %	0%	97.2 %	75.6 %	0%
			0.6	94.5 %	0%	86.4 %	0%	100 %	100 %	0%
			0.7	100 %	0%	100 %	0%	100 %	100 %	0%

Ethidium bromide was used as a curing agent according to the method described (19). The minimal inhibitory concentration of ethidium bromide was determined for the bacterial isolates in nutrient broth and the highest concentration permitting growth was used for plasmid curing. The results show that 700 µg/ml of ethidium bromide had no effect on curing of the plasmid DNA carrying all the antibiotics resistance genes, for all tested isolates and for different incubation periods (24, 48 and 72 h).

Ethidium bromide affects on coding of the plasmid DNA, tetracycline resisted with

different percentage, this result agrees with that of (20) where reported that low copy number plasmid was efficiently cured by EB. They assumed that differences in RNA polymerase and DNA polymerase occur due to depending on sensitivity of the bacterial strains, also on the differences in the degree of the antibiotic's penetration into cell wall of family Enterobacteriaceae. (21)

Exposure to EB causes the rate of elimination to decrease, and the resistance to EB to increase, and the resistance levels increase slightly after (24) hrs of growth in EB all that agreement with our results. The

plasmid that carry resistances antibiotics were not eliminated with EB. May be occur due to high copy number of resisted plasmids in these isolates. However, these results are in agreement with that found by (22) who found the percentage of cured plasmid DNA is (20%) or less.

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