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Curing of some antibiotic resistance by the action of sodium dodecyle sulphate and vitamin C in pseudomonas aerginosa isolate from horses wound and urine of human

A Graduation Project Submitted to the Department Council of the Internal and Preventive Medicine-College of Veterinary Medicine/ University of Al-Qadisiyah in a partial fulfillment of the requirements for the Degree of Bachelor of Science in Veterinary Medicine and Surgery.

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

(يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ)

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Certificate of Supervisor

I certify that the project entitled (**Curing of some antibiotic resistance by the action of sodium dodecyle sulphate and vitamin C in pseudomonas aerginosa isolate from horses wound and urine of human**)was prepared by **(Kawthar Muhammad Abdolkareem)** under my supervision at the College of Veterinary Medicine / University of Al-Qadisiyah.

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-- / -- / 2021

Certificate of Department

We certify that ----------has finished his/her Graduation Project entitled (----------) and candidate it for debating.

Instructor

Dr. Muthanna H. Hussain

-- / 06 / 2021

Head of Dept of Int. and Prev. Med.

-- / 06 / 2021

الإهــداء

يا من غرستُم في نفسي بذرة العلمِ الأولى
أهدي هذه الثمرة إليكما...

أُمي و أبي...
وفاءً وتقديراً...

كوثر محمد عبدالكريم

Dedication

SUMMARY

SUMMARY

Twenty-two isolates of *Pseudomonas aeruginosa* were collected from urine in Al-Diywania teaching hospital, eleven isolates from cowmeat and four isolates from wound horses.Theywere identified using theVitek 2, biochemical and morphologicalcharacteristics. All isolates had multiple resistance to Nalidixic acid, Oxacillin ,Vancomycin, Ampicillin, ,Ciprofloxacin and were sensitive toKanamycin ,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. Treatment with 700 µg/ml Vit.C, it was observed that no cured cells wereobtained for all antibiotics used, whereas in the treatment done for using SDS ata concentration of (1, 0.9, 0.8)% (W/V) had no effect on plasmid curing and complete lysis of treated bacterial cells, but appeared sensitive to (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,there was 0.0% sensitivity each toOxacillin 30mg,Ampicillin 10mg andCiprofloxacin 5mg.

Chapter one
(Introduction)

1.**Introduction**

1.1 *Pseudomonas aeruginosa* has been increasingly recognized for itsalmightiness to cause significant hospital-associated outbreaks of infection, particularly with the seemingof multidrugresistant strains. Outbreaks of multidrug-resistant *P. aeruginosa* colonization or infection have been reportedon urology wards, a burn unit, hematology/oncologyunits, and adult and neonatal criticalcare units (1,2).

*P.aeruginosa*is often a part of the normal flora (normalpopulation 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 nosocomialinfections. It is the causativeagent of about 10-15% ofthese infections.1 It exhibits highresistance to differentgroups of antibiotics by intrinsic or acquiredmechanisms.This remarkable resistance makes the treatment of *P.aeruginosa* infections very difficult (4,5).

It is of great value toinvestigate newdrugs to overcome such resistance either by use of these agents individually or incombination with antibiotics, drug resistance is an alarming problem worldwide and is spreading rapidly dueto overuse, self medication, and non-therapeutic useof antimicrobials (6).

Themechanisms of resistance to antibiotics include reduced cell wall permeability, production of chromosomal andplasmid mediated β-lactamases andan active multidrug effluxmechanism (7).

1.2.The aim of this study

1) Isolate *Pseudomonas aeruginosa* from urine of human , wound of horses and meat of cow .

2) Determine the sensitivity patterns (antibiograms) of these isolates to some selectedantibiotics.

3) Cure the isolates of their plasmid DNA using sodium dodecyl sulphate (SDS).

Chapter two
(Literature Review)

2. **literature Review**

The word " pseudomonas" from the Greek pseudo (“false”) + monas (“unit”). In 1894, German botanist Walter Migula coined the term Pseudomonas for a genus he described as, “Cells with polar organs of motility. Formation of spores occurs in some species, but it is rare.” Migula never clarified the etymology of the term (8).

In 1882 Carle Gessard, a chemist and bacteriologist from Paris, France, discovered Pseudomonas aeruginosa through an experiment that identified this microbe by its water soluble pigments that turned a blue-green when exposed to ultra-violet light (9)

2.1. *Pseudomonas aeruginosa* bacteria

Pseudomonas aeruginosa is a bacteria spread rapidly and increasing every day. It is usually associated with hospital injury outbreak, and existing breeds as multiple means resistance strains. Multiantiriprobial resistance of pseudomonas aeruginosa colonialism in units of blood diseases tumors, burning unit and urinary tract wings, also in neonatal units critical care (10, 11). p. aeruginosa is often part of the Ordinary Plants (Scientific Bacteria This is at all time) of the skin, mucus membranes and intestinal content for many health animals. It is also a reason for diseases in both livestock and companion animals, including ear inflammation and urinary tract infections in dogs, mastitis in dairy cows, endometritis in horses (12). Pseudomonas aeruginosa is very common in non-organized infections. It the causative agent of about 10-15% of these infections. It shows high resistance to different groups of antibiotic by intrinsic or acquired mechanisms. This remarkable resistance makes the treatment of p. aeruginosa infections are very difficult (13, 14). It is a great value to

achieve new drug to overcome this resistance either by using these agents individually or in combination with antibiotics, antibiotics resistance is a common problem spread all over the world and spread every day due to disuse of antibiotics (15). The Resistance to anti-microbial drugs may occur due to low cell wall permeability, β-lactamases production by chromosome and plasmid and an active multidrug efflux mechanism (16).

2.2. General Description of Pseudomonas bacteria:

Gram nagetive, Encapsulated, Facultatively aerobic, rod-shaped bacterium, blue-green color bacteria, Pseudomonas normally resides in the soil, marshes, and coastal marine habitats. It can survive under conditions that few other organisms can tolerate, it produces a slime layer that resists phagocytosis (engulfment), and it is resistant to most antibiotics. Pseudomonas can multiply in an extraordinary assortment of environments including eyedrops, soaps, sinks, anesthesia and resuscitation equipment, fuels, humidifiers and even stored distilled water.

2.3. Sodium dodecyl sulfate (SDS)

Sodium dodecyl sulfate (SDS) or sodium lauryl sulfate (SLS), sometimes written sodium laurilsulfate, is a synthetic organic compound with the formula CH3(CH2)11SO4Na. It is an anionic surfactant used in many cleaning and hygiene products. This molecule is an organosulfate and a salt. It consists of a 12-carbon tail attached to a sulfate group, that is, it is the sodium salt of dodecyl hydrogen sulfate, the ester of dodecyl alcohol and sulfuric acid. Its hydrocarbon tail combined with a polar "headgroup" give the compound amphiphilic properties and so make it useful as a detergent. Also derived as a component of mixtures produced from inexpensive coconut and palm oils, SDS is a common component of many domestic cleaning, personal hygiene and cosmetic, pharmaceutical, and food products, as well as of industrial and commercial cleaning and product formulations (17).

Chapter three (MATERIALS AND METHODS)

3.MATERIALS AND METHODS

3.1.Sample collection

fifty sample were collected from Urine in Al-Diywania teaching hospital, twenty five sample meat of cow and ten samples horses of wound. They were transferred to the laboratory and activated using brain heart infusion broth . After activation and inoculation of the isolates on the MacConKey and blood agar, Orientation chrome agar single colonies were selected for more purification, and oxidase test was done for positive isolates. Microscopically, Gram negative rod shape was identified provisionally as *Pseudomonas aeruginosa*, and sub cultured on nutrient agar slants. After incubation at 37°C for 24 h, it was stored at 4°C, till other bacteriological tests were carried out (18).The identification of the selected isolates was confirmed by Vitek 2.

3.2.Antibiotic sensitivity 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) guide lines .(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 sticks. The antibiotic disks were aseptically placed on the MH agar plates; and

these were incubated at 37^0C for 24 hrs. The inhibition zone diameters were measured to the nearest millimeter using meter rule (19).

3.3- Plasmid curing

Resistance curing was conducted on multidrug resistant isolates. This was done to determine whether the gene coding for resistance is carried in 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. The experiment was done according to a previously used methodology (20).

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. Nutrient broth was prepared and supplemented with(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 37C° for 24 h. After incubation, cultures were standardized and spread on Mueller Hinton agar and susceptibility testing was carried out on each of the cured isolates.

Curing with vit.C this method was described by (21), 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 10-7 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 at37°C for 24 h.

Chapter four
(Results and discussion)

4.Results and discussion

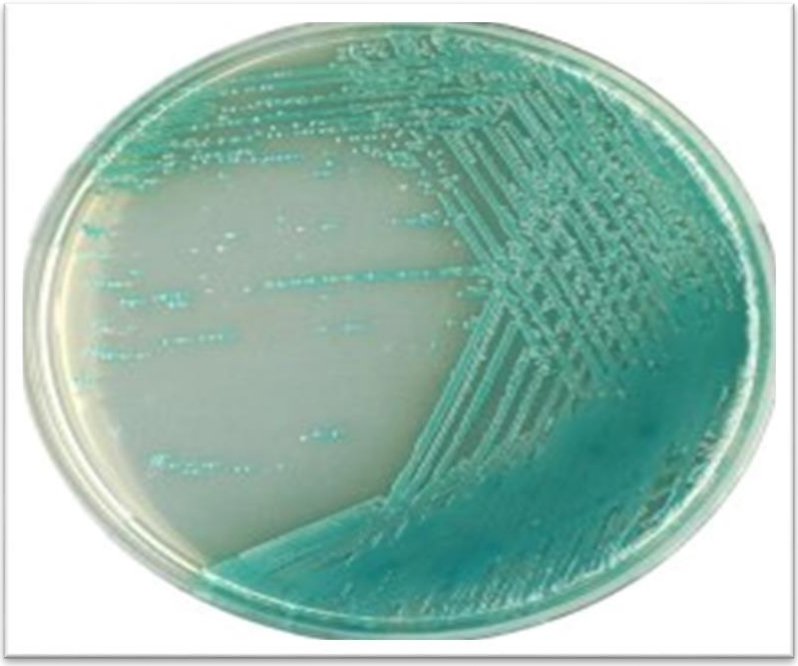
4.1. Isolation and identification of *P. aeruginosa*

About Twenty-two isolates of *P. aeruginosa* were collected from urine the laboratory of Al-Diywania teaching hospital, eleven isolates from meat of cow and four isolates from horses of wound table (1).

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

Pseudomonas aeraginosa 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

Cultural and morphological characteristics were used to characterize bacterial isolates by using nutrient, MacConkey's , blood agar and orientation chrome agar. The colonies of *P. aeruginosa* isolates had smooth, large and oval appearance, with flat edge and an elevated appearance. Thirty seven of these isolates produced pyocyanin (bluegreen pigment) figure (1), which is in accordance with that mentioned by (22). The bacterial cells from smear preparation are Gram-negative rods, arranged singly or in short chain. All bacterial produced colorlesscolonies and did not ferment lactose on MacConkey's agar. Furthermore, Vitek 2 were performed to support the results. All the isolates were oxidase and catalase positive, which is an important characteristic for all the bacteria. This result is in agreement with the results found by(23,24)



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

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

4.2Antibiotic susceptibility patterns of the bacterial isolate

The results of the antibiotic susceptibility of *P. aeruginosa* isolates included in this study for 37 antimicrobial agents .The results of the antibiotic susceptibility test showed higher number of multidrug resistance among *P. aeruginosa* isolates as shown in (table 2). All the isolates showed resistance to Nalidixic acid, Oxacillin, Vancomycin, Ampicillin and Ciprofloxacin, though some isolates were sensitive(24.3%) to Kanamycin and (45.9%)to Tetracycline (figure 3).

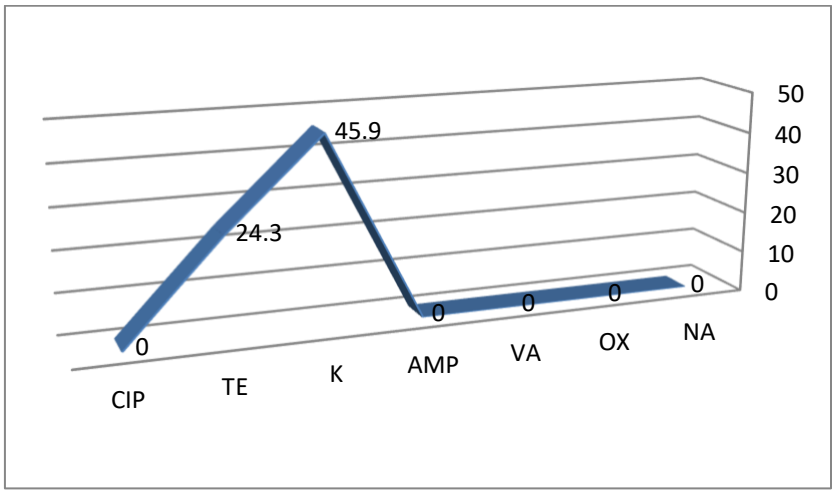


Figure (3). Percent rates of antibiotic susceptibility of the bacterial isolates.

Originally, *P. aeruginosa* harbored R-plasmid encoding multiple antibiotics resistances (25). The organism is generally resistant to numerous antimicrobial agents due to natural resistance in particular impermeability or mutations and acquisition of resistant determinants. Plasmid and integrin play a crucial role in acquisition of mobile elements (26).

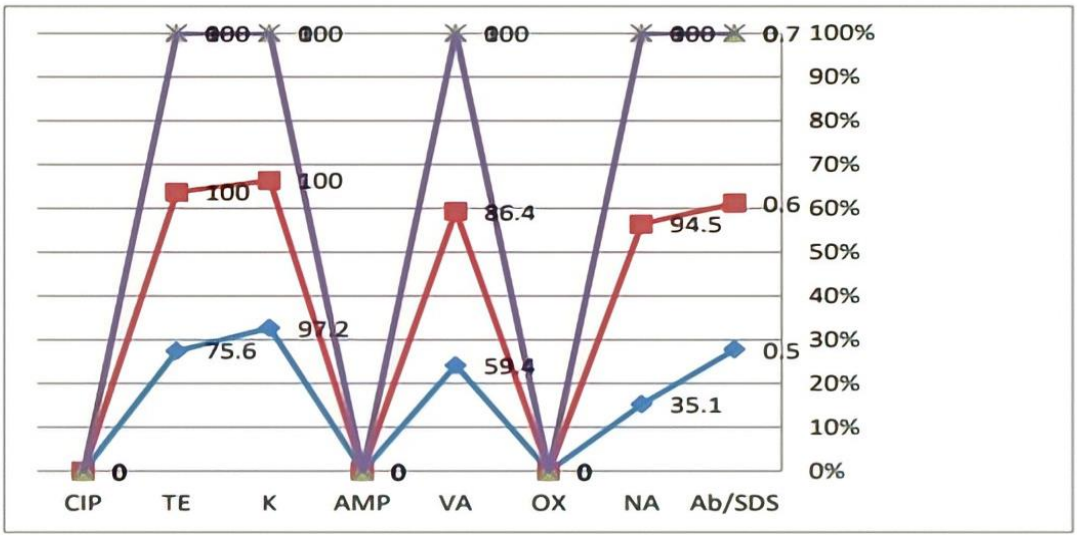
The results of antibiotic resistance study indicate that majority of *P. aeruginosa* showed antibiotic resistance to one or more antibiotics. Similar results were reported by(27).

4.3.Plasmid curing by sodium dodecyle sulphate (SDS)

Curing experiments with six concentrations of SDS (1 ,0.9, 0.8, 0.7, 0.6 and 0.5)%(W/V) were performed on the Pseudomonal isolates to determine changes in plasmid content associated with antibiotic resistance pattern.

The results obtained, indicated that SDS at concentration of (1, 0.9 and 0.8) % had no effect on plasmid curing and complete lysis of treated bacterial cells, but curing of antibiotics resistance was observed at(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)% (w/v) SDS as curing agent on the plasmid DNA of *P. aeruginosa* isolates. The results illustrated that all bacterial colonies appeared sensitive to(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 insensitivity due to removal of resistance plasmid DNA. There was 0.0% sensitivity each to Oxacillin 30mg,Ampicillin 10mg and Ciprofloxacin 5mg figure(4).



that the effectiveness of SDS may be related to plasmid copy number, or the amount of enzyme which inactivate antibiotics.(24)

Table (2): In vitro Antibiotic Susceptibility Patterns of Isolates strains before and after curing.

Source of isolation	No.of isolation	SDS		Antibiotic						
				NA 10mg	OX 30mg	VA 10mg	AMP 10mg	K 30mg	TE 30mg	CIP 5mg
Urine	22	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%

Vitamin C was used as a curing agent according to the method described by (29). The minimal inhibitory concentration of vit.C 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 vit.C 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).

In general, vit.C affects the plasmid DNA encoding, tetracycline resistances with various rates, though the antibiotic resistance genes maybe located on low copy number plasmid. This result agrees with that of (30)who reported that low copy number plasmid was efficiently cured by vit.C The agentscausing complete inhibition of plasmid replication like Acridine orange and vit. Cintercalate between base pairs in DNA. Furthermore, they suggested that differences in DNA polymerase and RNA polymerase sensitive are responsible for differences in EB sensitivity to bacterial strains due to differences in the rate of the agent's penetration into different strains of Enterobacteriaceae.(31)

speculated that further exposure to vit.C caused the rate of elimination to decrease and the resistance to vit.C to increase, and the resistance levels tended to increase slightly after 24 h of growth in vit.C. This finding agrees with the results obtained in this study. The previous results showed that the plasmids carrying antibiotic resistance genes were not eliminated with vit.C. This could be due to high copy number of these plasmids in these isolates. However, these results are in agreement with that documented by (32) who demonstrated that the percent of cured plasmid DNA is not more than 20% in optimal conditions in *P. aeruginosa.*

Chapter five
(References)

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