

**Elimination of resistance of some isolates of
Staphylococcus aureus to certain antibiotics by
chloroform and acetone extracts of the plant
*Olivera dicumbens***

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الخلاصة

من مجموع ٥٠ نموذجاً تم الحصول عليها من الإنسان ومن بيئات مختلفة، شخصت ١٨ عزلة منها على أنها تعود لبكتريا *Staphylococcus aureus*. اختبرت ٦ عزلات من هذه البكتريا بهدف ازالة مقاومتها للمضادات الحيوية بواسطة مستخلصات الكلوروفورم والاسيتون لنبات القزوح *Olivera dicumbens*. عوملت عزلات البكتريا بالتراكيز التشبيطية تحت الدنيا Sub-Minimum Inhibitory Concentrations (Sub-MIC). تم قياس نسبة المستعمرات التي فقدت مقاومتها للمضادات الحيوية. وجد ان المستخلص الكلوروفورمي بتركيز ٢٥٠ ملغم/مل كان الأكثر فاعلية في ازالة مقاومة البكتريا للمضاد الحيوي Ciprofloxacin، ووجد ان أعلى نسبة لفقدان المقاومة (٣٨%) في العزلة الخامسة. اما بالنسبة لفقدان المقاومة للمضاد الحيوي Ampicillin، فقد ظهرت أعلى نسبة مقاومة (٣٦%) للمضاد الحيوي في العزلة السادسة. من ناحية أخرى، ظهر اقل فقدان لمقاومة ضد المضاد الحيوي Nalidixic acid في العزلة الخامسة.

لم تلاحظ تأثيرات واضحة للمستخلص الاسيتوني، عليه يمكن الاستنتاج بان المستخلص الكلوروفورمي ربما يكون قد عمل مطفراً، مزيلاً لمقاومة بعض عزلات *Staph. Aureus* لبعض المضادات الحيوية.

ABSTRACT

Out of 50 samples obtained from humans of different environments, 18 were diagnosed as *Staphylococcus aureus*. 6 out of these diagnosed isolates were tested for elimination of resistance to antibiotics by chloroform and acetone extracts of the plant *Olivera dicumbens*.

Bacterial isolates were treated with subminimum inhibitory concentrations (sub-MIC) of the above mentioned extracts. Percentage of loss of resistance of colonies to antibiotics was measured. Chloroform extract at the concentration 250 mg/ml was found to be the most effective concentration inducing resistance of this bacteria to the antibiotic Ciprofloxacin. Highest percentage loss of resistance (38%) was found in the 5th isolate. For loss of resistance to the antibiotic Ampicillin, highest resistance (36%) was found in the 6th isolate. On the other hand, the least loss of resistance was found against the antibiotic Nalidixic acid in the 5th isolate. No clear effect was obtained with the acetone extract. It can, therefore, be concluded that the chloroform extract may have acted as mutagenic, eliminating the resistance of certain *Staphylococcus aureus* isolates to some antibiotics.

INTRODUCTION

Resistance, or acquisition of resistance, of bacteria to antibiotics is unclear and is still a matter of speculation. Researchers have tried to eliminate or weaken resistance of bacteria, other than *Staph. aureus*, to certain antibiotics, using chemical agents such as Acridine orange and sodium dodecyle sulphate (1), urea (2), Nalidixic acid (3), Acriflavin and Ethidium bromide (4) and Nitrosoquanidine (5); physical effectors such as Gamma irradiation (6); and heavy metals (7). Some authors, on the other hand, have mentioned that exposure of bacteria, such as *Salmonella* to multidoses of Cefalosporin increased sensitivity of the bacteria to the antibiotic, through induction of mutagenesis (8).

For plant extracts, previous authors presented some trials on effect of some extracts on sensitivity of some Gram-negative and Gram-positive bacteria (9), and effect of sub-minimum inhibitory concentrations (sub-MIC) of some plant extracts on sensitivity of *Staph. aureus* towards Tetracycline and Chloramphenicol (10).

Results of the above mentioned studies seem to have acted as a motive to investigate the possibility of elimination of resistance of the pathogenic bacteria, *Staph. aureus*, to certain antibiotics. As previous workers have documented that extracts of plants, especially those of medical importance, may well affect growth and development of different species of pathogenic bacteria, it is thought worthy to apply this idea on a plant which seems to have not tackled the thoughts of researchers in Iraq, that is *O. dicumbens*. This plant is terrestrial and is known, locally, as “deer gum”. It can be recognized by its strong smell and sharp taste of its compound flower.

MATERIALS AND METHODS

Isolates of bacteria

Out of 50 patients examined in Al-Salam hospital, 18 were found infected with *Staph. aureus*. Out of these 18 samples, 6 isolates were chosen according to their resistance to antibiotics.

Preparation of plant extracts

Chloroform and acetone extracts of *O. dicumbens* were prepared according to Makoto *et al.* (11). These extracts were tested at the concentrations of 500, 250, 125 and 62.5 mg/ml (12).

Antibiotics

Antibiotics used were Ciprofloxacin 300 µg/ml, Trymethoprim 40 µg/ml, Tetracyclin 15 µg/ml, Nalidixic acid 30 µg/ml, Ampicillin 50 µg/ml and Cephalexin 30 µg/ml. Antibiotic media were prepared according to Meckes *et al.* (13). Stock solutions and sub-MIC were prepared according to Ahmed (14).

Sub-MIC concentration of each of the above mentioned antibiotics was added to the nutrient agar (master plate) and kept in refrigerator until use.

Primary tests to eliminate resistance to antibiotics spontaneously

To acquire antibiotic-sensitive strain, colonies were transferred to master plates. Bacteria which failed to grow were reported (15,16).

Treatment of bacteria with plant extracts

Antibiotic-resistant isolates were subjected to chloroform and acetone extracts in master plates for 4, 12 and 18 hours. Colonies which failed to grow were reported (14,17).

Statistical analysis

Duncan's test was applied to express significance in differences between the means (18).

RESULTS AND DISCUSSION

Table (1) shows resistance and sensitivity of the six isolates of *Staph. aureus* test against different antibiotics. It is obvious from this table that all isolates are sensitive to Ciprofloxacin, whereas they differed in their sensitivity, or resistance, to other antibiotics tested. Isolates 1 and 5 showed highest resistance (83%) followed by isolates 4 and 6 (66%), isolate 3 (50%) and, finally, isolate 2 (17%). The only explanation available is that this difference could be attributed to a gene responsible

for eliminating an enzyme (or enzymes) which alter the resistance, or sensitivity of the bacteria to antibiotics (15).

When 100 colonies of bacteria, isolated from pharynx of patients were transferred from master plates to culture media containing antibiotics, some isolates seem to have lost resistance, spontaneously, to the antibiotics tested.

Table (1): Resistance and sensitivity of tested bacteria against different antibiotics

Nutrient agar media containing sub-MIC of antibiotics used						Bacterial isolates
Cip. (30 µg/ml)	TM (10 µg/ml)	TC (15 µg/ml)	Nal (30 µg/ml)	AP (50 µg/ml)	CF (30 µg/ml)	
S	R	R	R	R	R	1
S	S	S	R	S	S	2
S	S	R	S	R	R	3
S	R	S	R	R	R	4
S	R	R	R	R	R	5
S	R	S	R	R	R	6

R: Resistant

S: Sensitive

The order of spontaneous loss of resistance, from highest to lowest is Ciprof. (8-32%), followed by Tetra. (2-24%), Nalidix. (6-22%), Cephalex.(2-18%), Amp.(2-12%) and, finally, Trimethop.(2-4%) (Table 2).

Table (2): Spontaneous elimination of genes for the antibiotic resistance of *Staph. aureus* isolates

Nutrient agar media containing sub-MIC of antibiotics used						Bacterial isolates
Cip. (30 µg/ml)	TM (10 µg/ml)	TC (15 µg/ml)	Nal (30 µg/ml)	AP (50 µg/ml)	CF (30 µg/ml)	
18	2	2	12	6	4	1
16	0	12	22	0	6	2
8	0	S	6	S	2	3
12	0	S	10	S	2	4
32	4	24	S	12	18	5
S	0	S	8	2	S	6

S: Sensitive

These results agree with those obtained by previous works (16,17), who referred the resemblance in the spontaneous elimination of resistance to the resemblance in genetic contents of these isolates. Spontaneous loss of resistance has been explained by disorder in the distribution of plasmids carrying resistance genes (19,20).

When sub-MIC (250 µg/ml) of chloroform and acetone extracts of the plant were applied to the master plates containing colonies of *Staph. aureus* isolates, no result was obtained with the acetone extract. For chloroform extract, best results were obtained with the above mentioned

concentration at 12 hrs post-treatment. Resistance of two isolates (isolates 5 and 6) seems to be affected by the extract as sensitivity of these isolates to certain antibiotics increased.

Highest increase was obtained in isolate 6 which was 2% to Ampicillin (Table 2) and raised to 36% (Table 3), followed by isolate 1 which was 6% to Ampicillin as well (Table 2) and raised to 24%. Sensitivity to Ciprofloxacin was also raised in isolate 5 from 36% (Table 2) to 38% (Table 3).

Table (3): Elimination of antibiotics resistance of *Staph. aureus* by using chloroform in concentrations (250 µg/ml) as a mutagen

Nutrient agar media containing sub-MIC of antibiotics used						Bacterial isolates
Cip. (30 µg/ml)	TM (10 µg/ml)	TC (15 µg/ml)	Nal (30 µg/ml)	AP (50 µg/ml)	CF (30 µg/ml)	
22	0	12	0	24	16	1
6	0	0	0	6	6	2
12	S	0	0	10	0	3
14	S	S	0	S	0	4
38	4	0	0	24	12	5
16	S	0	0	36	S	6

S: Sensitive

In brief, results of the present study indicate that, highest degree of elimination of resistance occurred in case of Ampicillin. This alteration in the resistance of certain isolates to some antibiotics could either be related to the nature of the isolate or, as stated by previous authors (21,22), that the plant extract may contain some biological factors which may act as mutagens through induction of a change in the vital pathways of some enzymes in certain isolates.

However, since studies on *O. dicumbens* are rare, further investigations are needed to unravel the role which may be played by this plant on inducing positive changes in the resistance of other pathogenic bacteria to different antibiotics which will, in turn, give new clues in the fight against many diseases caused by pathogenic bacteria.

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