

Full length Research paper

Efficacy of some antibacterial agents on *Staphylococcus aureus* isolated from various burn cases

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The present study aimed to determine the major bacterial types from various burn cases and a study of antibacterial activity of plant extracts against *Staphylococcus aureus* isolated from burn cases in comparison with standard antibiotics. A total of 35 specimens were collected from burned patients (both sex, various degrees of burns) in burns unit of Al-Basrah General Hospital and Alsader Teaching Hospital in Basrah city during October 2006 to August 2007. Aqueous extracts from leaves and fruits of *Zizyphus spine christi* L. and *Olea europaea* L. were used as antibacterial agents against *S. aureus* isolated from burned patients in comparison with standard antibiotics. The predominant bacterial types are *Pseudomonas* sp. (74.19%) (23 isolates) followed by *S. aureus* (54.83%) (17 isolates), another types distributed in various percentages from burn cases. The greatest effect of plant extracts was for 1000 µg/ml of leaves of *Z. spain-christe* which gave 20 mm of inhibition zone; the fruit of *O. europaea*: 1000 Mg/ml gave 16 mm; while the standard antibiotics gave the following diameters of growth inhibition zones: kanamycin and gentamicin (26 mm), neomycin (25 mm), cephalexin (24 mm), fusidic acid (22 mm), tobramycin (16 mm). Various burns are highly infected with diversity of bacterial pathogens, the plant extracts shows a variable activity against *S. aureus*.

Key words: *Staphylococcus aureus*, antibiotics, burn cases

INTRODUCTION

Burns of skin are classified according to depth, as first, second, or third degree. While the depth of burn has a little effect on management within the first 48 h-post injury, it has a dramatic influence on wound care and overall mortality. It is, therefore, important to recognize the characteristics of the degree of injury in order to properly plan therapy and anticipate specific in patient requirements (Zuidema et al., 1979).

Experimentally and clinically, one can clearly delineate a sequence of events following thermal injury, which eventuates in the pathological and clinical entity known as "Burn wound sepsis". This is defined as the presence of organisms exceeding 100,000/gm of tissue in the burn tissues and actively invading the subjacent unburned tissue (Tieney et al., 1995).

Microbiology of the burn reveals that immediately after injury it is essentially sterile but in a matter of a few hours there is surface colonization with a variety of organisms in which the gram-positive cocci, primarily the staphylococci, predominate (Abele et al., 2000; Proctor and London,

1978).

At the period of three to five days following burn injury, the gram-negative organisms predominate and the more virulent strain begin an active invasion of the subjacent unburned tissues (Artz et al., 1979).

Two important aspects of the use of topical antibacterial therapy in the treatment of thermal injury must be constantly in mind; firstly, topical antibacterial therapy does not sterilize the burn wound. It simply results in the number of the bacteria. And secondly, since one is not eradicating the bacteria within the burn wound, control of the wound flora is merely a method of buying time during which every vigorous effort must be made to convert the open, dirty wound to a closed, clean one (Delalla, 1999). The fact that staphylococci are members of the indigenous microbial flora creates special problems in prevention and treatment of staphylococcus disease. These microorganisms also are noteworthy for the production of large number of exotoxins and other substances.

Some of that may play an important role in their capaci-

Table 1. Type of infection bacterial types is stated from 35 burn cases.

Mode of isolated Bacterial types	No of cases	%	No of isolates	%
Single	2	6.45	2	2.06
Double	8	25.8	16	16.49
Third	12	38.7	36	37.11
Over than Third	9	29.	43	44.32
Total no of positive cultured cases	31	88.57		
Total no of negative cultured cases	4	11.42		
Total no of isolates			97	

ty to cause disease (Fallon et al., 1999; Kolmos, 1999).

The present study aimed to determine the major bacterial types from various burn cases and a study of antibacterial activity of plant extracts against *Staphylococcus aureus* isolated from burn cases in comparison with standard antibiotics.

MATERIALS AND METHODS

Burn cases

A total of 35 specimens were collected from burned patients (both sex, various degree of burns) in burns unit of Al-Basrah General Hospital and Alsader Teaching Hospital in Basrah city during October 2006 to August 2007.

Bacteriology Study

Sterile swab tubes were used to collect the sample and inoculate in Brain Heart Infusion then streak on mannitol salt agar and Blood Agar.

The identification of various bacterial types and/or especially *in S. aureus* was carried out depending on routine laboratory techniques (Finegold and Boron, 1986). All culture are incubate aerobically in Incubator for 24 h on 37°C; All media sterilized by autoclave (1.5 pond/cm³) for 15 min; All glassware sterilized by oven 180-200°C for 2 h.

Plant extracts

Two plants were used in this study *Zizyphus spina-christi* L. (Rhamnaceae) leaves and fruit of *Olea europaea* L. Oleaceae leaves and fruit that are wide and in common distribution in all regions of Al-Basrah City.

Aqueous extracts from leaves and fruits of each plants – sterilized by Millipore filter technique (0.45 µm, 25 mm) - were used to test antibacterial agents and made various concentration from crude extract (100, 250, 500, 750 and 1000 mg/ml).

The antibacterial activity of plant extracts was compared with eight standard antibiotics:

Penillin G (P) (10 units), Kanamycin (K) (30 Meg), Cephalixin (CE) (30 Meg), Tetracyclin (TE) (30 Meg), Neomycin (N) (30 Meg), Genetamicin (CN) (10 Meg), Fusidic acid (FA) (30 Meg) and Tobramycin (TOB) (10 Meg).

Assay of antibacterial activity

Two methods were used to study the antibacterial activity of plant extracts against *S. aureus*. They are written below:

1) Plate (Agar diffusion) method used to determine the diameter of growth Inhibition zones (GIZ) (mm) by using Mueller Hin-ton Agar (MHA) (oxid).

2) Tube (dilution) method used to determine the minimal Inhibitory Concentrations (MICS) (Mg/ml) (MIC: are the lowest concentrations that kill 95% of bacterial cell. For plant extracts only using Brain-Heart Infusion

RESULTS

Table 1 demonstrates the mode of isolated bacterial types from 35 burns cases. We found that mode of three isolate from one case is predominant in percentage 38.7% of cases followed by over three isolates (29%), double (25.8%), and isolate single bacterial type from 6.45% of positive cultured cases. Total numbers of negative cultured cases are 11.42% from all cases.

Total numbers of isolates are 97 bacterial types identified in the second Table.

Table 2 shows the main bacterial types isolated from all positive cases 31(88.57%), the predominant bacterial types are *Pseudomonas* sp. (74.19%) (23 isolates) followed by *S. aureus* (54.83%) (17 isolates). Other types are distributed as follow:

Staphylococcus epidermidis (11 isolates) (35.48%), *Proteus* and β-hemolytic streptococi (9 isolates) (28.03 %). *E. coli* (8 iso-lates) (25.8%). *Klebsiella* and α-hemolytic streptococci (7 isolates) (22.28%). *Bacillus subtilis* (4 isolates) (12.9%) and finally *Enterobacter* (2 isolates) (6.45%).

The comparison of antibacterial activity of plant extracts and antibiotics (determine by agar method) is shown in Table 3. MICs value-by tube method-of plant extracts in Table 4, the greatest effect of plan extracts for 1000 Mg/ml of leaves of *Z. spain-christe* gave 20 mm I.Z., 750 Mg/ml (12 mm) and 500 Mg/ml (9 mm). Other concentrations not effective MIC of these extracts were between 180-200 Mg/ml.

Other extracts give the following diameters in each concentrations. Fruit of *Zizyphus* 1000 Mg/ml (18 mm), 750 Mg/ml (10 mm), 500, 250, 100: not effective MIC: 250, 300 Mg/ml. Fruit of *Olea eurooaee*: 1000 Mg/ml (16 mm), 750 Mg/ml (13 mm), 500 Mg/ml (6 mm), and 50, 100 Mg/ml not effective leaves of MIC: 250-260 same plant 100 Mg/ml (14 mm), 750 Mg/ml (8 mm), 500, 250 and

Table 2. Bacterial types isolated from (31) positive burn cases.

Bacterial types	No of cases	% from+ ve cases (31)	% of isolated from total isolates (97)
<i>Pseudomonas</i> sp.	23	74.19	23.71
<i>Escherichia coli</i>	8	25.8	8.24
<i>Proteus</i> sp.	9	29.03	9.29
<i>Klebsiella</i>	7	22.58	7.21
<i>Enterobacter</i>	2	6.45	2.06
<i>Bacillus subtilis</i>	4	12.9	4.12
<i>S. aureus</i>	17	54.83	17.52
<i>S. epidermidis</i>	11	35.48	11.34
Hemolytic <i>Streptococcus</i>	7	22.58	7.21
β .hemolytic <i>Streptococcus</i>	9	29.03	9.27
Total no of isolated types	97		
Total no of +ve burn cases	31		

Table 3. A comparison of antibacterial agents susceptibility (Plant extracts and antibiotics) against *S. aureus* isolated from burn cases (plate (Agar diffusion) methods).

I- extracts	
Diameter of Inhibition zone (mm)	
(A) <i>Zizyphus</i>	
Fruit	Results
1000	18
750	10
500	Not effective
250	Not effective
100	Not effective
Leaves	Results
1000	20
750	12
500	9
250	Not effective
100	Not effective
(B) <i>Olea</i>	
Fruit	Results
1000	16
750	13
500	6
250	Not effective
100	Not effective
Leaves	Results
1000	14
750	8
500	Not effective
250	Not effective
100	Not effective
II: antibiotics	
Antibiotic	Diameter of inhibition zone (mm)
Penicillin G 10 μ (P)	Not effective
Kanamycin 30 mg (K)	26

Table 3. contd.

Penicillin G 10 µ (P)	Not effective
Kanamycin 30 mg (K)	26
Cephalexin 30 mg (CE)	24
Tetracyclin 30 mg (TE)	Non effective
Neomycin 30 mg (N)	25
Gentamicin 10 mg (GN)	26
Fusidic acid 30 mg (FA)	22
Tobramycin 10 mg (TB)	16

Table 4. Determination of Minimal Inhibitory concentration (MIC) of studied extracts on *S. aureus* isolated from burn cases

<i>Zizyphus</i>		<i>Olea</i>	
Type	MIC mg/ml	Type	MIC mg/ml
Fruit	250-300	Fruit	250-260
Leaves	180-200	Leaves	400-450

100 Mg/ml not effective MIC: 400, 450 Mg/ml.

In comparison with standard antibiotics, the diameters of growth inhibition zones as follow: Kanamycin and Gentamicin (26 mm), Neomycin (25 mm), Cephalexin (24 mm), Fusidic acid (22 mm), Tobramycin (16 mm), Penicillin and Tetracyclin not effective.

DISCUSSION

Our results evidenced highly infected burns with various species of anaerobic and facultative anaerobic bacteria at the same place and same time and the metabolic activity of these bacteria may be able to hydrolyse carbohydrates and lysis of necrotic burned tissue aerobically and anaerobically that help various bacteria to continue in growth at the infected area (Able et al., 2000; Case well, 1998).

The isolation of numerous bacterial types in the present study confirmed the results of previous study that showed that the *P. aeruginosa*, *S. aureus*, and others may be able to proliferate rapidly in and invade through the non-viable burned tissue, and can multiply also in the wet dressing, and the risks of infection are greater in especially in burns unit that when burned patients were nursed in general units. Through the facility for expert treatment and research made burns units highly desirable in every other respect (Rodgers et al., 1997; Proctor and London, 1978).

Most antibiotic in our study are effective on growth of *S. aureus*. Same results showed in the study of Delalla (1999; Fallon et al. (1999) that showed the benefits of use of synergistic antibiotics combination and/or in single antibiotic dose for treatment of burned patients caused by pseudomonal and staphylococcal infections.

In this investigation plant extract from *Zizyphus* and *Olea* give excellent effects on *S. aureus* growth in optimal concentration 750 and 1000 mg/ml. In spite of, some concentrations of various extracts are not effective. This fact for benefits of our plant extracts as a chemotherapeutic agents without antibiotic may reflect reality importance. This idea is taken from prophetic and Arabs medicine that recommend used leaves extracts of *Zizyphus* as a preservative sterilize solutions for dead human being, and for preservation postmortem body from decomposition degradation, and another biotic process. And that is the important view as we show in results of the present study as new treatment approach for therapy of accidentally burn cases (Abele et al., 2000; Fallon et al., 1999; Faoagali et al., 1997).

Finally isolation of *S. aureus* as a sub major types from burn cases accompanied results of the same other studies which demonstrated that *S. aureus* infections may spread by extrusion to contagious tissues, or by way of lymphatic and then blood. Metastasis lesions are produced in a wide variety of tissues (Kolmos, 1999; case-well, 1998; Rodgers et al., 1997).

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