

Effects of some plant extracts and antibiotics on *Pseudomonas aeruginosa* isolated from various burn cases

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ABSTRACT

Objectives: To determine the major groups of bacteria associated with burn infections, isolation of *Pseudomonas aeruginosa* from burn cases, and the testing of antibacterial activity of some plant extracts in comparison with the standard antibiotics.

Methods: A total of 92 burn cases, covered with tetracycline (40 cases) and non-covered with tetracycline (52 cases) from both sex and various ages, were collected from various hospitals in Basrah City, Iraq on the year 2001. Bacteriological investigation for isolation of bacterial pathogens especially *Pseudomonas aeruginosa* was carried out. Two methods were used to evaluate the anti-bacterial activity of various concentrations of aqueous extracts of leaves of *Myrtus communis* and Eucalyptus with comparison to 6 antibiotics, these methods were to determine the growth inhibition zones and minimal inhibitory concentration.

Results: From a total 92 cases, 78 (84.8%) gave positive cultures. One hundred and fifty eight isolates were identified as bacterial pathogens. *Pseudomonas*

aeruginosa was the predominant bacterial pathogen from 35.9% of the tetracycline covered burns and 42.3% of the non-tetracycline covered burns followed by other bacterial types in various percentages. Aqueous leaves extracts of *myrtus communis* and eucalyptus gave an excellent effect on bacterial growth and their effects were located within the limits of antibiotic effects.

Conclusions: *Pseudomonas aeruginosa* was the predominant bacterial isolate followed by *Staphylococcus aureus*, then other type of bacterial was isolated in various percentage from each tetracycline covered burns and non-tetracycline covered burns. Most concentrations of the studied plants showed a high antibacterial activity against *Pseudomonas aeruginosa*, and showed significant differences between susceptibility of *Pseudomonas aeruginosa* isolated from each tetracycline covered burn and non-tetracycline covered burn.

Keywords: Burns, bacteria, medicinal plants, antibiotics.

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In the last 2 decades, *Pseudomonas aeruginosa* (*P. aeruginosa*) has become the most important causes of nosocomial infections. It is replacing *Staphylococcus aureus* (*S. aureus*) in causing septicemia and death in patients with burns, and being isolated frequently from intractable urinary tract infections and infected surgical wounds.^{1,2}

Pseudomonas aeruginosa is a gram negative aerobic or facultative anaerobic bacilli. It is found sporadically in most areas of the skin and in the intestinal tract of approximately 10% of healthy individuals as part of the resident microflora,³ also, it is an opportunist, able to cause infections mainly in immuno-compromised hosts.⁴

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Since it is usually resistant to all but few antibacterial agents are often predominates when sensitive organisms are suppressed.⁵ Infections of burns present a confused picture due to various factors, the most obvious one being difficulty in telling whether the pathological changes are caused by infection itself caused by the initial trauma. Confusion also arises from the fact that many burns heal well even when heavily colonized by bacteria.^{6,7} In Iraqi hospitals, as in other hospitals, *P. aeruginosa* presents a problem, especially, in the urological and burn units.¹ Although, *P. aeruginosa* produces a variety of enzymes and toxins, in addition to a slim polysaccharide and endotoxin, the mechanisms by which it produces disease remain unclear. It produces proteases that inactivate components of complements. Its exotoxin promotes cellular damage, tissue invasions and it is toxic for macrophages.^{4,8} The aims of this study are determination of the major groups of bacteria which are involve in burn cases, isolation of *P. aeruginosa* from burn cases and to test the antibacterial activity of some plant extracts in comparison with the standard antibiotics.

Methods. Burns cases. A total of 92 specimens were collected from various burned patients from both sexes and various ages. Various burned concerned 2 types tetracycline covered burns (TCBs) and non-tetracycline covered burns (non-TCBs).

Bacteriological study. Sterile swab tubes saturated with brain-heart infusion were used to collect the samples and incubated for 18-24 hours in 37°C before streaking on nutrient agar (NA), blood agar (BA) base, MacConkey agar (MA), then identify the various bacteria and *P. aeruginosa* depend on routine laboratory techniques³ Cultures were incubated aerobically at 37°C for 18-24 hours.

Plant extracts and antibiotics. Two plants were used in this study, *Myrtus communis* (*M. communis*) and *Eucalyptus species*, these 2 genera belonged to the family of *myrtaceae*. Various concentrations (50, 100, 250, 500, 750, and 1000 mcg/ml) (mcg=µg) were made from aqueous extracts of leaves of each plant, to test the antibacterial activity and compare it with a standard antibiotic (as antibiotics disc): Gentamicine (10mcg), neomycin (30mcg), tetracycline (30mcg), cephalixin (30mcg) kanamycin (30mcg) and penicillin (10 unit) supplied from Oxoid Company, England.

Evaluation of activity. Two techniques were used to determine the antibacterial activity of plant extracts and antibiotics.^{9,10} 1. Plate (agar diffusion) method - used for determination of growth inhibition zones (IZ) (mm) by using Muller-Hinton agar (MHA) from Oxoid Company, England. 2. Tube (dilution) method - used for determination minimal inhibitory concentrations (MICs) (mcg/ml) of plant extracts by using Brain-Heart infusion (Difco).

Results. In **Table 1**, a total of 92 burn specimens were cultured, 78 cases (84.8%) yielded positive culture, and 58 isolates of bacterial pathogens from 43 non-TCB and 34 TCBs were identified. We also found that isolates of double bacterial pathogens played a predominant mode of isolation from 19 (24.35%) cases of non-TCBs and 23 (29.5%) cases of TCBs, followed by other modes of isolation. **Table 2** illustrates the major bacterial types isolated from various burned patients. Evaluation of antibacterial activity of plant extracts and antibiotics on growth of *P. aeruginosa* isolated from TCBs and non-TCBs cases is shown in **Table 3**. In general, only plant extract of 1000mcg/ml gave an inhibitory effect, while concentrations of 50, 100 and 250 mcg/ml (in some) showed no effect on bacterial growth. The largest diameter of IZs (20mm) resulted from the effect of 1000mcg/ml of Eucalyptus leaves extracts and the MIC value for this extract was between 100-200mcg/ml on *P. aeruginosa* from non-TCBs. In comparison with standard antibiotics, we also summarized that anti-pseudomonal activity evaluated by IZs of growth of *P. aeruginosa* isolated from non-TCBs and TCBs in **Table 3**.

DISCUSSION. This study isolates the major bacterial pathogens associated with various types of burns, and proved that *P. aeruginosa* is the predominant pathogen in these cases followed by *S. aureus*, then the other bacterial types. We can identify the 158 isolates from TCBs and non-TCBs. Our results confirmed the result of previous studies that suggested a highly infected burn might be due to the presence of aerobic and facultative anaerobic bacteria in the same place and same time, also the metabolic activity of these bacteria can hydrolyze the carbohydrates and lysis the necrotic burned tissues aerobically and anaerobically and this will help various bacterial types to grow on the infected area.^{1,4,11} Dexter, Lowbury and Phair⁶⁻⁸ shown that *P. aeruginosa* proliferate rapidly in and invades through the non-viable burned tissue. Also, *P. aeruginosa* had the ability to proliferates in the vascular eschar, which served as a cultured media. The organisms would spread through the eschar and enter into the sub-eschar space, which demarcates the non-viable from viable tissue. If proliferation proceeded uninhibited, and the bacterial density exceeded 10⁵organisms/g of tissue, viable tissue was likely to be invaded.^{2,11} In this study, plant extract from *M. communis* and eucalyptus gave an excellent effect on growth of *P. aeruginosa*, while the same concentrations of other various extracts are not effective. These results showed the importance of plant extracts as a chemotherapeutic agent and support the old prophetic and Arabic medicine which recommend the use of the leaf extract of *Zizyphus* as a preservative and a sterilization solution for the human body after death and for preservation of

Table 1 - Numbers and percentage of infectious bacterial pathogen isolates from 92 burn cases.

Mode of isolated bacterial types	Non tetracycline covered burns				Tetracycline covered burns			
	N of cases	N of isolations	Total +ve cases (78) %	Total N of isolations (158) %	N of cases	N of isolations	Total +ve cases (78) %	Total N of isolations (158) %
Single	9	9	11.5	5.7	12	12	15.4	7.6
Double	19	38	24.5	24.1	23	46	29.5	29.1
Third	11	33	14.1	20.9	-	-	-	-
Over than 3	4	20	5.1	12.7	-	-	-	-
N +ve cultured cases	43	-	46.7	-	35	-	38.0	-
N -ve cultured cases	9	-	9.8	-	5	-	5.4	-
N isolates	-	100	-	63.3	-	58	-	36.7
Total N of +ve cultured cases 78 (84.8%), total N of cases 92, total N of -ve cultured cases 92 (15.2%), total N of isolates 158, N - number, +ve - positive, -ve - negative.								

Table 2 - Numbers and percentage of infectious bacterial types isolated from each burn case.

Bacterial types	Non tetracycline covered burns			Tetracycline covered burns		
	N of cases	Total +ve cases (78) %	Total N of isolates (158) %	N of cases	Total +ve cases (78) %	Total N of isolates (158) %
<i>Pseudomonas aeruginosa</i>	28	35.9	17.7	33	42.3	20.9
<i>E. coli</i>	10	12.8	6.3	-	-	-
<i>Klebsiella</i>	7	9	4.4	-	-	-
<i>Proteus</i>	5	6.4	3.2	-	-	-
<i>B. Subtilis</i>	6	7.7	3.8	-	-	-
<i>Staphylococcus Aureus</i>	19	24.3	12	25	32.1	15.8
<i>Staphylococcus epidermidis</i>	9	11.5	5.7	-	-	-
<i>∂-hemolytic streptococcus</i>	9	11.5	5.7	-	-	-
<i>β-hemolytic streptococcus</i>	7	9	4.4	-	-	-
N - numbers, +ve - positive, -ve - negative, E - <i>escherichia</i> , B - <i>Bacillus</i> .						

Table 3 - A comparison of antibacterial activity of various concentrations of plant extracts and antibiotics on *Pseudomonas aeruginosa* isolated from various burn cases, evaluated by determination of inhibition zone diameters (mm) and minimal inhibitory concentrations (mcg/ml).

<i>Pseudomonas aeruginosa</i>	<i>Myrtus communis</i> concentration (mcg/ml)						Eucalyptus concentrations (mcg/ml)						Antibiotics							
	Diameter of IZ (mm)					MIC	Diameter of IZ (mm)					MIC	Penicillin G	Kanna-mycin	Cepha-lexin	Tetra-cyclin	Neumycin	Genta-micin		
	1000	750	500	250	100		50	1000	750	500	250								100	50
Non-Tetracycline covered burns	14	11	5	NE	NE	NE	140 - 150	20	14	8	6	NE	NE	100 - 120	NE	14	20	8	23	28
Tetracycline covered burns	12	9	NE	NE	NE	NE	300 - 320	16	11	7	NE	NE	NE	150 - 160	NE	8	16	NE	20	19

NE - not effective, MIC - minimal inhibitory concentration, IZ - inhibition zones.

postmortem body from decomposition and degradation. Also, we have shown in our results a new treating approach for burn cases.¹²⁻¹⁵ Our results confirmed the results of other studies¹⁶⁻¹⁹ that indicated the efficient activity of *M. communis* and eucalyptus extract against various types of bacteria in vitro and in vivo levels. We recommended that a study for the presence of resistance bacteria to antibiotics, sensitivity test for all bacterial types isolated from burn cases must be carried out before giving an antibiotic for burned patients and using plants extracts used in this study as a lotion for treating burns when the other commercial ointments are absent or useless.

In conclusion, the severe infected burns showed various types of bacterial growth. The predominant mode of isolation was more than 2 or 3 bacterial pathogens. *Pseudomonas aeruginosa* was the predominant bacterial isolate followed by *S. aureus*, then other types show the various percentages from each TCBs and non-TCBs and extracts of *M. communis* and eucalyptus gave excellent effects on bacterial growth and their is effect located within the limits of antibiotics effects.

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