

INCIDENCE AND RESISTANCE PATTERN OF BACTERIA ASSOCIATED WITH BURN WOUND SEPSIS

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ABSTRACT

In the present study 100 bacterial isolates from burn wound sepsis were tested *in vitro* for their resistance pattern against twenty antimicrobial agents. The study indicated that *Pseudomonas aeruginosa* and *Staphylococcus aureus*, were the commonest organisms isolated from burn wound infections followed by *Klebsiella* species, *Proteus* species, *Echerichia coli* and *Staphylococcus epidermidis*. With reference to sensitivity/resistance pattern, it was concluded that front line antibiotics for treating burn wound infections should include ceftriaxone (cephalosporins), amikacin and tobramycin (aminoglycosides) and enoxacin (Fluoroquinolones).

INTRODUCTION

Burns provide a suitable site for bacterial multiplication. It is richer and more persistent source of infection than the surgical wounds because a readily accessible damaged tissue and nutrient rich exudates of burns constitute an excellent bacterial culture medium.

Moreover the larger area of tissue is exposed for a longer time that renders patients prone to invasive bacterial sepsis. In extensive burns when the organisms proliferate in the eschar, and when the density exceeds 100,000 organisms per gram of tissues, they spread to the blood and cause a lethal bacteremia. Therapy of burn wound infections is therefore aimed at keeping the organisms burden below 100,000 per gram of tissues which increases the chances of successful skin grafting (Robson *et al.*, 1992).

To evaluate newer therapies for wound infections, it becomes necessary to qualify and quantify bacteria that invade burn wounds. A number of clinical studies carried out to determine the flora of burn wounds in different parts of the world indicated that the general

bacterial flora of burn wounds may be different in different clinical settings and it depends on such factors as pre existing illness, types of antimicrobial used and indigenous flora that inhabit the burn unit. (Lawrence 1985; Phillips *et al.*, 1989; Fleming *et al.*, 1991; Neely *et al.*, 1991, Zellweger *et al.*, 1993; Donati *et al.*, 1993; Kalayi and Mobammad 1994 and Wu and Liu, 1994). Dodd and Stutman, 1991 discussed the current issues related with the management of burn wound sepsis. They emphasized on appropriate precautions that included meticulous washing and the use of gloves when handling the wound site and prophylactic application of topical antibacterial agent. When sepsis ensued despite these measures, systemic antibiotics should be started empirically as an adjunctive therapy to surgical debridement.

Pruitt and McManus 1992 studied the changing epidemiology of infection in burn patients. They noted that topical chemotherapy, prompt excision and timely closure of the burn wound significantly reduced the occurrence of invasive burn wound infection and its related mortality. Infection control

Table-1
Percent of resistance pattern of organisms isolated from burn wound infections

S. No.	Antimicrobial agents	Resistance pattern of wound isolates					
		<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Proteus species</i>	<i>Klebsiella species</i>	<i>Staphylococcus epidermidis</i>
1	Ampicilin	80	85.7	100	100	100	66.7
2	Amoxycillin	80	50	100	100	100	66.7
3	Amoxycillin/Clavulanic acid	88.7	42.9	50	42.9	66.7	33.3
4	Ampicillin/Cloxacillin	73.3	50	100	100	100	33.3
5	Cafazolin	93.3	42.9	87.5	42.9	66.7	66.7
6	Cephadrine	86.7	35.7	50	28.6	66.7	0
7	Cefaclor	80	14.3	37.5	28.6	33.3	33.3
8	Ceftizoxime	86.7	78.8	87.5	42.9	100	33.3
9	Cofuroxime	73.3	42.9	87.5	28.6	86.7	33.3
10	Ceftriaxone	33.3	50	37.5	14.3	86.7	0
11	Ceftazidime	26.7	42.9	50	14.3	33.3	33.3
12	Aztreonam	26.7	84.3	25	0	33.3	66.7
13	Amikacin	13.3	0	0	14.3	33.3	33.3
14	Gentamicin	33.3	14.3	37.5	0	33.3	33.3
15	Tobramycin	6.7	0	0	0	33.3	0
16	Doxycycline	100	28.6	100	85.7	100	33.3
17	Erythromycin	80	14.3	87.5	100	66.7	33.3
18	Enoxacin	0	14.3	37.5	0	33.3	0
19	Ofloxacin	0	28.6	50	14.3	33.3	0
20	Ciprofloxacin	0	35.7	37.5	0	33.3	0

procedures including surveillance of wound cultures, strict enforcement of patient and staff hygiene and patient monitoring had been effective in eliminating endemic resistant organisms and preventing the establishment of newly introduced resistant organisms.

Rosenblatt 1991 suggested that antimicrobial susceptibility tests were very

useful in guiding antimicrobial therapy and they should be performed primarily on clinically significant isolates from critical specimens by standard methods. A number of other reports on the epidemiology and successful management of burn wound infections indicated that effective and safe antibiotic control combined with surgical measures was the mainstay of the management

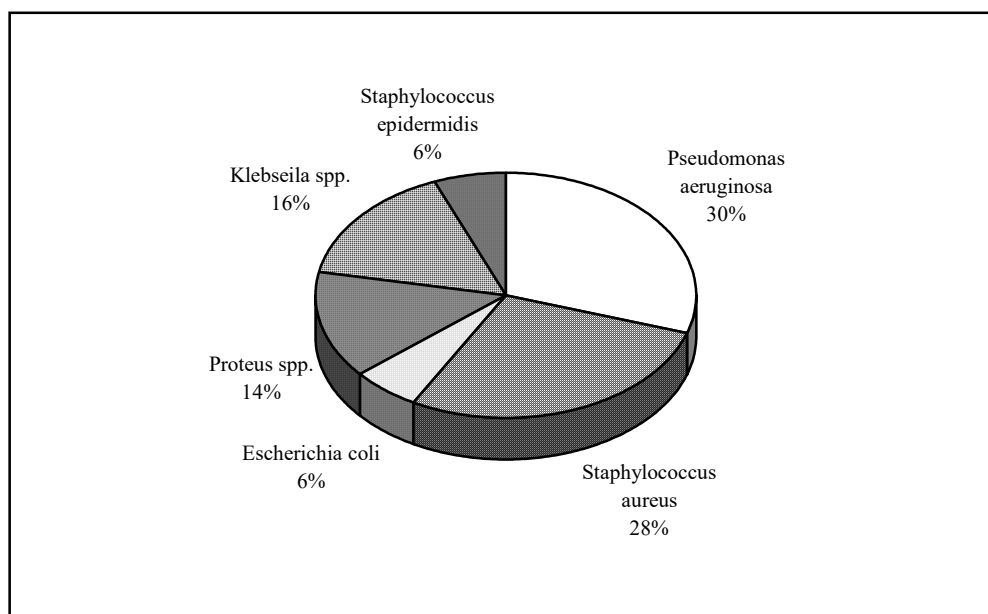


Fig. 1: Percentage of organisms isolated from burn infections (BW 1)

of serious burn wound sepsis (Polk *et al.*, 1983; Theron and Nel, 1983; Withelm, 1991; Steen, 1993; Tumidge and Grayson, 1993).

Since the current scientific literature does not indicate any report on the etiology and management of burn wound sepsis from this part of the world, the present study is carried out to determine the microbial flora of burn wounds in native setting and to study their resistance pattern against selected antibiotics.

EXPERIMENTAL

During present studies hundred clinical isolates causing burn wound infections belonging to different genera were collected, identified and their resistance/sensitivity pattern to 20 antibacterial agents was determined.

Collection of samples

The specimens were collected from two public sector hospitals of Karachi. In case of burn wounds the specimen was a piece of infected tissue obtained by tissue biopsies from wound sites collected in normal saline in

screw capped bottles at the time of surgery in operation theaters. The specimens were homogenized in homogenizer for 1 minute at a speed of 800 rps.

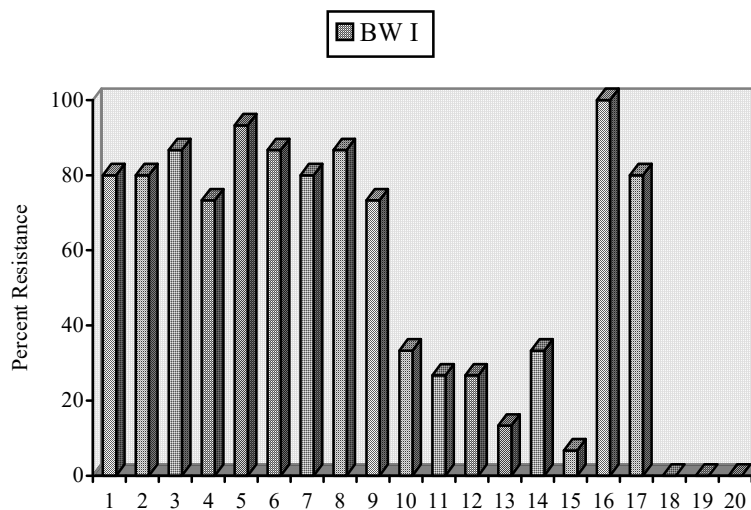
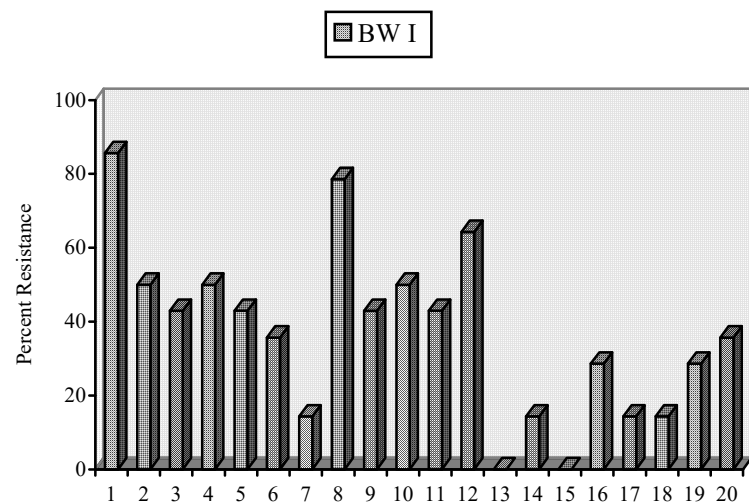
Isolation and identification of organisms

The homogenized specimens were streaked on to the surface of nutrient agar, MacConkey's agar and blood agar plates and incubated at 37°C for 24 hours. The colonies were isolated and selected for further morphological, cultural and biochemical examination for their prompt identification.

Antimicrobial testing

Mueller Hinton Agar (MHA) was used for determining the sensitivity of bacteria by single disk diffusion method of Kirby Bauer against different antimicrobial agents (Muller-Hinton 1941; Bauer *et al.*, 1966).

Each isolate was grown in 5 ml Mueller – Hinton broth at 37°C for 2-8 hours till the turbidity reached or exceeded that of a 0.5 MacCferland standard. If the standard was exceeded, the suspension was diluted with broth till it was visually comparable to the

Fig. 2: Antimicrobial resistance pattern of *Pseudomonas aeruginosa*.Fig. 3: Antimicrobial resistance pattern of *Staphylococcus aureus*.

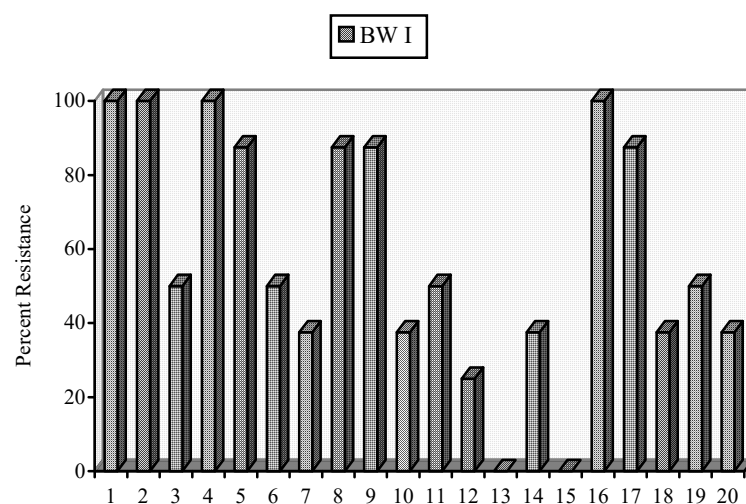
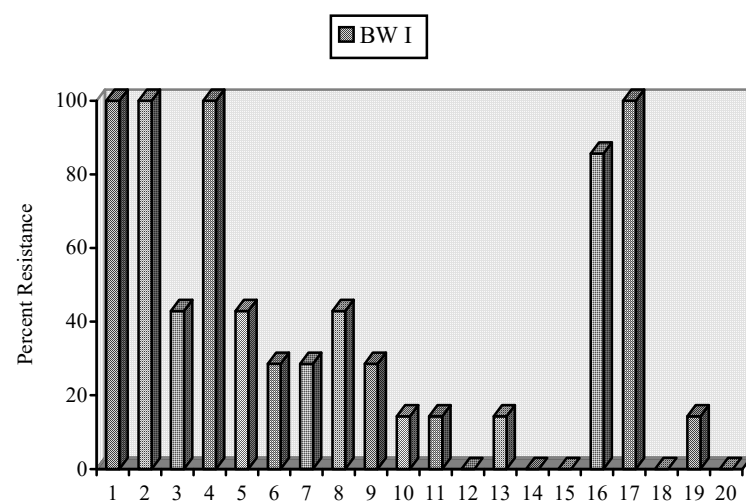
1 Ampicillin	2 Amoxycillin	3 Amoxycillin/Clavulanic acid	4 Ampicillin/Cloxacillin
5 Cafazolin	6 Cephadrine	7 Cefaclor	8 Ceftizoxime
9 Cefuroxime	10 Ceftriaxons	11 Ceftazidime	12 Aztreonam
13 Amikacin	14 Gentamicin	15 Tobramycin	16 Doxycycline
17 Erythromycin	18 Enoxacin	19 Ofloxacin	20 Ciprofloxacin

standard. The broth culture was used for streaking the Mueller – Hinton agar plates which were then allowed to dry for 10-15 minutes. The appropriate antibiotic discs were placed on the agar plates with the help of sterile pair of forceps. The plates were incubated at 35-37°C for 16-18 hours. After

incubation, the diameter of clear zones around the antibacterial discs were measured to the nearest millimeter.

RESULTS AND DISCUSSIONS

In the present study 100 specimens from

Fig. 4: Antimicrobial resistance pattern of *Escherichia coli*.Fig. 5: Antimicrobial resistance pattern of *Proteus* species.

1	Ampicillin	2	Amoxycillin	3	Amoxycillin/Clavulanic acid	4	Ampicillin/Cloxacillin
5	Cafazolin	6	Cephadrine	7	Cefaclor	8	Ceftizoxime
9	Cefuroxime	10	Ceftriaxons	11	Ceftazidime	12	Aztreonam
13	Amikacin	14	Gentamicin	15	Tobramycin	16	Doxycycline
17	Erythromycin	18	Enoxacin	19	Ofloxacin	20	Ciprofloxacin

burn wounds were studied with respect to their resistance pattern against twenty antimicrobial agents. Out of 100 burn wound isolates, 52 (52%) were collected from Abbasi Shaheed Hospital and 48 (48%) were obtained from

Civil Hospital. Tissue specimens were collected from the operation theaters of hospitals through wound biopsy culture technique followed by the homogenization of the specimen. The isolated organisms were

Pseudomonas aeruginosa (30 isolates – 30%), *Staphylococcus aureus* (28 isolates – 28%), *Klebsiella* species (16 isolates – 16%), *Proteus* species (14 isolates – 14%), *Escherichia coli* (6 isolates – 6%) and *Staphylococcus epidermidis* (6 isolates – 6%) (Fig.1).

Antibiotic sensitivity of the isolated organisms was determined by standard disk diffusion method recommended by National committee of clinical laboratory standards (Table-1). As indicated in Table-1, *pseudomonas aeruginosa*, the most common

burn wound isolates exhibited high resistance to ampicillin (80%), amoxycillin (80%), cefazolin (93.3%), cephradine (86.7%), cefacolor (80/o), erythromycin (80%), and doxycycline (100%). However the third generation cephalosporins, aminoglycosides and fluoroquinolones proved to be very effective against *Pseudomonas aeruginosa* (Table-1, Fig. 2)

Staphylococcus aureus isolates from burn wounds exhibited highest susceptibility against amikacin and tobramycin 100% and 100% respectively. Other agents were also effective except ampicillin, amoxycilin and aztreonam. Against these agents burn wounds isolates indicated a resistance pattern of 85.7%, 50.0% and 64.3% respectively (Table-1, Fig. 3).

Burn wounds isolates of *Escherichia coli* exhibited 100% resistance to ampicillin, amoxycillin, ceftizoxime and doxycycline. However cefaclor, aztreonam, aminoglycosides and fluoroquinolones were found to be highly effective agents. (Table 1, Fig. 4). Similarly, burn wound isolates of *Proteus* species indicated high resistance to ampicillin, amoxycillin, doxycycline, and erythromycin (Fig 5), and moderate sensitivity to first and second generation cephalosporins. However they were highly susceptible to third generation cephalosporins, monobactam, aminoglycosides and fluoroquinolones (Table-1, Fig. 5).

The *Klebsiella* species isolated from burn wounds indicated 100% sensitivity to amikacin and tobramycin and moderate sensitivity to cephalosporins, aminoglycosides, monobactam and fluoroquinolones (Table-1, Fig. 6).

The *Staphylococcal epidermidis* isolates showed high sensitivity to cephradine, cefaclor, ceftriaxone, aminoglycoside and fluoroquinolones. Enoxacin proved to be the most effective antibiotic exhibiting 100% sensitivity for burn wound isolates (Table-1, Fig. 7). Comparative studies of most effective antibiotics i.e. aminoglycosides (amikacin,

gentamicin and tobramycin) and fluoroquinolones (enoxacin, ciprofloxacin and ofloxacin) indicated that among aminoglycosides, amikacin proved to be most effective agent for bacteria causing burn wounds infection. Similarly among fluoroquinolones, enoxacin was the most effective agent against burn wound isolates followed by ciprofloxacin and ofloxacin.

Conclusion that can be drawn from the present studies are:

Collectively, Gram negative bacteria are more frequently involved in burn wound infection than Gram positive bacteria. This is in complete conformity with the results of Basak *et al.*, 1992; Akhtar and Aziz, 1995 and Bhutta, 1996. They reported that Gram negative aerobic bacilli were the most frequent organisms isolated from sepsis. Fleming *et al.*, 1991 hypothesized bacterial translocation as a source of burn wound contamination.

They pointed out that ischemia and reperfusion injury of Gut mucosa after severe burn injury led endogenous gastrointestinal organisms to pass into septic areas.

In present project *Pseudomonas aeruginosa* and *Saphylococcus aureus* were the commonest organisms isolated from burn wound infections followed by other Gram negative bacteria. A number of reports on burn wounds infection from different parts of the world indicated that both organisms were the most frequent isolates from different types of sepsis including burn wound (Smith and Thompson, 1992; Frame *et al.*, 1992; Kalayi and Mohammad, 1994 and Donati *et al.*, 1993).

With reference to the resistance pattern of antibiotics, the aminoglycosides and fluoroquinolones are noted to be very effective agents for treating burn wounds with lowest percent resistance. Hence it can be concluded that front line antibiotics for treating burn wound infections should include ceftriaxone (Cephalosporins) amikacin and tobramycin

(aminoglycosides) and enoxacin (fluoroquinolones). Thron and Nel, 1983 reported the beneficial role of third generation cephalosporins in the management of extensive burn wound sepsis. However Stone *et al.*, in 1983 indicated that third generation cephalosporins were equal if not superior to the combination of gentamycin plus clindamycin in the treatment of polymicrobial surgical sepsis.

In another study using gentamicin and tobramycin only for the treatment of severely burned patients, both antibiotics appeared to have substantial effect on eschar microbiology (Polk *et al.*, 1983). Since in previous reports on the management of burn wound sepsis, very limited number of antibiotics were used in single study, it was not possible to compare at length the present work with the previous studies on the subject.

Finally the study emphasized judicious limitation in the use of antimicrobials, use of the antibiotics in appropriate doses and where possible, avoidance of drugs to which resistance had been shown to emerge rapidly in a specific clinical setting. This will help to minimize the evolution and spread of resistant bacterial species.

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