

Full Length Research Paper

Bacteriology of burns and antibiogram in an Iranian burn care center

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Burn patients face an obviously high risk for infections due to the immunocompromising effects of their injury. This study aimed to detect and identify bacteria isolated from patients and hospital environment in the burn unit and determine their antibiogram pattern in response to commonly used antimicrobial agents; in order to give recommendations for management of bacterial infections and drug-resistance. Materials of this study were 100 samples of burn wounds and multiple swab samples of different hospital environments. One hundred and twelve isolates were analyzed, from which there was a single agent in the majority of cases (73.3%). *Pseudomonas aeruginosa* was the most common isolate (32.2%), followed by *Enterobacter* spp. (16.9%), coagulase-negative staphylococci (12.5%), *Acinetobacter* spp. (11.7%), *Klebsiella* spp. (8.9%), *Staphylococcus aureus* (7.2%), -hemolytic streptococci (4.4%), and others (6.2%). The most commonly detected isolate from hospital environment was *P. aeruginosa* (35%) followed by *Enterobacter* spp., *Klebsiella* spp., coagulase-negative staphylococci. *P. aeruginosa* was the most resistant to third- and fourth-generation cephalosporins (100%), whereas other gram-negative bacteria were resistant to ciprofloxacin and cephalosporin (70 to 100%). Restriction in the abuse of antibiotics and establishment of an infection control unit will help lower the incidence of infection.

Key words: Bacteriology, burn wounds, antibiogram, bacterial infections.

INTRODUCTION

Open and large wounds, including those containing necrotic tissues, make burn patients more susceptible to infection. In particular, immunosuppression caused by impaired neutrophil function and the cellular and humoral immune system can facilitate multiplication and colonization of burn wounds by different microorganisms (Oncul et al., 2002). The immunocompromising effects of burns, especially in patients who stay in the hospital for a long time and may be subjected to endotracheal intubation, blood vessel and bladder catheterization, or air and other environmental contaminants, put such patients at a high risk of infection (Andrade et al., 2009; Macedo and Santos, 2006; Oncul et al., 2002; Savas et al., 2004).

Bacterial infections in burn patients are widely reported,

and the change of normal flora from gram-positive to gram-negative types of bacteria is usually noticed within 4 to 10 days after hospitalization (Macedo and Santos, 2006; Oncul et al., 2002). Numerous reports have demonstrated that hospital environment surfaces are a source of antibiotic-resistant bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter* spp., *Klebsiella pneumoniae*, *Clostridium difficile*, and methicillin-resistant *Staphylococcus aureus* (MRSA). Thus, contamination of wound surfaces by these agents occur frequently (Hota et al., 2009; Macedo and Santos, 2006). This is particularly true for *P. aeruginosa*, which may grow on the moist surface of burn wounds and is highly pathogenic in thermally injured, immunosuppressed patients (Gang et al., 1999).

In addition, health-care workers whose hands become contaminated after touching these surfaces can transfer bacteria to a patient or other sites in the environment (Hota et al., 2009). Various antimicrobial agents used for infection control may cause bacteria such as *P. aeruginosa*, other gram-negative bacteria, and normal flora to develop resistance to antibiotics (Ozkurt et al.,

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Abbreviations: MRSA, Methicillin-resistant *Staphylococcus aureus*; ESBL, extended-spectrum -lactamase.

Table 1. Organisms isolated from sample cultures.

Organisms	Number	Percentage
<i>P. aeruginosa</i>	36	32.2
<i>Enterobacter</i> spp.	19	16.9
Coagulase-negative staphylococci	14	12.5
<i>Acinetobacter</i> spp.	13	11.7
<i>Klebsiella</i> spp.	10	8.9
<i>S. aureus</i>	8	7.2
-Hemolytic streptococci	5	4.4
Others	7	6.2
Total	112	100

2005). Drug-resistant bacteria are easily transferred from one patient to another. Overcrowding in burn units is another important factor for cross-infection (Douglas et al., 2001; Komolafe et al., 2003). The gains of established infection control measures are now being felt in the developed countries with a purpose built burns unit. However, in developing countries, establishing such measures is hindered by poverty, ignorance, poor management and lack of personnel (Macedo and Santos, 2006). Therefore, the study of environmental transmission of resistant bacteria is one additional approach in an effort to find an effective set of infection control precaution to help combat these organisms and periodic monitoring of bacterial species and their antibiogram is necessary.

MATERIALS AND METHODS

This study was a quasi-experimental and carried out over a 1-year period at the Imam Mousa Kazem burn center in Esfahan, Iran. Sampling was conducted with 100 patients including 65 males and 35 females, but for personal reasons, 14 patients exited the study at different stages. Patients ranged in age from 0 to > 70 years of which most of them (30%) were between 21 to 30 years old. As such, swabs were obtained from only 86 individuals with burns on 10% total body surface area (TBSA) and localized deep burns at 2 and 3 degrees 5 days after admission. The swabs were dipped in trypticase soy broth (TSB) and transferred immediately to the laboratory.

Multiple swab samples from different hospital environments were inoculated on the aforementioned media as well. All samples were inoculated on blood agar, Mc Conkey agar, and eosin methylene blue agar within 2 h of collection for selective isolation of gram-positive and negative bacteria. After incubation at 35°C for 18 h, colonies were stained and identified by classical biochemical method (Forbes et al., 2007). We used specific tests such as growth at 42°C in brain-heart infusion agar, oxidative tests, and oxidative fermentation tests for carbohydrate utilization (Kaushik et al., 2001) for the isolation of *P. aeruginosa*. Antibiotic susceptibility was determined by disk diffusion and interpreted according to guidelines set by the National Committee for Clinical and Laboratory Standards (NCCLS, 2002). Silver sulphadiazine was used topically and the dressing was changed daily. Ceftazidime, kanamycin, and imipenem were administered as prophylactic antibiotics from the first day of admission. Statistical method used

was chi-square test.

RESULTS

We isolated 112 bacterial strains from 86 patients; single and multiple isolates were detected at the following prevalence rates: 73.3% (N = 63) and 26.8% (N = 23). The most common organism isolated was *P. aeruginosa* (32.2%), followed by *Enterobacter* spp. (16.9%), coagulase-negative staphylococci (12.5%), *Acinetobacter* spp. (11.7%), *Klebsiella* spp. (8.9%), *S. aureus* (7.2%), -hemolytic streptococci (4.4%), and others, including *Escherichia coli*, *Salmonella* spp., enterococci, and -hemolytic streptococci (6.2%).

We used SPSS software for statistical analysis, and a significant difference was observed between the frequency of isolates ($P = 0.001$) as shown in Table 1. The most commonly detected isolate from hospital environments was *P. aeruginosa* (35%), followed by *Enterobacter* spp. (30%), *Klebsiella* spp. (20%), and coagulase-negative staphylococci (15%) ($P = 0.572$) as shown in Table 2. Table 3 displays the resistance rate of the organisms against different antibiotics.

DISCUSSION

Despite advances in topical and parenteral antimicrobial therapy, bacterial infection remains a critically important issue in burn patients. A defective immune system, necrotic and moist wounds, transduction of infectious agents from the gastrointestinal system and severe colonization of bacteria, prolonged hospitalization, and invasive diagnostic and therapeutic procedures all contribute to infections (Macedo and Santos, 2006). We studied the bacteriology of wounds, and evaluated bacterial resistance toward antibiotics and hospital environment contamination. Our results show that 26.8 and 73.3% of cases involved multiple and single isolates, respectively, which is in agreement with other studies (Kaushik et al., 2001; Komolafe et al., 2003; Ozumba and

Table 2. Organisms isolated from hospital environment.

Organism	Number	Percentage
<i>P. aeruginosa</i>	7	35
<i>Enterobacter</i> spp.	6	30
<i>Klebsiella</i> spp.	4	20
Coagulase-negative staphylococci	3	15
Total	20	100

Table 3. Pattern of antibiotic resistance of isolates.

Antibiotic	<i>P. aeruginosa</i> (N = 36)	<i>Enterobacter</i> spp. (N = 19)	<i>Acinetobacter</i> spp. (N = 13)	<i>Klebsiella</i> spp. (N = 10)	<i>S. aureus</i> (N=8)
Ticarcillin (%)	36 (100)	19 (100)	13 (100)	8 (80)	8 (100)
Ceftazidime	36 (100)	14 (73.72)	9 (69.2)	10 (100)	8 (100)
Ceftizoxime	36 (100)	14 (73.72)	13 (100)	7 (70)	NT
Ciprofloxacin	27 (75)	15 (78.96)	9 (69.20)	7 (70)	8 (100)
Ceftriaxon	36 (100)	13 (68.48)	13 (100)	10 (100)	NT
Co-amoxiclav	36 (100)	14 (73.72)	13 (100)	10 (100)	5 (62.5)
Amikacin	30 (83.3)	13 (68.48)	NT	10 (100)	NT
Imipenem	23 (61.1)	NT	NT	NT	NT
Vancomycin	NT	NT	NT	NT	5 (62.5)
Methicillin	NT	NT	NT	NT	8 (100)

N= Number, NT= Not tested, percentages are given in parentheses.

Jiburum, 2000).

The most frequently isolated organism from burn wounds was *P. aeruginosa*, followed by *Enterobacter* spp., *Acinetobacter* spp., and *Klebsiella* spp., which is in agreement with the findings of other studies (Karimi et al., 2002; Kaushik et al., 2001; Rastegar and Alaghebandan, 2000; Warren and Fraser, 2001). Gram-positive bacteria such as coagulase-negative staphylococci and -hemolytic streptococci were isolated with lower frequency, whereas in other studies, they were recognized as predominant agents of infection (Izquierdo-Cubas et al., 2008; Komolafe et al., 2003; Rastegar and Alaghebandan, 2000; Revathi and Jain, 1998).

Multidrug-resistant (MDR) *P. aeruginosa* is one of the most common nosocomial pathogens and is often a major problem in burn centers, and its development of resistance to new antibiotics is much faster than the rate of invention and development of new antibiotics (Karimi et al., 2002; Ozkurt et al., 2005).

In our study, *P. aeruginosa* was 100% resistant to third-generation cephalosporins (ceftazidime), but resistance rates of 48.9% (Savas et al., 2004) and 42 to 45% (Ullah et al., 2005) have been reported. The resistance rate of *P. aeruginosa* to imipenem, ciprofloxacin, and ticarcillin was 61.1, 75 and 100%, respectively. Khosravi et al. (2008) reported that the resistance of *P. aeruginosa* strains to imipenem was 41%, of which 38% were

multidrug-resistant strains. Among these strains, 19.5% appeared to produce metallo-lactamase. *P. aeruginosa* strains that contain -lactamase are more resistant to penicillin, aminoglycoside, cephalosporins, and carbapenems (Ullah et al., 2009). *P. aeruginosa* frequently demonstrates resistance to multiple antimicrobial agents, and serious infection due to drug-resistant *P. aeruginosa*, is a major problem (Savas et al., 2004). Among the gram-negative bacteria, the sensitivity rate of *Klebsiella* spp. was 0 to 10% toward third-generation cephalosporins, amikacin and co-amoxiclav, whereas sensitivity rates of 16.7 to 39.1% (Revathi et al., 1998) and 31.3 to 87.5% (Ozumba et al., 2000) have also been reported. *Acinetobacter* spp. was not sensitive to ciprofloxacin, third- and fourth-generation cephalosporins and amikacin, but the findings of Revathi et al. (1998) showed that the sensitivity rate was between 18.1 and 36.6%. Upon comparison with our results, it is concluded that drug resistance in bacteria appears to have increased.

In our burn center, MDR gram-negative bacteria and methicillin- and vancomycin-resistant *S. aureus* (MRS, VRS) were isolated from wounds in which they had caused acute problems which was similar to other studies (Oncul et al., 2002). A primary cause of drug resistance in gram-negative bacteria is their ability to generate extended-spectrum -lactamase (ESBL) enzymes, that can inactivate penicillin and cephalosporins, which are

necessary in treatment of infections. Antibiotic resistance can emerge after prolonged antibiotic consumption, and the organisms that are resistant to one drug are likely to become resistant to others. Cross-resistance and genetic loci are important factors in this problem (Neely and Holder, 1999). The length of hospitalization is another factor for drug resistance in bacteria (Oncul, 2002; Ozkurt et al., 2005; Savas et al., 2004).

Nowadays, *P. aeruginosa* and other gram-negative bacteria (Enterobacter, Klebsiella) are responsible for nosocomial infections in burns. These agents are found frequently in hospital environments, and burn wounds are an ideal medium for their survival. Such bacteria are inherently resistant to common antibiotics, even surviving common antiseptics; therefore, eradication of organisms from patients and the environment is difficult (Oncul et al., 2002). Colonized patients and staff are a major source of cross-contamination of other patients (Ozkurt et al., 2005). Therefore, recognition of infectious persons is necessary to prevent transmission of infection. The results of our study indicated that several factors, such as failure to prevent cross-transmission in hospital and the use of broad-spectrum antibiotics, were responsible for increased drug resistance, bacterial colonization, and some infections in burn patients. In addition, other contributing factors were long periods of hospitalization, procedures for hospital infection control, and the number of carriers. Therefore, we must implement a comprehensive education campaign for all health care workers and establish more effective infection control practices and policies in the burn units. Results reported in recent studies have determined that being exposed to heavy antibiotic use, high-density patient population that is in frequent contact with health-care staff, and the attendant risk of cross-infection are important factors in the issue of antibiotic resistance.

In conclusion, we give the following recommendations for the prevention and control of bacterial infections and drug resistance:

1. Disinfection of environmental surfaces in hospitals;
2. Reduction of broad-spectrum antibiotic usage;
3. Improvement of barrier nursing, personnel's personal hygiene, and restriction of staff traffic;
4. Regular microbiological analysis of the hospital environment, staff, and burn patients.

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