Evaluation of Bacterial Infection of Burn Wounds in a Burn Center, Qom, Iran

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Background & Aims of the Study: In spite remarkable progress in the anti-microbial treatment in the last 60 years and despite all the care in the health system, infectious diseases especially burn wound infection is a major problem and one of the most important causes of morbidity and mortality for burn patients. Certainly, the identification, review and updating of bacterial infections and their antibiotic resistances have an important role in the control, prevention, and correct treatment of burn patients. This study aimed to determine burn wounds and antibiotic resistance in patients hospitalized at the Nekoei Burn Center, Qom, Iran.

Material & Methods: A cross-sectional study was performed on patients referred to a Burn Center in Qom, Iran, for a three-year period (from May 2012 to November 2014). After sampling, the identification of the isolates was done by conventional biochemical tests. Disk diffusion method was performed for testing antibiotic resistance according to CLSI guideline.

Results: Among a total of 793 patients, 45 patients (19.82%) were positive culture. Pseudomonas aeruginosa was the most dominant microorganism in patients (66%), followed by coagulase-negative staphylococci (16%) and Staphylococcus aureus (12%). In the evaluation of antibiotic resistance, Pseudomonas aeruginosa showed the most resistant to ciprofloxacin (93.3%) and ceftriaxone (86.6%). Among the used antibiotics, meropenem and cephalaxin had a better effect than other antibiotics.

Conclusion: Based on the obtained results, Pseudomonas aeruginosa was the most isolated common microorganism of burn wounds at our hospital and it seems that treatment of common antibiotics in this center has not enough effectiveness. Thus, it is essential to use effective antibiotics correctly and to prevent the extension of resistant bacteria.

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Background

Skin is an essential component of the nonspecific immune system that protects the host against pathogens (1-3). Intact skin is a physical barrier and protects human from pathogenic microorganisms (4). Contact with fire, hot water, steam, electricity, flame, radiation, or caustic chemicals can cause skin damages and interrupt the immune status, resulting loss of a competent antimicrobial barrier (5,6).

Burns has always been a major problem in many parts of the world and it is estimated that 75% of the total deaths from burns caused by wound infection. Physical damages can destroy the body's defenses that normally prevents invasion of microorganisms (6-10).

Due to disorder of the skin's mechanical integrity and suppresses the immune system, burn patients are more prone to different...
infections. In these patients, microorganisms especially bacteria and fungi rapidly colonize skin wound surface after damaging. Then, systemic spread may occur which can lead to inflammatory responses and life-threatening complications (11).

Despite the considerable progress of antimicrobial therapy in the last 60 years, the bacterial infections in the burn patients are increasing (12). Although the risk of infection in burns is well known, but in recent decades antibiotic-resistant isolated strains of burn patients have been reported. Thus, the distribution of drug-resistant bacteria has become a critical problem in different parts of the hospitals (13).

The prevention of infection in burn patients is an important challenge because continuous management of the burn wound must be performed (14). In uncontrolled or untreated cases, invasive infections may result in the death of the burned patient (Church, Elsayed et al. 2006).

In a burn wound, the combinations of patient factors (such as age, burn depth, and immune system) and virulence factors related to microorganisms (such as enzymes, toxins, etc.) had an important role in the infection of burn site. Different microorganisms can cause burn infection, but *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Enterococcus* spp., *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumonia*, and *Enterobacter* spp. are the most common Gram-positive and Gram-negative pathogens identified in burn patients (11,15).

**Aims of the study:**
The aim of this study was to identify the most isolated bacterial agents from burn patients referred to a burn center of Qom city and the evaluation of the antibiotic resistance of the isolates.

**Sample collection and isolation of the bacteria**
This study was a cross-sectional study that conducted among 793 patients admitted to Nekoei Burn Center of Qom, Iran, during a three-year period (from May 2012 to November 2014). After obtaining informed consent, sampling was done by wet sterile cotton swabs. The swabs were putted in 5cc of Brain Heart Infusion broth (Merck, Germany) and were immediately sent to the microbiology laboratory. The BHI tubes were incubated at 37°C for 24 hours and next sub-cultured on blood agar, MacConkey agar, mannitol salt agar, and nutrient agar media (Merck, Germany) at 37°C for 18-24 hours.

**Identification of isolated bacteria**
Preliminary identification of the bacterial isolates was done using conventional methods e.g. Gram staining, colony morphology, pigment production, and haemolysis pattern on blood agar, DNase, coagulase, oxidase and catalase tests. Conventional biochemical tests for Gram-negative bacteria e.g. TSI (Triple Sugar Iron), SIM (Sulfide, Indole, Motility), citrate, MR-VP and oxidation-fermentation test of the isolates were also performed to confirm the genus and species of isolates.

**Antimicrobial resistance assay**
Antibiotic resistance of bacteria was evaluated according to CLSI guideline (16). The antibiogram test of the isolates was carried out using antibiotic disks (PadtanTeb, Iran), including amikacin (30µg), ceftizoxime (30µg), ticarcillin (75µg), ceftriaxone (30µg), cefotaxime (30 mg), ciprofloxacin (5µg), cephalothin (30µg), trimethoprim-sulfamethoxazole or SXT (25µg), imipenem (10µg), gentamicin (10µg), ampicillin (10µg), piperacillin (10µg), nalidixic acid (30µg), carbenicillin (100 µg), meropenem (10µg), cephalaxin (30µg), oxacillin (1µg), penicillin (10U), vancomycin (30µg), amoxicillin/clavulanic acid (30µg), and
tetracycline (30µg) on muller-hinton agar (Merck, Germany) at 37°C for 24h.

Results

In this study, 793 patients were included which 84% and 16% of them were male and female, respectively. The highest burns equal to 60% was related to the male with an age range of 19-45 years. The distribution of various age groups is shown in Table 1.

Table 1) The percent of various age groups under study

| Age group         | Percentage |
|-------------------|------------|
| Up to 12          | 16         |
| 13 to 18          | 16         |
| 19 to 45          | 58         |
| More than 45      | 10         |

The lowest and highest degrees totally, during 3 years 45 patients (19.82%) were positive culture. Of the total 301 patients admitted in 2012, 16 cases (5.32%) of them had burn wound infection. In 2013, from 331 patients, 11 cases (3.32%) and in 2014 of 161 cases, 18 patients (11.18%) were identified as positive culture (Fig. 1).

The most common bacterial isolates isolated were *Pseudomonas aeruginosa* (66%), coagulase-negative *staphylococci* (16%), *Staphylococcus aureus* (12%) and *Klebsiella* spp. (6%) (Fig. 2).

The results of antibiotic resistance showed that the most resistance was observed among isolates of *Pseudomonas aeruginosa*. High level of resistance was detected against ciprofloxacin at a rate of 93.3% and ceftriaxone amounted to 86.6%. The lowest resistance was also identified against meropenem (6.6%) and cephalexin (6.6%). Full results of the antibiotic resistance pattern of *Pseudomonas aeruginosa* can be seen in Table 2. Other isolated species were sensitive to evaluated antibiotics.
Infectious diseases are major causes of human mortality. Today drug resistance of the microorganisms has been reported as a serious threat in health systems around the world (17). The incorrect use of first-line antibacterial treatment has led to detection of the resistant bacteria. With failure of therapy, other options may be used which are more toxic, less effective and often expensive for patients. The studies have shown that the hospitalization and the risk of death in patients with resistant infections are progressively greater (18,19).

This project was focused to determine the prevalence of the bacterial pathogens causing infection in burn wounds and to detect antibacterial resistance in the isolated species. *Pseudomonas aeruginosa* was dominant species in 793 patients studied, followed by coagulase-negative staphylococci, *Staphylococcus aureus*, and Klebsiella spp. In performed study by Rezaei et al. shown that *P. aeruginosa* and Klebsiella were the most common Gram-negative bacteria and *S. aureus* was the most common Gram-positive organism recovered from the burn patients (20). In another work in Ghana, the predominant Gram-negative and Gram-positive organisms isolated were *Pseudomonas* spp. (30.2%) and *Staphylococcus aureus* (2.3%), respectively (21). As shown by studies, *P. aeruginosa* is well-known as an opportunistic pathogen and with minimal requirements for survival can adapt to a wide range of environmental niches.

The organism infected high-risk groups include neonates, patients with cystic fibrosis, burned patients, etc. (22).

In current study, antibacterial susceptibility patterns showed that *Pseudomonas aeruginosa* were more resistant to antibiotics than other bacteria. Antibiotic-resistant bacteria are difficult or impossible to treat and increasingly have become a global crisis (23). Mechanisms of antibiotic resistance in bacteria are varied and include Efflux pumps and outer membrane (OM) permeability, antibiotic inactivation, target modification, peptidoglycan and protein structure alteration. The complexity of these mechanisms may be used by *P. aeruginosa* against antibiotics (18,24). So, reports have shown that *P. aeruginosa* is difficult to control by some antibiotics (25-27). The most resistance among isolated *P. aeruginosa* was related to ciprofloxacin and ceftriaxone, 93.3% and 86.6%, respectively. Ranjbar et al. in Tehran reported that the *Pseudomonas aeruginosa* strains isolated from burned patients were resistant to ciprofloxacin (65%), ceftriaxone (60%) (28). Among the hospitalized burn patients, Sorkh also detected the *Pseudomonas aeruginosa* with resistance to ceftriaxone (94.66) and ciprofloxacin (84%) (29).

The lowest resistance was also identified against meropenem and cephalaxin. In agreement with our study, Bayram et al. showed that meropenem, amikacin, ciprofloxacin, and cefepime were the most effective drugs against *Pseudomonas aeruginosa* (13).

### Discussion

Infectious diseases are major causes of human mortality. Today drug resistance of the microorganisms has been reported as a serious threat in health systems around the world (17). The incorrect use of first-line antibacterial treatment has led to detection of the resistant bacteria. With failure of therapy, other options may be used which are more toxic, less effective and often expensive for patients. The studies have shown that the hospitalization and the risk of death in patients with resistant infections are progressively greater (18,19).

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In another study in India reported that *Pseudomonas aeruginosa* resistance to carbapenems (imipenem, meropenem) was 13-19% (30). High-level resistance to amikacin and gentamycin was detected. The performed work by Othman in Sulaymaniyah, Iraq, determined that Pseudomonas were resistant to gentamicin with 85.3% resistance and to amikacin with 57.3% resistance (11).

**Conclusion**

*P. aeruginosa* is an alarming increase in resistance to antibiotics in our hospital especially in the Burn Center. A guideline on stewardship of the antibacterial agents should be developed for reducing and preventing of the spread of resistance.

**Footnotes**

**Conflict of Interest:**
The authors declared no conflict of interest.

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