Original Article

Drug resistance of isolated strains of Pseudomonas Aeruginosa from burn wound infections to selected antibiotics and disinfectants

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ABSTRACT

Background and Objectives: **Infection is the most common problem following burn injury. Selection and dissemination of intrinsic and acquired resistance mechanisms increase the probability of burn wound colonization by resistant species including Pseudomonas aeruginosa. Multi-drug resistant Pseudomonas aeruginosa has frequently been reported as the cause of nosocomial outbreaks of infection in burn wards or as colonizers of the wound of burned patients. Therefore, this research study was conducted to compare the activity of various antibiotics and disinfectants against clinically important strains of P. aeruginosa.**

Materials and Methods: **One hundred strains of P. aeruginosa were obtained as clinical isolates from burn wound infections. The antimicrobial activity of antibiotics was tested by disk diffusion method of Kirby-Baur. For disinfectants, 30 µl of each of them was placed on sterile blank disk and studied by disk diffusion method.**

Results: **The frequency of resistant strains to kanamycin, tobramycin, amikacin, cefotaxime, carbenicillin, ceftazidime, ceftizoxime, cefixim, ciprofloxacin, cefazolin, cephalexine, and ceftriaxone was 100, 93, 95, 81, 84, 95, 94, 100, 99, 100, 100, and 92 respectively. The averaged diameter of inhibition zone for chlorhexidine (0.2%), povidione iodine (10%), cetrimide-C (3.5%), dekosept, hypochlorite (10%), micro 10^+ (2%), deconex 53^+ (2%), and ethanol (70%) was 14.4 ± 1.9 mm, 10.6 ± 1.3 mm, 9.1 ± 2.6 mm, 8.6 ± 2.2 mm, 26.9 ± 5.2 mm, 6.58 ± 1.5 mm, 8.3 ± 2.2 mm, and 6 ± 0.0 mm respectively.**

Conclusion: **The high frequency of resistance to antibiotics and sensitivity to a few disinfectants suggests to restrict the spread of P. aeruginosa and to limit administration of these antibiotics and to use of hypochlorite and chlorhexidin as disinfectant as a preventive treatment.**

Key words: **Pseudomonas aeruginosa, Burn infection, Antimicrobial resistance**

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Introduction

Burns remain a huge public health issue, at least in terms of morbidity and long term disability throughout the world, especially in the developing countries. Burn injuries still produce a significant morbidity and mortality in Iran (1). Burn-wound infection is one of the most common causes of death and serious problem after burn injury (2, 3). Burn predisposes the body to infection by damaging the protective barrier function of the skin, thus facilitating the entry of pathogenic microorganisms and by inducing systemic immunosuppression. In this respect, Pseudomonas aeruginosa (P. aeruginosa) is an opportunistic pathogen that produces a number of unique virulance factors. Extracellular toxin, proteases, hemolysins, and exopolysacharides are a few types of virulance factors that have been implicated in the pathogenicity of P. aeruginosa (4 – 6). In addition, P. aeruginosa is a major cause of burn injury colonization and serious wound infection. Furthermore, P. aeruginosa remains the leading pathogen causing wound infection in the Tohid burn center (Tehran, Iran), and the frequency of its infection is higher than reported from other countries (7, 8). Multi-drug resistance of P. aeruginosa is another complication in affected patients (9).

Therefore, the purpose of this study was to determine the susceptibility of clinically isolated P. aeruginosa from burn infection to selected antibiotics and disinfectants.

Materials and Methods

One hundred strains of P. aeruginosa were obtained as clinical isolates from burn wound infections during a period of 10 months from Tohid burn center (Tehran, Iran). P. aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923 were included as control strains to verify the accuracy of the antibiotic susceptibility test procedure. The antimicrobial activity of kanamycin (30 µg), tobramycin (10 µg), amikacin (30 µg), gentamicin (10 µg), cephotaxim (30 µg), carbenicillin (100 µg), ceftazidime (30 µg), ceftizoxime (30 µg), cefixime (5 µg), ciprofloxacin (5 µg), cefazoline (30 µg), cephalexine (30 µg), and ceftriaxon (30 µg) (Padtan Teb Co, Iran) against P. aeruginosa were tested by disk diffusion method of Kirby-Baur. Bacterial suspensions with 106 CFU/ml were spread on the surface of Muller-Hinton agar plates. Within 15 minutes after the surface of the agar has been inoculated, antimicrobial disks were applied with sterile forceps. The plates were inverted and incubated at 35 ˚C for 18 h and then diameter of each zone of inhibition was measured (10).

The antimicrobial activity of chlorhexidine (0.2%), povidione iodine (10%), cetrimide-C (3.5%), dekosept, hypochlorite (10%), micro 10^+ (2%), deconex 53^+ (2%), and ethanol (70%) were tested by disk diffusion method. For disk diffusion method, 30 µl of each of above disinfectants was placed on sterile blank 6 mm-diameter disk. Then, the disks were manipulated with sterile forceps. Thereafter, plates were incubated at 35 ˚C for 18 h and then diameter of each zone of inhibition was measured (11).

Results

From 100 isolated strains of P. aeruginosa, the frequency of resistant strains to kanamycin, tobramycin, amikacin, cefotaxime, carbenicillin, ceftazidime, ceftizoxime, cefixim, ciprofloxacin, cefazolin, cephalexine, and ceftriaxon were between 84 and 100 (Table 1).

The results of antimicrobial effects of disinfectants had shown that mean of diameter of inhibition zone for chlorhexidine (0.2%), povidione iodine (10%), cetrimide-C (3.5%), dekosept, hypochlorite (10%), micro 10^+ (2%), deconex 53^+ (2%), and ethanol (70%) were 14.4 ± 1.9 mm, 10.6 ± 1.3 mm, 9.1 ± 2.6 mm, 8.6 ± 2.2 mm, 26.9 ± 5.2 mm, 6.6 ± 1.5 mm, 8.3 ± 2.2 mm, and 6 ± 0.0 mm respectively by disk diffusion method. Inhibition zone for ethanol was not seen and all strains were completely resistant to this disinfectant (Table 2).

Table 1. The results of antibiogram for isolated strains of P. aeruginosa

	Number of strains of:		
Antibiotics	**R**	**I**	**S**
Kananycin	100	0	0
Toramycin	93	5	2
Amikacin	95	2	3
Gentamicin	96	2	2
Cefotaxime	81	17	2
Carbenicillin	84	9	7
Ceftazidime	95	2	3
Ceftizoxime	94	2	4
Cefixime	100	0	0
Ciprofloxacin	99	5	6
Cefazoline	100	0	0
Cephalexine	100	0	0
Ceftriaxon	92	6	2

R=Resistant I= Intermediate S= Sensitive

Table 2. Means of inhibition zone of disinfectants in disk diffusion method

Disinfectants	Means of inhibition zone (mm)
Chlorhexidine	14.4 ± 1.9
Povidone iodine	10.6 ± 1.3
Cetrimide- C	9.1 ± 2.6
Dekosept	8.6 ± 2.2
Hypochlorite	26.9 ± 5.2
Micro 10$^+$	6.6 ± 1.5
Deconex 53$^+$	8.3 ± 2.2
Ethanol	6 ± 0.0*

Discussion

It has been reported that P. aeruginosa remains the leading pathogen causing wound infection in Tohid burn center (Tehran, Iran) (8). The incidence of P. aeruginosa infection in Tohid burn center has been higher than reported from other countries (7). In addition, an increasing trend in its resistance is a major problem in Pseudomonas infections. In this study, 100% of isolated strains were resistant to the kanamycin, cefixime, cefazoline, and cephalexine. In a similar study by Rastegar Lari et al in 1995, it had been shown that the rates of resistance for amikacin, ciprofloxacin, and gentamicin were 48.9%, 45.2%, and 88.5% respectively (7). In our study (in 2002), we found that resistance to the mentioned antibiotics increased to 95%, 99%, and 96% respectively.

We also used the zone of inhibition method to test the efficacy of various disinfectants against isolated strains. It was found out that the most effective disinfectants were hypochlorite, povidone iodine, chlorhexidine, cetrimide- C and deconex 53$^+$. These results have also been confirmed by other studies. In this regard, Sterla et al and Leonardo et al had shown that chlorhexidin is effective against P. aeruginosa (12, 13). Meanwhile, Chogawala et al (1990) had shown that povidone iodine (1%) is effective on P. aeruginosa (14). A few studies have also proved the efficacy of commercial sodium hypochlorite against P. aeruginosa (13, 15-16). The important point to consider is that ethanol 70% has not been effective in this respect, although it has been commonly used as disinfectant. The high incidence and wide spread of P. aeruginosa and its high resistance to antibiotics as mentioned above indicates the necessity for urgent measures to be taken to restrict the spread of this pathogen and to limit administration of these antimicrobial agents. Also, we suggest using disinfectants hypochlorite and chlorhexidin as a preventive treatment.

Conclusion

considering the high frequency of resistance of P. aeruginosa to the current antibiotics, it is possible that the infections of this pathogen may be common, and so, molecular epidemiology for determining its clone in Tohid burn center is clinically very essential.

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