

CLINICAL INVESTIGATION

The Prevalence and Resistance Patterns of *Pseudomonas aeruginosa* in Intensive Care Units in a University Hospital

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Abstract: The intensive care units (ICUs) are burdened with a high frequency of nosocomial infections often caused by multiresistant nosocomial pathogens. *Pseudomonas aeruginosa* has emerged as one of the most problematic Gram-negative pathogens. The objective of this study was to identify frequency of *Pseudomonas aeruginosa* from the various clinical samples in ICUs, and to investigate resistance patterns against various antibiotics widely used for treatment. This study was carried out between September 2000-September 2002. Antimicrobial susceptibility testing was performed by the disc diffusion method according to NCCLS (National Committee for Clinical and Laboratory Standards) guidelines. The following antibiotics were tested: imipenem, meropenem, aztreonam, ciprofloxacin, ceftazidime, cefepime, piperacillin, norfloxacin and the aminoglycosides (gentamicin, netilmicin, tobramycin, and amikacin). *Pseudomonas aeruginosa* were isolated from 16.4 % (152/928) of the patients in ICUs. The highest *Pseudomonas aeruginosa* isolation was obtained in the burns unit (26.9%, 78/290) followed by, cardiovascular surgical ICU (17.6%, 13/74) general surgical ICU (24/164, 14.6 %), internal ICU (17/180, 9.4%) and coronary ICU (20/220, 9.1%). There is a statistically significant difference between surgical ICU and medical internal ICU ($P < 0.05$). The most effective antibiotics were carbapenems (imipenem and meropenem) and the resistance rates were detected as 15% and 20.4%, respectively among 152 *Pseudomonas aeruginosa* strains.

In conclusion, the frequency of *Pseudomonas aeruginosa* was found to be high in patients treated at ICUs. The results demonstrate that the resistance rates are alarmingly high. To reduce the emergence and spread of antimicrobial-resistant pathogens in ICUs, monitoring and optimisation of antimicrobial use should be considered carefully. These findings suggest that the resistance rates of aminoglycosides, 3th generation antibiotics and quinolone are increasing progressively in Turkey.

Key Words: *Pseudomonas aeruginosa*, prevalence, ICUs, resistance, antibiotic

Introduction

Pseudomonas aeruginosa, the primary human pathogen in the genus *Pseudomonas*, is widely distributed in nature. It may colonize healthy humans without causing diseases, but is also a significant opportunistic pathogen, and a major cause of nosocomial (hospital-acquired) infections. *Pseudomonas aeruginosa* is regularly a cause of nosocomial pneumonia, nosocomial urinary tract infections, surgical site infections, infections of severe burns, and infections patients undergoing either chemotherapy for neoplastic diseases or antibiotic therapy (1,2).

Pseudomonas aeruginosa is one of the most common nosocomial pathogens in humans and is often a major problem. Though rare in the normal flora of humans, it is isolated frequently from hospitalized patients. *Pseudomonas aeruginosa* is one of the most important microorganisms which causes problems clinically as a result of its high resistance to antimicrobial agents¹. The widespread occurrence of antibiotic resistant strains of *Pseudomonas aeruginosa* in hospitals is a matter of growing concern. Unfortunately, there are no specific measures to prevent nosocomial pseudomonal infections. Despite the availability of a variety of effective

antimicrobial agents, treatment of pseudomonal pneumonia is often challenging (3,4).

Pseudomonas aeruginosa remains an important cause of hospital-acquired infections especially in intensive care units (ICUs). The diversity of clinics and the regional variations in antibiotic protocols result in the different resistance profiles (2,3,4).

Patients hospitalised in ICUs are at particular risk of acquiring nosocomial infections due to serious underlying disease, compromised membrane and skin barriers following the use of invasive devices, and extended length of hospital stay, among other factors. Exposure to various antimicrobial agents may further complicate such hospitalisation and create conditions conducive to resistance selection among host bacterial flora or nosocomially-transmitted pathogens. Studies have demonstrated that rates of antimicrobial resistance are greater in bacteria isolated from ICUs compared with other hospital wards and outpatient clinics (5).

Antimicrobial resistance is a growing problem worldwide, especially in hospitals, where resistant organisms are often first detected in ICUs. *Pseudomonas aeruginosa* frequently displays resistance to multiple antimicrobial agents (6). Serious infection due to strains of *Pseudomonas aeruginosa* that exhibit resistance to all common antipseudomonal antimicrobials is an increasingly serious problem (7).

In this study we aimed to establish the prevalence of *Pseudomonas aeruginosa* in the ICUs of our hospital and to compare their antibiotic susceptibility patterns.

Materials and Methods

This study was conducted in the ICUs and burns unit of a teaching hospital over a period of 24 months. The study was undertaken to find the prevalence of hospital-acquired *Pseudomonas aeruginosa* infection in Baskent University Hospital in Adana, Turkey between September 2000- September 2002. In this study, 152 *Pseudomonas aeruginosa* were isolated from 928 patients hospitalized in four different ICU (coronary ICU, surgical ICU, internal ICU, cardio vascular surgical ICU) and burns unit.

Pseudomonas aeruginosa were identified by colonial morphology, a positive oxidase reaction, pyocyanin production on Mueller-Hinton agar (Difco), motility and growth at 42 °C on cefrimide agar. Colonies which

displayed a positive oxidase reaction were subcultured, and characterised using a commercial biochemical identification kit (API NE). One sample was studied from each patient. The sensitivity of *Pseudomonas aeruginosa* strains to imipenem (10 mg/disc), meropenem (10 mg/disc), aztreonam (30 mg/disc), ciprofloxacin (5 mg/disc), ceftazidime (30 mg/disc), netilmicin (30 mg/disc), cefepime (30 mg/disc), gentamicin (10 mg/disc), tobramycin (10 mg/disc), amikacin (30 mg/disc), piperacillin (100 mg/disc) and norfloxacin (10 mg/disc) was investigated by the Kirby-Bauer disc diffusion method according to NCCLS (National Committee for Clinical and Laboratory Standards) criteria (8). Mueller-Hinton broth was used as the growth medium. The final bacterial inoculum concentration was approximately 1.5×10^8 colony-forming units/ml (cfu/ml). Before the antibiotic discs were placed, the Mueller-Hinton plates were inoculated with swabs submerged in the final inoculum concentration and streaked over the entire surface of the plates. Plates were incubated aerobically at 35-37 °C for 18-24 hours. All antibiotic discs were obtained from Oxoid.

As a control strain, ATCC 27853 was used for identification and susceptibility tests.

Statistical analysis

Statistical analysis was performed using a chi square test and P values less than 0.05 were considered statistically significant. The statistical analyses were performed using Statistical Package for Social Sciences (SPSS, ver 10.0) software.

Results

Over a period of twelve months (between September 2000-September 2002), a total of 152 *Pseudomonas aeruginosa* strains were isolated from hospitalized patients in different ICUs and burns unit. The highest isolation rates of *Pseudomonas aeruginosa* strains were observed among the following departments in descending order: burns unit (26.9%), cardiovascular surgical ICU (17.6%), surgical ICU (14.68%), medical internal ICU (9.4%), and coronary ICU (9.1%) (Figure 1). There was no difference in isolation rate between medical internal ICU and coronary ICU ($P > 0.05$), but there was a higher isolation rate in burns unit than the others ICUs ($P < 0.05$). The same important difference was detected between surgical ICUs (cardiovascular surgical ICU,

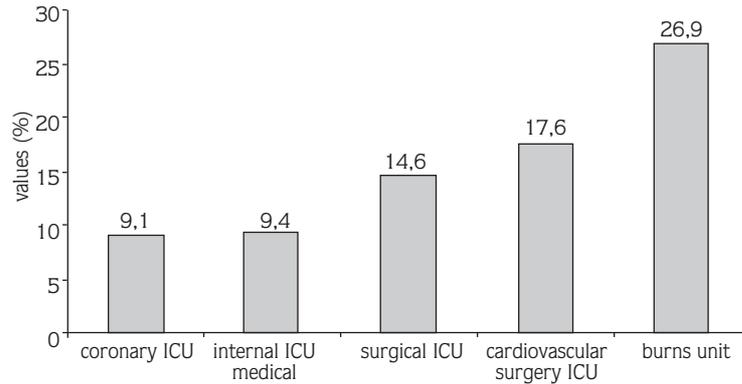


Figure 1. Isolation rates *Pseudomonas aeruginosa* according to departments.

surgical ICU) and medical internal ICUs (medical internal ICU, coronary ICU), ($P < 0.05$).

In this study, *Pseudomonas aeruginosa* was isolated from 152 out of 928 patients (16.4%) from whom various clinical samples (such as, urinary, surgical site infections, burns, tracheal aspiration samples) were taken.

The antibiotic resistance patterns of isolates are presented in Figure 2. The most effective antibiotics were carbapenems (imipenem and meropenem) and the resistance rates were detected as 15% and 20.4%, respectively among 152 *Pseudomonas aeruginosa* strains. Against norfloxacin, ciprofloxacin, and piperacillin however, resistance rates of *Pseudomonas aeruginosa* strains were 25.5%, 27.4% and 28.7, respectively. Apart from these antibiotics, the most effective antibiotics were netilmicin (30.1%), cefepime (39.0%), amikacin (42.2%), cefotaxime (48.9%), aztreonam (63.1%), tobramycin (65.5%) and gentamicin (70.7)%.

Discussion

Pseudomonas aeruginosa is a major cause of nosocomial infection. Despite advances in sanitation facilities and the introduction of a wide variety of antimicrobial agents with antipseudomonal activities, life-threatening infections caused by *Pseudomonas aeruginosa* continue to be hospital infections. ICU patients are particularly susceptible to nosocomial infection because the normal skin and mucosal barriers to infection are commonly compromised by the use of invasive devices (9). The distribution of isolates is significantly affected by

the type of hospital (general, teaching or specialized). It is reported that isolation due to nosocomial infection changes from 3% to 16% in multi-center studies. *Pseudomonas aeruginosa* is the most common pathogen in nosocomial infections. It is the leading cause of nosocomial respiratory tract infections (2,3,10).

Intensive care patients especially create an environment for infection because of the debilitating effect of a prolonged hospitalisation and the application of medical equipment (airways, catheters etc) (10).

In this study, the highest *Pseudomonas aeruginosa* isolation rate (26.9%) was obtained in the burns unit which was followed by cardiovascular surgical ICU (17.6%), surgical ICU (14.68%), medical internal ICU (9.4%), and coronary ICU (9.1%). There was a statistically significant difference between surgical ICUs and medical internal ICUs ($P < 0.05$, Figure 1). The burns units are a very susceptible habitat for bacterial colonization (11). ICUs are generally considered epicenters of antibiotic resistance and the principal sources of outbreaks of multi-resistant bacteria. The most important risk factors are obvious, such as excessive consumption of antibiotics exerting selective pressure on bacteria, the frequent use of invasive devices and relative density of a susceptible patient population with severe underlying diseases (12).

Nowadays, the prevalence of *Pseudomonas aeruginosa* and the new resistant strains continue in both community-acquired pathogens and hospital originated infections (13).

In a study carried out in Turkey, Inan et al. isolated 68% of *Pseudomonas aeruginosa* strains and 60-83% of

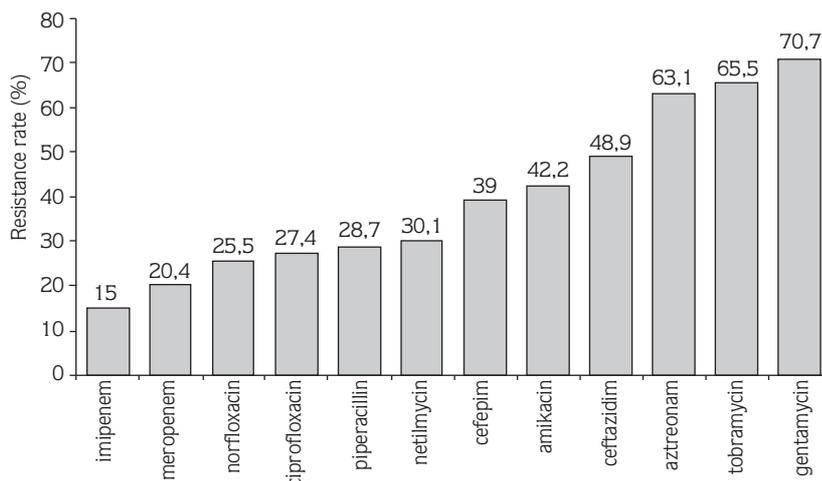


Figure 2. Resistance rates of *Pseudomonas aeruginosa*.

the antibiotics resistant strains from ICU patients. In the same study, resistance was detected against ceftazidime 34%, imipenem 26%, gentamicin 67%, and amikacin 26% (14).

Recently, increased resistance has been observed against 3th generation cephalosporins for gram negative bacilli, especially *Pseudomonas aeruginosa* (16). Cefepimee and Ceftazidime are the commonest 3th generation antibiotics in ICU protocols. Ceftazidime has a key role in resistance detection in ICUs. However in ICUs, antibiotic therapy protocols are different in almost all countries and there are also regional differences (15). Resistance to Cefepimee and Ceftazidime are significant in our study (30.1-48.9%). Previous studies suggest that the selective pressure from the use of antimicrobial agents is a major determinant for the emergence of resistant strains (9,12,15).

One of the significant resistant groups detected against aminoglycosides was *Pseudomonas aeruginosa* (17). Resistance of gram-negative aerobic bacteria to aminoglycoside antibiotics differs according to region and country. Resistance to aminoglycosides was higher in Southern Europe than in Central and Northern Europe (17). Reports of the susceptibility of *Pseudomonas aeruginosa* to gentamicin and tobramycin have ranged from as low as 49.8% and 77.7%, in Greece, to as high as 96.6% and 99.2%, respectively, in the United Kingdom (17). Previous studies reported that antipseudomonal effects of amikacin were greater than those of gentamicin (18,19). In the present study, the

rate of aminoglycoside resistance was found to be relatively high (resistance to amikacin; 42.2%, netilmicin; 30.1%, tobramycin; 65.5% and gentamicin; 70.7%). However, in 1988, it was reported that 54% of gram-negative bacilli in Turkey were resistant to gentamicin, 35% to tobramycin, and only 0.9% to amikacin (18). Consistent with these findings, resistance to amikacin of *Pseudomonas aeruginosa* was still lower than to tobramycin or gentamicin. However, this data suggests that resistance to amikacin is increasing progressively in our country.

In various studies, it was reported that increased resistance rates have been detected against to carbapenems, quinolons and third-generation-cephalosporins for *Pseudomonas aeruginosa* worldwide (20-22). In our study, resistance rates against imipenem and morenepem from carbanemem groups were determined as 15% and 20%, respectively.

Additionally, in our study presented here, the resistance rate was found as 28% against piperaciline from ureipenicillin group, and 63% for monobactam from aztreonam group.

In the literature, it was reported that resistance to imipenem was 14% in Spain (23), 19.3% in Italy (24), and 68% in Saudi Arabia (25). The National Nosocomial Infections Surveillance (NNIS) system reported the incidence of imipenem resistance as 18.5% among isolates of *Pseudomonas aeruginosa* from ICU patients (26).

The Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) study group reported an incidence of 19% in 10 medical centres (27). In a later study, it was reported that *Pseudomonas aeruginosa* strains have developed relatively high resistance levels against to quinolone group (28).

The resistance of *Pseudomonas* to the antibiotics in the quinolone group is variable in different centers (28,29). In a prospective study, resistance to ciprofloxacin in ICU was reported as 8-31% (30). In this study, resistance rates against ciprofloxacin and norfloxacin were found as 27.4%, 25.5%, respectively. Quinolone resistance in our study is the same as these reports.

Ciprofloxacin resistance rate was 23% in Spain (23), 31.9% in Italy (24), and 26.8% in Latin America (31). Contrary to ciprofloxacin, our isolates were highly resistant to ceftazidime (48.9%). According to different reports, resistance to ceftazidime was 15%-22% in the world (23,24,31). Resistance to piperacillin was higher, similar to ceftazidime. While piperacillin resistance rate was 10% in Spain (23), 12% in Italy (24), 14% in Latin America (31), it was found as 28.7 in our study. Resistance rates of anti-pseudomonal antibiotics were quite low in the United Kingdom: 5% for ceftazidime, 7% for piperacillin, 10% for ciprofloxacin, and 11% for imipenem (32).

As a result, the resistance to antipseudomonal antibiotics was found to be higher than in the previous studies reported in different countries in the isolated *Pseudomonas aeruginosa* strains from ICU patients in our hospital (5,10,12,24,25,28).

Thus, in ICUs, empirical antibiotic treatments should be avoided and treatment should be carried out using antibiotic susceptibility tests. ICUs should be regularly inspected for *Pseudomonas* colonization which shows a strong resistance pattern against the various antibiotics. Colonization of ICU patients with antimicrobial-resistant pathogens can lead to clinical infection because of breakdown of normal host defenses.

In conclusion, we recommend regular screening of ICU patients to give an early warning of the presence of antimicrobial-resistant pathogens and allow the assessment of barrier and infection control techniques. Such monitoring can also aid infection control in determining how to focus efforts on reducing the emergence and spread of antimicrobial resistant pathogens.

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