Clinical, microbiologic, and epidemiologic characteristics of *Pseudomonas aeruginosa* infections in a University Hospital, Malatya, Turkey

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Background: *Pseudomonas aeruginosa* strains are generally resistant to many antibiotics, and nosocomial infections because of this species are one of the major problems in many hospitals. Molecular typing provides very useful information about origin and transmission of the strains. The aims of the present study were to investigate clinical and microbiologic characteristics of the nosocomial infections caused by *P aeruginosa* strains in a medical center and to bring up the cross-transmission level of this opportunistic pathogen in a university hospital by analyzing the clonal relationship among the isolates.

Methods: A total of 105 *P aeruginosa* strains had been identified among the 80 inpatients in a 1-year period from August 2003 to August 2004. Demographic, clinical, and epidemiologic data of the patients were prospectively recorded. The standardized disk-diffusion method was used to determine resistance of the strains to imipenem, ceftazidime, aztreonam, amikacin, gentamicin, mezlocillin, cefepime, tobramycin, meropenem, ceftriaxone, and ciprofloxacin. Clonal relatedness of the strains was investigated by pulsed-field gel electrophoresis (PFGE).

Results: Of the 105 *P aeruginosa* strains identified, 45 (43%) were isolated from the patients hospitalized in intensive care units. Thirteen patients had repeated pseudomonas infection (total 38 infections/13 patients); 26 of these repeated infections in 9 patients showed the same localization. Half of the patients had at least 1 underlying disease such as burn (48%), chronic illness (32%), and malignancy (20%). Fifty-seven patients (71%) had urinary and/or other catheterization. Urinary tract infection (35%) was the most frequent infection encountered, followed by respiratory tract infection (34%) and sepsis (13%). Resistance to the antibiotics tested was in the 12% to 88% range; amikacin was the most effective and ceftriaxone was the least effective antibiotic. The PFGE typing method showed that 28 of the 80 patients' isolates were clonally related, including 23 indistinguishable or closely related strains (29%), and 5 possibly related strains (6%). Epidemiologic data of the 16 patients (20% of the patients) confirmed a clonal relationship among the strains. Of the 26 isolates of the 9 patients having repeated infection in the same location, 18 (69%) were in the clonally related groups, whereas 11 of the 12 strains isolated from repeated infections on different body sites were clonally different.

Conclusion: Our results indicated that *P aeruginosa* infections in our hospital mainly affected the patients hospitalized in intensive care units and those having catheterization, burn, and/or chronic illness. Amikacin was the best antibiotic as far as bacterial resistance was considered. Although lack of major PFGE type confirmed no *P aeruginosa* outbreak, typing results showed that cross transmission and treatment failure are the 2 main problems, which should be consider together to prevent this bacterial infection in medical centers. (Am J Infect Control 2006;34:188-92.)

Pseudomonas aeruginosa is a frequent nosocomial pathogen that is often responsible for the infections in patients with cystic fibrosis; bronchiectasias; neutropenia; AIDS; and/or burn and patients with metabolic, hematologic, or malignant diseases.[1,2] This bacterium is mainly responsible for ventilator-associated pneumonia, surgical site infection, urinary tract infection, and sepsis in intensive care unit patients.[3] Most of the strains are commonly resistant to many antibiotics including imipenem and meropenem; therefore, it is usually a problematic pathogen in therapy.[4]

P aeruginosa is generally acquired from the environment, and person-to-person spread occurs rarely.[5] Contaminated respiratory care equipment, irrigating solutions, catheters, infusions, cosmetics, dilute antiseptics, cleaning solutions, and even soaps have been reported as vehicles of transmission.[6-8]

Data about clonal relation among the strains isolated from severe infections can help to improve more effective control precautions to prevent spread of these resistant strains from patients to patients in a hospital. International epidemiologic surveillance requires reliable techniques that can differentiate unrelated strains from clonally related ones. Pulsed-field gel electrophoresis (PFGE) has been accepted as a ''gold standard''

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for confirming relatedness among *P aeruginosa* strains.[3,9,10]

Previous molecular epidemiologic studies confirmed many outbreaks because of *Klebsiella species*,[11] *Acinetobacter baumannii*,[12] *Chryseobacterium meningosepticum*,[13] and *Stenotrophomonas maltophilia*[14] in our medical center, which has an 850-bed capacity and central climate and aeration systems. Until now, in our hospital, there had been no reported outbreak of *P aeruginosa*, which is one of the most common bacteria present in hospital environment. Here, we aimed to analyze clinical and epidemiologic characteristics of the nosocomial *P aeruginosa* infections and to emphasize the spreading degree of this opportunistic pathogen in a university hospital by testing clonal relationship among strains isolated from inpatients in our hospital.

METHODS

Patients and bacterial strains

During a 1-year period from August 2003 to August 2004, a total of 105 strains were isolated from 105 infections of 80 patients hospitalized in a teaching hospital having an 850-bed capacity and 8 intensive care units. Definitions of nosocomial infection by *P aeruginosa* included signs and symptoms of the infection and isolation of this species from clinical samples as a unique pathogen.[3] Nosocomial infection for these patients were defined according to the Centers for Disease Control and Prevention (CDC) definitions.[15] Distribution of patients in the services was as follow: 45 patients from intensive care units (ICU), 14 from urology, 6 from nephrology, 6 from chest disease, 5 from surgery, 5 from gastroenterology, 5 from cardiovascular and thoracic surgery, 4 from neurology, 4 from hematology, 4 from pediatrics, and 7 from other clinics. All patients were daily examined at bedside, and data including demographic characteristics, prior hospitalizations, used antibiotics, clinical diagnosis, risk factors, location of the patients in the hospital, and invasive applications were recorded.

P aeruginosa was identified by its characteristic metabolic test results, and ability to grow at 42°C. The isolates were further identified by the Remel RapID NF Plus System (Remel Inc., Lenexa, KS), and those that could not be identified by this system were retested by API 20 NE test (bioMerioux, Lyon, France).

Antibiotic susceptibility testing

Antibiotic susceptibility tests were performed with the standardized disk-diffusion method on Mueller-Hinton agar, according to the Kirby-Bauer method and following the criteria of the Clinical Laboratory Standards Institute (CLSI).[16] *P aeruginosa* ATCC 27853 was used as an internal control. The antibiotic disks (Oxoid) used were imipenem (10 μg), meropenem (10 μg), ceftazidime (30 μg), ciprofloxacin (5 μg), aztreonam (30 μg), amikacin (30 μg), gentamicin (10 μg), mezlocillin (75 μg), cefepime (30 μg), tobramycin (10 μg), and ceftriaxone (30 μg). The diameters of inhibition zones were measured for each antibiotic, and the results were showed as resistant or sensitive according to the criteria of CLSI.

Molecular typing of the *P aeruginosa* strains

PFGE typing was performed on 105 strains isolated from 80 inpatients. Isolation and deproteinization of the genomic DNA were done following the protocol of Barth and Pitt, with minor modifications.[17] Briefly, *P aeruginosa* colonies on nutrient agar were suspended in 1 mL sodium chloride/ethiylenediamine tetraacetic acid (SE) buffer (75 mmol/L NaCl, 25 mmol/L EDTA [pH 8.6]), and the optical density was adjusted to 0.7 (λ = 590 nm). The cells were embedded into low (2% wt/vol) melting agarose. After digestion of the cells and washing of the plugs, genomic DNA in the agarose plugs was restricted by 20 U of *Xba*I (Fermantas Corporation) for 6 hours at 37°C in a water bath. DNA fragments were separated on 1.2% pulse-field certified agarose (Bio-Rad Laboratories, Nazareth, Belgium) gels run in 0.5X Tris-borate-EDTA buffer (44.5 mmol/L Tris, 44.5 mmol/L boric acid, 1 mmol/L EDTA [pH 8.6]) by using a CHEF-DR II system (Bio-Rad Laboratories). The electrophoresis conditions were 11°C at 6 V/cm^2 for 30 hours. The initial and final switch times were 5 seconds and 25 seconds, respectively. The gel was stained with ethidium bromide (5 μg/mL) and photographed under UV light. The DNA band profiles were analyzed with GelCompar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium). According to the interpretative criteria of Tenover et al, isolates were classified as indistinguishable (cluster), closely related, possibly related, or different.[18]

RESULTS

Patients

A total of 105 *P aeruginosa* infections were observed in 80 inpatients in the study period. Of the patients, 19 (24%) were female, and 61 (76%) were male. Fifty-seven patients (71%) arrived at our hospital from their home and 23 (29%) from different hospitals. Half of the patients had at least 1 underlying disease that would predispose the patient to *P aeruginosa* infections. Burns (48%), chronic illness (32%), and malignancy (20%) were the most common diseases recorded. Thirty-three patients (40%) underwent surgery, and 57 (71%) had urinary tract and/or intravascular catheterization. Other medical applications were oral feeding in 47

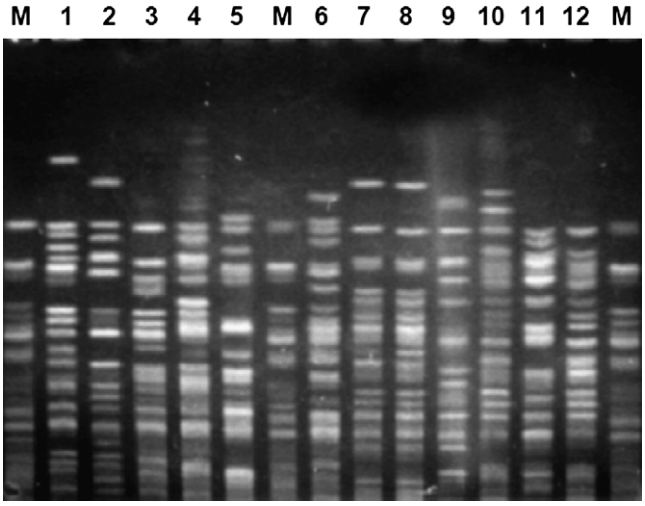

Fig 1. A representative PFGE typing results of 12 *P aeruginosa* strains. The typed strains had at least 10 bands, which are preferable for evaluation of the PFGE results. Lines 7 and 8 included 2 indistinguishable (cluster) strains; lines 1 and 2 and lines 11 and 12 showed the probable related strains; lines 3-6 and 9 were different strains. Lines M, the band profile of *P aeruginosa* ATCC 27853 strain restricted by 20 U of *Xba*I.

patients (59%), nasogastric tube in 18 patients (22%), and total paranteral nutrition in 15 patients (19%). Only 34 patients (42%) stayed in single-patient room; the other 46 patients shared a room with at least 1 other patient. Seventeen patients (21%) shared the same devices with the patients in different services.

Of the 105 *P aeruginosa* infections detected in 80 patients, 37 (35%) were urinary tract infection, 36 (34%) respiratory tract infection, 16 (15%) bacteremia or sepsis, 10 (9%) wound infections, 3 (3%) pancreatitis, 2 (2%) peritonitis, and 1 (1%) empyema. *P aeruginosa* infection repeated in 13 patients (total 38 infections in 13 patients). Of these infections, 26 in 9 patients were localized in the same anatomic sites; the remaining 12 infections in the other 6 patients localized to different body sites. Twelve of the 26 recurrent infections having the same localization were pneumonia (in 5 patients), and 14 were urinary tract infections (in 4 patients).

Strains

The 105 *P aeruginosa* strains were isolated from 105 clinical specimens, including 37 (35%) urine; 25 (24%) tracheal aspirates; 16 (15%) blood; 6 (6%) sputum; 8 (8%) wound swabs; 10 (9%) peritoneal, pleural, and abscess aspirates; and 3 (3%) bronco-alveolar lavage. The resistance rates of the *P aeruginosa* strains were 34% to ciprofloxacin, 29% to imipenem, 33% to meropenem, 27% to ceftazidim, 88% to ceftriaxon, 35% to cefepime, 57% to aztreonam, 11% to amikacin, 49% to gentamicin, 31% to tobramycin, and 48% to mezlocillin.

Typing results

All 105 isolates were genotyped by using PFGE. Figure 1 shows the representative PFGE typing results of 12 strains. When only 1 isolate from each patient was evaluated, we found that 23 of the 80 strains (29%) were indistinguishable or closely related, 5 (6%) were possibly related, and 52 strains (65%) were unique. A total of 28 strains was found to be clonally related, and 12 of these strains were indistinguishable divided in 6 groups; range of group was 2 to 3 strains. There was no epidemiologic relationship among the 12 strains showing clonal similarity. Epidemiologic data of the remaining 16 patients (20% of the patients) confirmed clonal relationship among the strains. Of the twenty-six isolates of the 9 patients having more than 1 infection in the same location, 18 (69%) were in the clonally related groups, whereas 11 of the 12 strains isolated from repeated infections of different body sites were clonally different.

DISCUSSION

As an opportunistic pathogen, *P aeruginosa* mainly causes infection in patients hospitalized for long periods, who underwent medical application (bronchoscopy or endoscopy and others), and having underlying diseases.[1,2,19] Patients treated in intensive care units are under increased risk because invasive devices are often used in these patients.[20,21] In agreement with these data, half of our patients were in intensive care units and had at least 1 underlying disease; 32 (40%) had surgery operation, and 57 (71%) had urinary tract and/or intravascular catheterization. Because main sources of this bacterium are contaminated equipment and as a result of intensive use of catheterization, urinary tract infections were frequently found in our patients.

When a specific strain was repeatedly isolated from the same patient over a relatively long period of time, the patient was considered to be persistently infected with that strain. The strains were repeatedly isolated from either the same or different sites infections of these patients.[22] In our hospital, 38 (36%) of the 105 *P aeruginosa* infections were repeated infections, and 26 of these affected the same body sites. *P aeruginosa* is the third most common pathogen when recurrent infections are complicated by obstruction, catheters, or stones.[6] In up to 60% of cases of recurrent urinary tract infection (UTI), the second infection is caused by a strain identical to that which caused the prior infection.[23] In agreement with these data, 12 (86%) of the 14 strains isolated from the repeated UTIs were

clonally related, and all 4 patients having repeated UTI had clonally indistinguishable first and second isolates.

Multidrug-resistant strains of *P aeruginosa* have become increasing problematic in certain hospitals. In our study, susceptibility rates were found as 66% to ciprofloxacin, 71% to imipenem, and 67% to meropenem. We found that amikacin was the most effective antibiotic, with a resistance rate of 11%, and ceftriaxone was the least effective agent (resistance rate, 88%). Rates of the resistance to cephalosporins and aminoglycosides were in the range of 27% to 88% and 11% to 34%, respectively. Approximately half of the strains were resistant to aztreonam and mezlocillin. Similar to our results, in a previous study, the rates of resistant *P aeruginosa* strains were 33.3% to ciprofloxacin, 26.3% to imipenem, 31.6% to ceftazidime, 42.1% to aztreonam, 11.4% to amikacin, 34.2% to gentamicin, 40.4% to piperacillin, 28.1% to tobramycin, 81.1% to ceftriaxone, 40.5% to norfloxacin, and 63.6% to carbenicillin.[24]

Investigations of the *P aeruginosa* clones and resistance patterns are particularly useful in patient management and maintenance of infection control procedures. PFGE typing results indicated that approximately one third (28/80 patients) of the patients having *P aeruginosa* infection in our hospital were clonally related. When the epidemiologic data of these patients were considered, no link was found among the 12 patients having clonally related strains. The remaining 16 patients having related strains by PFGE had been found epidemiologically related, supporting that 20% of patients in our hospital may have obtained *P aeruginosa* by cross contamination. Cross transmission from patient to patient may occur via the hands of the health care staff or through contaminated materials and reagents.[8] As indicated previously,[3] the existence of the same clone in different clinics may reflect horizontal transmission via the hands of personnel and/or medical devices. Unfortunately, there were no attempts to find the sources of the *P aeruginosa* infections in our study. Although some investigators found a single multidrug-resistant *P aeruginosa* clone from several patients and thought that a molecular investigation of these strains would be a guide for the infection control program,[25] we could not find a predominant PFGE typing pattern among *P aerations* isolates; the range of the indistinguishable groups varied between 2 and 3 strains.

In conclusion, *P aeruginosa* infections in our hospital mainly affected the patients hospitalized in intensive care units and those having catheterization, burn, and/or chronic illness. Resistance to the antibiotics tested was in the 12% to 88% range; amikacin was the most effective and ceftriaxone was the least effective antibiotic in in vitro conditions. Lack of a major PFGE type confirmed no *P aeruginosa* outbreak, and both the clonally and epidemiologic relationship found in one fifth of the patients supports cross transmission and problems in infection control in our hospital. High clonal relationship among the strains causing repeated infection indicated inefficiency of treatment regimes.

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