An outbreak of *Pseudomonas aeruginosa* because of inadequate disinfection procedures in a urology unit: A pulsed-field gel electrophoresis-based epidemiologic study

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Background: *Pseudomonas aeruginosa* is an opportunistic pathogen causing nosocomial infections in many hospitals. We aimed to investigate the source of urinary tract infections by determining clonal relationship of *Pseudomonas aeruginosa* strains with pulsed-field gel electrophoresis (PFGE).
Methods: During a 2-month period, all postoperative infections because of *P aeruginosa* were investigated in the Urology Department. Patient data were collected from medical records. Surveillance samples were obtained from various places in urological operating rooms. PFGE typing was performed for all *P aeruginosa* isolates.
Results: A total of 14 *P aeruginosa* strains (12 from patients and 2 from environmental samples) were isolated. PFGE typing of these 14 strains yielded 2 possibly related clones, which differed from each other by 4 major bands. Ten of the patient isolates were clonally identical with the strains of 2 forceps.
Conclusion: Typing results confirmed that inadequately disinfected surgical devices can be the source of outbreak. After institution of infection control measures and education, no further clusters of *P aeruginosa* infection were detected in the Urology Department. (Am J Infect Control 2008;36:33-8.)

Pseudomonas aeruginosa is an important gram-negative nosocomial pathogen that exists in humid environment. It can cause a broad spectrum of infections involving the respiratory, gastrointestinal, and urinary tracts as well as wound infections, sepsis, and others.[1] *P aeruginosa* contributes to high morbidity and mortality among the patients in intensive care units, oncology departments, burn units, and surgery wards.[2] Tap water,[1] medical equipment,[3-5] hospital water systems,[6] inadequate disinfection procedures,[3] hospital personnel,[7] and other patients[8,9] are possible sources of *P aeruginosa* infection in hospitals.

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P aeruginosa usually affects the urinary tract through ascending infection and adheres strongly to bladder uroepithelium.[10] Nosocomial urinary tract infection (UTI) usually results from surgical intervention or instrumentation of the urinary tract involving the prostate gland and urinary bladder. If a cluster of cases is detected, a common source probably associated with urologic instrumentation should be suspected.[2,3,11]

Molecular typing is an important tool to follow transmission routes of microbial pathogens that can be used in clinical settings to discriminate ongoing epidemics of an infectious agent from incidentally increased rates.[12] Even though there are some polymerase chain reaction (PCR)-based typing methods such as arbitrary-primed PCR and amplified fragment-length polymorphism, which are very quick, pulsed-field gel electrophoresis (PFGE) is accepted as the ''gold standard.''[12-14] PFGE has been widely used for clonal analysis of *P aeruginosa* isolates either in a specific setting or population-based research.[8,11-14]

Recently, we noticed that surgery-associated *P aeruginosa* infections increased in the urology ward. Therefore, this study was conducted to trace the source of infections, investigate clonal relationship of strains, and reveal effectiveness of PFGE on taking effective control measures to terminate the outbreak.

METHODS

Setting

This study was carried out in the ward and operating rooms of the Urology Department in a tertiary teaching hospital with 800 beds, 10 intensive care units, and 16 operating rooms. This hospital serves as a referral center for a population of approximately 1,400,000 people. The Urology Department has a ward of 30 beds located on the eleventh floor and 2 central operating rooms located on the subbasement. The urologic team performs approximately 25 elective operations in a week. More than 80% of these operations require endoscopic instrumentation.

Epidemiologic surveillance

Data collection. From March 1 to April 18, 2005, *P aeruginosa* strains were isolated in urine samples of 8 postoperative urology patients, and this situation was described as an outbreak by the Hospital Infection Control Committee. A subcommittee started a surveillance program in the Urology Department on April 18, 2005. Data including demographics, underlying disease, date and type of surgery, surgical procedures, operating room, surgeon and assistants, peri- and postoperative antibiotics, clinical evaluation, type of infection, and outcome were collected from medical records of patients with positive urine cultures for *P aeruginosa*. From April 18 to April 27, 4 additional *P aeruginosa* strains were isolated. Definition of nosocomial infection was made according to the Centers for Disease Control and Prevention definitions.[15]

Environmental sampling and antibiotic susceptibility profiles. By previously informing the urology staff, a total of 22 surveillance samples were obtained from various places of the 2 operating rooms: 14 from equipment including tap outlet of cystoscope and ureterorenoscope, resectoscope loops, inner surface of the pneumatic lithotripter, and forceps and the remaining 8 from the surface of the operation table, vaseline-impregnated gauze, and disinfectant solutions. Samples were taken from both outer surfaces by using moistened cotton swabs and inner surfaces or working channels by flushing with 20 mL of sterile saline. Ten milliliters of each saline wash sample was inoculated into Bactec blood culture bottles (Bactec 9120 System; Becton Dickinson, Sparks, MD) and incubated at 37°C until culture positivity or for 7 days. The moistened swab samples were inoculated directly onto blood agar and eosin-methylene-blue agar and incubated at 37°C for 24 to 48 hours. Two milliliters of disinfectant solution was inoculated directly into Bactec blood culture bottles.

Cultures showing bacteriologic growth were identified on the basis of different conventional biochemical tests. Gram-negative, oxidase-positive, and nonfermentative rods were considered as *Pseudomonas* species. Antimicrobial susceptibility tests for environmental and patients' isolates were performed on Muller Hinton agar using Kirby Bauer methods. The following antimicrobial disks (Oxoid) were used: amikacin (30 μg), gentamicin (10 μg), netilmicin (30 μg), tobramycin (10 μg), mezlocillin (75 μg), aztreonam (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), cefepime (30 μg), meropenem (10 μg), ciprofloxacin (5 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), carbenicillin (100 μg), imipenem (10 μg), and piperacillin (100 μg). *P aeruginosa* ATCC 27853 was used as an internal control. The Clinical and Laboratory Standards Institute criteria were used to determine susceptibility to antimicrobial agents.[16] Identification and antimicrobial susceptibility of the strains were confirmed by an automated system (Phoenix; Diagnostic Instrument Systems; Becton Dickinson).

PFGE

PFGE typing was performed by the method optimized previously by Yetkin et al.[13] Bacterial isolates were grown on nutrient agar overnight, at 37°C. The cells were suspended in 1 mL sodium-EDTA buffer (75 mmol/L NaCl, 25 mmol/L EDTA [pH 8.6]), and the optical density was adjusted to 0.7 ($\lambda = 590$) in spectrophotometer. The cells were embedded into low melting point agarose. After digestion of the cells and washing of the plugs, genomic DNA in the agarose plugs was restricted by 20 U *Xba*I (MBI Fermantas, Hanover, MD) for 6 hours at 37°C in water bath. The separation of DNA fragments was performed in 1.2% pulsed-field certified agarose gel (Bio-Rad Laboratories, Nazareth, Belgium) 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 ultraviolet light. According to the interpretative criteria of Tenover et al,[17] isolates were classified as indistinguishable (cluster), closely related, possibly related, or different.

RESULTS

A total of 81 patients underwent endourologic procedures at the urology clinic during the 2-month period, of whom 12 had *P aeruginosa* infections. The patients had a mean age of 51.4 ± 14.8 (range, 23-78) years.