Short Communication

Pulsed-Field Gel Electrophoresis of Multidrug-resistant and -sensitive strains of *Pseudomonas aeruginosa* from a Malaysian Hospital


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**SUMMARY:** Over a period of 6 months from January to June 2002, an unusual increase in the isolation of highly resistant *Pseudomonas aeruginosa* strains was observed in the various wards and intensive care units of a large general hospital in Johor Bahru, Malaysia. An equal number of multidrug-resistant (MDR) and drug-susceptible strains were collected randomly from swabs, respiratory specimens, urine, blood, cerebral spinal fluid, and central venous catheters to determine the clonality and genetic variation of the strains. Macrorestriction analysis by pulsed-field gel electrophoresis showed that the 19 MDR strains were genetically very homogenous; the majority showed the dominant profile S1 (n = 10), the rest very closely related profiles S1a (n = 1), S2 (n = 4), and S2a (n = 3), indicating the endemity of these strains. In contrast, the 19 drug-sensitive strains isolated during the same time period were genetically more diverse, showing 17 pulsed-field profiles (P = 0.50 - 1.00), and probably derived from the patients themselves. The presence of the MDR clone poses serious therapeutic problems as it may become endemic in the hospital and give rise to future clonal outbreaks. There is also the potential for wider geographical spread.

*Pseudomonas aeruginosa* is cosmopolitan in its distribution and can be isolated from soil, water, plants, and animals including humans. The minimal nutritional requirements of *Pseudomonas*, as evidenced by its ability to grow in distilled water and its tolerance of a wide variety of physical conditions including temperature, contribute to its ecologic success and ultimately to its role as an effective opportunistic pathogen (1). *P. aeruginosa* is accountable for 10-30% of all hospital-acquired infections, a site-specific prevalence that may vary from study to study (2). Multidrug-resistant (MDR) *P. aeruginosa* isolates have increased in frequency, and subsequently pose serious therapeutic problems.

Discriminative subtyping techniques can determine the clonal relationship of *P. aeruginosa* as it is an important cause of nosocomial outbreaks (3). *P. aeruginosa* cannot be serotyped completely due to lack of rough lipopolysacharides, but molecular methods such as ribotyping, pulsed-field gel electrophoresis (PFGE), PCR-based technique, and amplified fragment length polymorphism (AFLP) can subtype almost all strains (4-6). Among these methods, PFGE is considered to be the reference method for the majority of nosocomial pathogens because of its high discriminatory ability, reproducibility, easy interpretation of banding profiles, and universal applicability (5-7).

Over a period of 6 months from January to June 2002, an unusual increase of highly resistant *P. aeruginosa* was observed in the various wards and intensive care units (ICUs) of a large general hospital in Johor Bahru, Malaysia. During this period, 1,114 strains of *P. aeruginosa*, 19 (1.7%) of which were found to be resistant to all commercially available drugs except the rarely used colistin-polymyxinB (unpublished observation) were isolated. PFGE was applied to differentiate, below species level, resistant and sensitive strains of *P. aeruginosa* isolated from various specimens in the above-mentioned hospital. The objective was to determine the association of different genotypes with the antibiograms in order to establish the presence or absence of clonality, which information may be useful in tracking the spread of nosocomial infections caused by genetically related strains.

Nineteen each of resistant and sensitive strains of *P. aeruginosa* were collected randomly from swabs, respiratory specimens, urine, blood, cerebral spinal fluid (CSF), and central venous catheters from 38 patients in the various wards in the hospital during the study period of 6 months (January-June 2002). No attempt was made to differentiate carriage, colonization, or clinical infection. The isolates were identified by standard laboratory tests and confirmed by BD BBL Crystal Enteric/Nonfermenter Identification System (Franklin Lakes, N.J., USA), and susceptibility to antimicrobials was carried out using the Kirby-Bauer disc diffusion method (8) according to the National Committee for Clinical Laboratory Standards (8). Antimicrobials tested were piperacillin (100 μg), ceferazone (75 μg), ceftazidime (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), amikacin (30 μg), imipenem (10 μg), and colistin (300 μg). *P. aeruginosa* ATCC 27853 was included as a control. Eight of the 10 strains from the orthopedic ward (W2) were MDR. The remaining strains were from surgical, medical, and children’s wards. Three of 14 strains from the ICUs were also MDR. Isolates came from 30 males and 8 females, their ages ranging from 1-83 years. Fifteen strains came from swabs, 12 from respiratory specimens, 8 from urine and the rest from blood, CSF, and central venous catheter tips (see Fig. 2).

Genomic DNA for PFGE was prepared according to the protocol previously described (9). DNA banding patterns were interpreted according to the criteria of Tenovar et al. (10). To compare the macrorestriction patterns, the Dice coefficient