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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 endemicity 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 (F = 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 techniques, 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 application (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 according to the National Committee for Clinical Laboratory Standards (8). Antimicrobials tested were piperacillin (100 μg), cefeperazone (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

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of similarity, *F*, which expresses the proportion of shared DNA fragments in two isolates, was employed. Clustering was based on the unweighted pair group method of averages algorithm (UPGMA) and was performed with GelCompar Version 3.1 software (Applied Maths, Kortrijk, Belgium).

Based on the GC content of *P. aeruginosa*, *Spe*I was a suitable restriction endonuclease because the banding patterns consisted of 13 - 20 DNA fragments each and the pulsed-field profiles (PFPs) were easily distinguished and analysed. There were 22 *Spe*I-PFPs among the 38 resistant (n = 19) and sensitive (n = 19) strains. Reproducibility of the PFGE profiles was established by repeated testing of the same isolate on separate occasions. Stability was confirmed by the identical restriction patterns produced on multiple passages.

The MDR strains were genetically very homogenous, and the majority showed the dominant profile S1 (n = 10); others showed the very closely related profiles S1a (n = 1), S2 (n = 4), or S2a (n = 3). These profiles differed in less than three bands (F > 0.92) (Fig. 1). One MDR strain (PR12) had a distinct and unique profile S3 (Fig. 2). Cluster analysis showed that all the MDR strains were in one distinct clade based on 80% similarity, and were well distinguished from the drug-sensitive strains (Fig. 2). Based on published guidelines (10), all the MDR strains could be considered as closely related

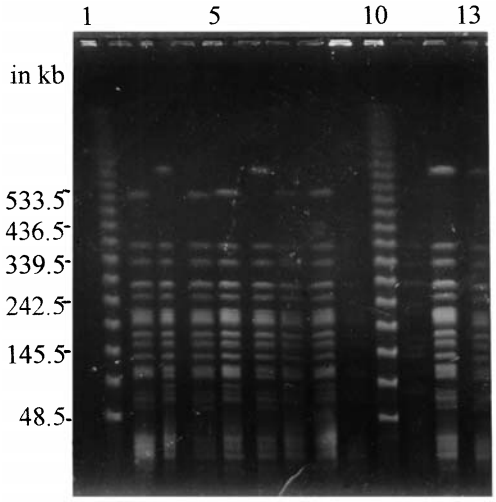

Fig. 1. Representative *Spe*I profiles of MDR *P. aeruginosa*. Lanes 1 - 13: M, S2a, S1a, S2a, S1, S2, S1, S1, D, M, D, S2, S2. M: lambda DNA concatemer marker; D: bands not distinct. Repeated digestion showed distinct banding profiles.

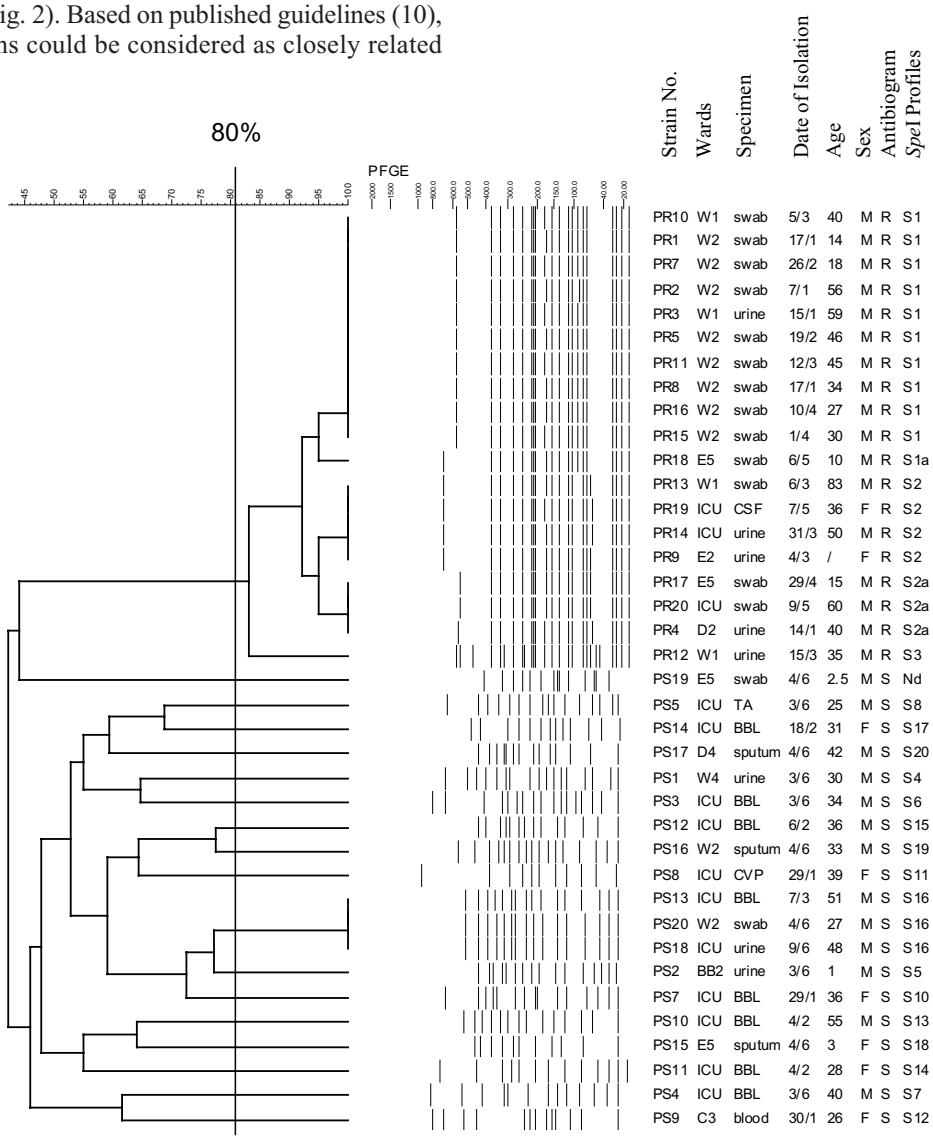

Fig. 2. Dendrogram showing cluster analysis of 38 strains of *Spe*I digested *P. aeruginosa*, generated by GelCompar program using the UPGMA method, based on the *F* values. BBL: bronchio-alveolar lavage; TA: trachial aspirate; CVP: central venous catether tip; M: male; F: female; R: resistant; S: sensitive.

and most probably derived from a distinct clone. This clone was probably endemic, given that strains with S1/S1a/S2 profiles were isolated from various specimens (swab, urine, CSF) taken from different wards/locations (ward W1, W2, E2, E5, ICU), suggesting nosocomial spread. The same MDR strains of *P. aeruginosa* indicated by the identical PFGE subtype were from orthopedic wards, and 45% of the total MDR came from these wards. This may reflect cross-infection or acquisition from a common or point source. However, no conclusion can be drawn regarding the source of infection, given that environmental specimens were not available for testing.

In contrast, drug-sensitive strains were genetically more diverse, showing 17 PFPs ($F = 0.50 - 1.00$) (Fig. 2). Excepting three strains, all others had separate and unique profiles. This cluster of three sensitive strains with similar *Spe*I profiles (S16) came from three different individuals and from different types of specimens (bronchial aspirate, tracheal aspirate, sputum) (Fig. 2). The genomic diversity of the drug-sensitive strains indicates that most of the *P. aeruginosa* were endogenous; that is, from the patients themselves.

P. aeruginosa has emerged as an important nosocomial pathogen and frequently colonizes hospitalized patients at rates may exceeding 50%, and colonization often presages invasive infection (11). Its high intrinsic resistance to antimicrobials and ability to develop MDR pose serious therapeutic problems (12). In order to trace the source and prevent further spread of this organism, epidemiologic investigation by the use of markers is necessary to discriminate among strains, based on the fact that bacterial isolates of the same transmission route would be clonally related. The discriminatory power of typing methods used in epidemiological investigations is critical to conclusions drawn regarding outbreaks caused by single or multiple strains (3). Studies have shown that PFGE has a better discriminatory power than other molecular-based methods such as ribotyping (4,13,14). Although we have demonstrated that the MDR strains most probably arose from an endemic clone in the hospital wards, no environmental samples were available for testing. In any case, the presence of the MDR strain poses a serious threat as it may become persistent or endemic in the hospital environment and circulate from patient to patient. The persistence of MDR strains in the hospital environment has been demonstrated by many researchers (15,16).

In conclusion, *P. aeruginosa* infection caused by MDR strains was probably due to an endemic clone, isolates of which were genetically more homogenous. On the other hand, infections caused by the drug-susceptible strains were genetically more diverse and polyclonal, and most probably originated from individual patients themselves. As this is the first study on the molecular subtyping of *P. aeruginosa* in our hospital, the DNA fingerprints obtained will provide a baseline for tracking the spread of this endemic clone.

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