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The Use of SDS Polyacrylamide Gel Electrophoresis of Periplasmic Proteins to Subtype *Pseudomonas aeruginosa* Pyocin Type 10/b Clinical Isolates

Summary: The periplasmic protein banding patterns (PPBPs) of thirteen strains of *Pseudomonas aeruginosa* pyocin type 10/b implicated in two nosocomial outbreaks in the neonatal unit of Rafeidia Hospital, Nablus, Palestine, were examined. In addition, five strains from sporadic cases from the same unit occurring in 1996 and 1997 were also studied. Despite different sources of the strains, PPBPs generated by PAGE suggested a clonal nature of the strains obtained during each of the two outbreaks. Although they had very similar PPBPs, the two outbreak clones were not identical. In contrast, sporadic strains of *P. aeruginosa* pyocin type 10 appeared to be much more heterogeneous than those of the two outbreaks. PPBP analysis appeared to be a useful tool that may be of value for epidemiological purposes.

Introduction

Discrimination of *Pseudomonas aeruginosa* strains is important in terms of a hospital infection control policy. A variety of typing methods has been proposed, including biotyping, antibiotyping, pyocin typing, phage typing, serotyping and plasmid profile typing [1–8]. Nevertheless, molecular methods such as DNA restriction fragment length polymorphism using pulsed field gel electrophoresis and ribotyping are considered to be the best typing methods for detailed epidemiological studies [6–9]. These methods, however, are cumbersome and restricted to specialized laboratories. SDS-PAGE of whole-cell proteins has also been used for many years to examine genetic relatedness among strains of *P. aeruginosa*, but in general, it appears to lack sufficient sensitivity to discriminate between different strains, especially those coming from the same hospital [5, 6]. Periplasmic protein banding patterns (PPBPs) have been established as a useful tool in epidemiologic studies on *Serratia* strains [10]. We report the successful use of this typing method as an epidemiological tool to analyze *P. aeruginosa* pyocin type 10/b strains isolated from two outbreaks and from sporadic cases in the neonatal unit of our hospital.

Materials and Methods

Bacterial strains: Thirteen strains of *P. aeruginosa* pyocin type 10/b were studied. They were recovered during two outbreaks in the neonatal unit of our hospital in 1995. The first outbreak (outbreak I) occurred between 23 July and 29 August 1995. Six infants were infected or colonized with *P. aeruginosa* pyocin type 10/b. Of the six infants three had throat colonization; one had an umbilicus infection and two developed cutaneous infections. The second outbreak (outbreak II) occurred later between October and November 1995, where four infants had developed cutaneous infections; two had respiratory infection and one had throat colonization. All infants had received at least one course of antibiotic treatment (not the antibiotic for which resistance has become apparent); this measure was associated with the disappearance of the organism. In addition, five strains isolated sporadically between 1996 and 1997 from the same unit were also included for comparative purposes. Strains were identified as *P. aeruginosa* based upon typical morphology, positive oxidase reaction, pigment production, biochemical properties (triple sugar iron and ability to oxidize carbohydrates) and growth at 42°C. Pyocin typing was performed according to the method of *Fyfe* et al. [11].

Preparation of periplasmic proteins (PPs): PPs were released using the method described by *Ames* et al. [12]. Two milliliters of culture grown overnight in minimal salts medium with 0.4% glucose was centrifuged for 10 min at 1,100g, and then supernatant was decanted. Two microliters of chloroform was added. After brief vortexing, the tubes were maintained at room temperature for 15 min, and then 200 µl of 0.01 M tris hydrochloride (pH 8.0) was added. Intact bacteria and debris were removed by centrifugation at 6,000g for 20 min, and the supernatant containing the PPs was carefully withdrawn and electrophoresed.

SDS-polyacrylamide gel electrophoresis: Gel electrophoresis was carried out in vertical SDS-polyacrylamide gels containing 0.1% SDS [13], with a stacking gel of 6.0% acrylamide and a running gel of 12.5% acrylamide. Electrophoresis was performed for 6–8 h at 10 mA. The standard protein markers (Sigma Chemicals, USA) were included. Gels were fixed and stained with silver staining as previously described [14]. Photographs of each gel were taken by gel documentation system (UVP, UK). The plot profiles based on peak positions for various strains were constructed by Macintosh analysis system (NIH image version 1.60), and consequently, compared using Dice coefficient based on peak position [15]. This coefficient, F, expresses the proportion of shared protein bands in two strains and was calculated by using the formula $F = 2n_{xy}/(n_x+n_y)$, where n_x is the total of protein bands from isolate X, n_y is the total number from isolate Y, and n_{xy} is the number of bands identical in the two isolates. An F val-

Received: 8 June 1998/Revision accepted: 14 November 1998

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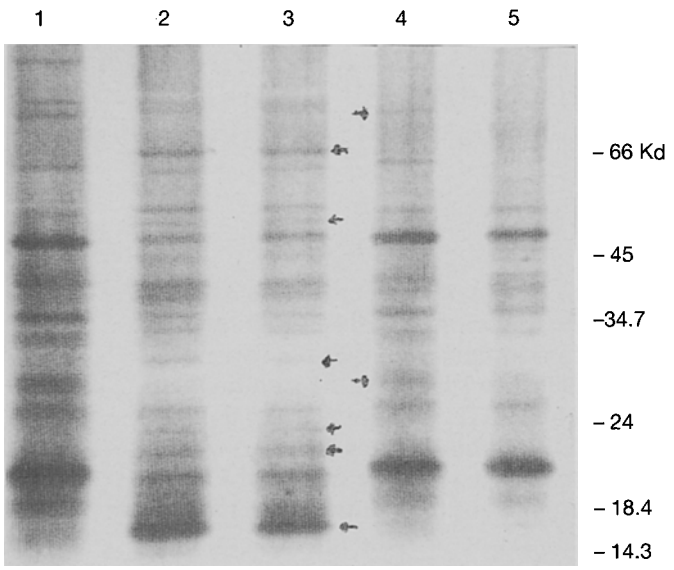

Figure 1: SDS-PAGE of periplasmic proteins of *Pseudomonas aeruginosa*: lanes: 1, *P. aeruginosa* ATCC 27853; 2 and 3, strains from outbreak I; 4 and 5, strains from outbreak II. The numbers on the right refer to the positions of standard protein marker bands. Arrowheads indicate the distinguishable bands between strains 3 and 4.

ue of 1.0 indicates that the two isolates have identical periplasmic protein patterns, and a value of 0 suggests complete dissimilarity.

Results

Four different PPBPs were noted among the five sporadic isolates tested, with an F value ranging from 0.41 to 0.61 (data not shown). In contrast, the strains from outbreak I appeared to be genetically identical (F = 1.0) (Figure 1, lanes 2 and 3). Similarly, the PPBPs of the strains from outbreak II showed three different, but almost similar strains, differing by only one or two bands (F = 0.91 to 1.0) (Figure 1, lanes 4 and 5). Although the strains from these two outbreaks appear to have similar PPBPs, they are not identical and analysis on the same gel indicated the difference by more than four bands (F = 0.45 to 0.81). (Figure 1, lanes 3 and 4); in this example, F is approximately 0.50. All the isolates were resistant to ampicillin, carbenicillin and streptomycin but susceptible to ceftazidime, cefotaxime, gentamicin, tobramycin and amikacin. To determine the degree of reproducibility and stability of the PPBPs, a sporadic strain was tested five times in a single electrophoretic run. A mean F of > 0.96 was found. Figure 2 shows the plot profile of the isolate twice; in this example, F = 0.96. Moreover, the stability of the periplasmic proteins and the reproducibility of electrophoretic technique were tested on three occasions about 2 months apart. Five strains including *P. aeruginosa* ATCC 27853 as a control were subcultured on Trypticase soy agar and then examined for PPBPs. The majority of protein banding patterns were both stable and reproducible for the same strains tested (F>0.96).

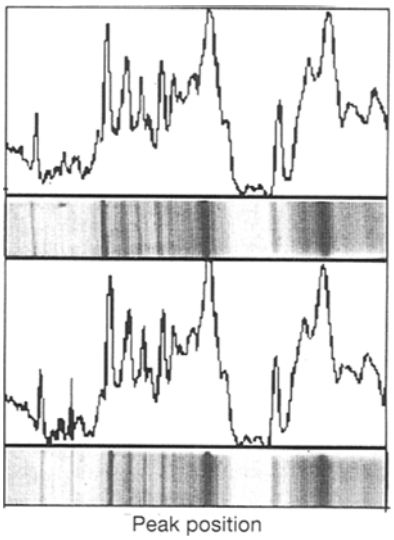

Figure 2: Comparison of the plot profile of electropherogram of periplasmic protein from a sporadic strain processed twice.

Discussion

Periplasmic protein analysis had been proposed in this study to circumvent the problems of *P. aeruginosa* typing raised by antibiogram typing and enzyme profiling previously carried out [16]. The advantage of this new approach lies in the fact that periplasmic proteins, located in the periplasmic space, can be released by a simple and rapid method which is based on the exposure of cells to chloroform [12]. Periplasmic proteins represent approximately 10 to 15% of the total cell protein [17]. Although extraction of the periplasmic proteins from other cellular proteins achieves approximately a 10-fold reduction in the number of test proteins resulting in a potential loss of information [17], this loss is counterbalanced by the possibility of using Dice coefficient based on peak position that gives more discriminative information about the degree of similarity between patterns [10]. This new typing system has been applied to a few bacterial species including *Serratia* species [10] and *Bacteroides fragilis* [18]. The results presented here have provided valuable information relating to the epidemiology of *P. aeruginosa* pyocin type 10/b in Rafeidia Hospital. Analysis of the periplasmic protein patterns generated by PAGE suggested the clonal nature and close identity of the strains obtained during each of the two outbreaks. Although they had very similar periplasmic protein patterns, the two outbreak clones were not identical. The results also suggest that multiple clones of *P. aeruginosa* pyocin type 10/b are endemic to our hospital and coexist simultaneously, causing sporadic cases of pseudomonal infection throughout the year. The present study, however, does not allow any conclusion to be drawn about whether distinct strains are responsible for a particular outbreak or whether the outbreaks are caused by similar strains which had undergone a mutation in a particular site for a periplasmic protein. It has been argued that

strains with similar patterns are clonally related [10, 18]. In conclusion, our results demonstrate the importance of periplasmic protein patterns as a diagnostic tool for the identification of the isolates and thus suggest that periplasmic protein banding patterns may be useful in epidemiological studies.

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