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Heterogeneity of Class I β-Lactamase Expression in Clinical Isolates of *Pseudomonas aeruginosa*

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Expression of chromosomal β-lactamase was examined in 85 clinical isolates of *Pseudomonas aeruginosa*. β-Lactamase assays with and without cefoxitin induction revealed four phenotypes of enzyme expression: low basal, inducible; moderate basal, inducible; moderate basal, constitutive; and high basal, constitutive. The isoelectric points of the major β-lactamase bands were 9.4, 9.2, and 8.4. These results indicate that there is a limited heterogeneity in expression of chromosomal β-lactamase of *P. aeruginosa*.

Virtually every strain of *Pseudomonas aeruginosa* produces a chromosomal β-lactamase that belongs to Richmond-Sykes class I (10). Under normal conditions, this enzyme is expressed at a very low basal level; however, it can be induced to much higher levels with such compounds as benzylpenicillin, cefoxitin, or imipenem (1, 6, 9–11, 13). Mutants of *P. aeruginosa* altered in their expression of class I β-lactamase have also been described. These include mutants partially or fully derepressed for enzyme expression, those with little or no enzyme, and mutants expressing moderate levels of β-lactamase constitutively (2–6, 11, 14). These altered states of enzyme expression are often associated with changes in susceptibility to various β-lactam antibiotics. To date, most studies concerning the production of class I β-lactamase by *P. aeruginosa* have examined very few strains, usually from narrow geographic locations. Therefore, the current study was designed to assess the expression of class I β-lactamase in a large number of clinical isolates of *P. aeruginosa* collected from diverse geographic locations.

A total of 85 clinical isolates of *P. aeruginosa* were collected from various laboratories in the United States and Europe. These included 18 isolates from London, England; 13 from Omaha, Nebr.; 4 from Chicago, Ill.; 6 from Cincinnati, Ohio; 18 from Houston, Tex.; 5 from Detroit, Mich.; 4 from Minneapolis, Minn.; 3 from Miami, Fla.; and 14 from Brussels, Belgium. These were not random isolates but rather isolates that displayed or developed resistance to a variety of β-lactam antibiotics. They represented 45 distinct strains, since many were multiple isolates recovered from a single patient during a course of antimicrobial therapy. A second isolate from the same patient was included only if it was altered in its expression of β-lactamase in comparison to the first isolate or was a different strain. Strain identity was based upon serotype (serotyping was kindly performed by Charles Zierdt, National Institutes of Health, Bethesda, Md.).

β-Lactamase expression in each of the 85 isolates was examined in sonic extracts prepared with or without 2 h of induction with 100 μg of cefoxitin per ml (3). The isoelectric point (pI) of each enzyme was determined before and after induction as described previously (12). In each isoelectric focusing run, controls were included to monitor run-to-run variations. These controls consisted of sonic extracts prepared from fully derepressed mutants of *P. aeruginosa* 164 (3), *Enterobacter cloacae* P99 (kindly provided by L. Koupal of Merck Sharp & Dohme, West Point, Pa.), and various gram-negative bacteria possessing well-characterized plasmid-mediated β-lactamases (kindly provided by A. A. Medérios of Brown University, Providence, R.I.). No plasmid-mediated β-lactamases were detected in any isolate, and each band detected on isoelectric focusing gels displayed characteristics indicative of a class I enzyme (12).

TABLE 1. β-Lactamase expression in 85 clinical isolates of *P. aeruginosa*

Phenotype	No. of isolates	β-Lactamase activity[a]	
		Uninduced	Induced
Low basal, inducible	36	5 ± 3 (1–20)	277 ± 53 (37–681)
Moderate basal, inducible	10	260 ± 135 (89–591)	1,078 ± 174 (731–1,622)
Moderate basal, constitutive	25	304 ± 74 (62–748)	515 ± 91 (222–946)
High basal, constitutive	14	1,679 ± 432 (810–3,198)	1,914 ± 390 (1,142–3,185)

[a] Reported as nanomoles of cephalothin hydrolyzed per minute per milligram of protein. Values are means ± 2 standard deviations (ranges).

The 85 clinical isolates could be separated into four distinct phenotypes on the basis of the level of basal (uninduced) enzyme expression and inducibility by cefoxitin (Table 1). The first phenotype consisted of 36 isolates with low basal levels of enzyme expression which could be induced to a higher level with cefoxitin (referred to as low basal, inducible isolates). Ten isolates showed moderately elevated basal levels of enzyme expression which could still be induced by cefoxitin (referred to as moderate basal,

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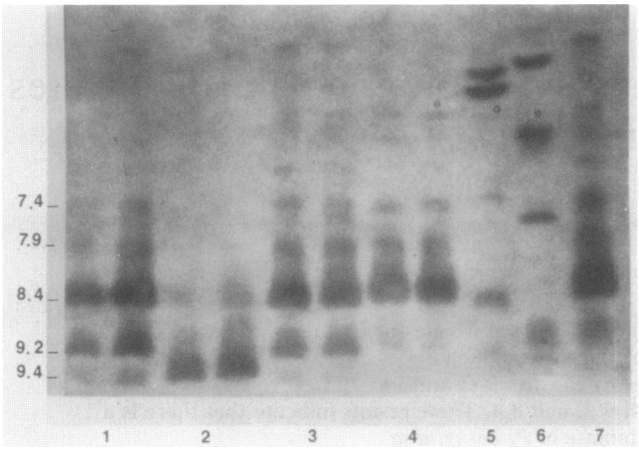

FIG. 1. Examples of isoelectric focusing patterns of β-lactamases from *P. aeruginosa*. Lane pairs 1 to 4 contain sonic extracts from four strains of *P. aeruginosa* before (lane to the left of number) and after (lane to the right of number) induction by cefoxitin. The strains possessed either a moderate level, inducible phenotype (lane pairs 1 and 2) or a moderate level, constitutive phenotype (lane pairs 3 and 4). Lanes 5 to 7 contain controls. Lane 5 (top to bottom), TEM-1, TEM-2, OXA-3, and the chromosomal band of the host strain; lane 6 (top to bottom), HMS-1, PSE-2, SHV-1, and the chromosomal band of the host strain; lane 7, *P. aeruginosa* 164CD (3). Numbers at left are pIs.

inducible isolates). A third phenotype was found in 25 isolates with moderately elevated constitutive enzyme expression (referred to as moderate basal, constitutive isolates), while a fourth was found in 14 isolates with extremely high constitutive enzyme expression (referred to as high basal, constitutive isolates).

Among the 45 distinct strains represented by the 85 isolates, only three pIs were represented by the major β-lactamase band observed on isoelectric focusing gels; 18 strains had a major band of pI 8.4, 23 strains had a major band of pI 9.2, and 4 strains had a major band of pI 9.4. Additional minor bands were observed in uninduced sonic extracts from most moderate basal, inducible strains and those expressing enzyme at moderate or high levels constitutively. Additional minor bands were observed in sonic extracts from most low basal, inducible strains only after induction with cefoxitin. For those low basal, inducible isolates with isogenic counterparts within the other phenotypes, the additional bands appearing after induction were similar to those found in sonic extracts of their moderate basal, constitutive; moderate basal, inducible; or high basal, constitutive mutants. In only 2 of the 21 distinct low basal, inducible strains did the pI of the major band change after induction with cefoxitin. However, the band(s) apparent before induction was still present. A limited number of similar pIs were represented by both major and minor bands for all isolates examined (Fig. 1). These were 9.4, 9.2, 8.4, 7.9, and 7.4, although no strain had a major band at the latter two pIs. There was no association between the pI of the major β-lactamase band and the geographic source of the strains or serotype. Serotypes 1, 2, 3, 5, 6, 10, 11, 15, 16, and 17 were represented among the strains studied.

The relationship between class I enzyme expression and β-lactam resistance was assessed in disk diffusion assays performed and interpreted in accordance with the recommendations of the National Committee for Clinical Laboratory Standards (8). For this analysis, results from two isolates recovered from patients with cystic fibrosis were excluded. These strains grew very poorly on Mueller-Hinton agar and thus produced very large zones (>30 mm) that were not truly indicative of antibiotic susceptibility. For the remaining 83 isolates, there was a clear association between the basal level of class I enzyme expression and resistance to mezlocillin, piperacillin, carbenicillin, and ceftazidime (Table 2). There was no such association with imipenem resistance. Of the 36 low basal, inducible isolates, 17 were susceptible to the penicillins and ceftazidime. Only two other isolates showed a similar susceptibility profile. Both were moderate basal, constitutive class I enzyme producers. All high basal, constitutive isolates were resistant to the three penicillins and ceftazidime.

The results of this study confirm and expand upon previously published information concerning the class I β-lactamase of *P. aeruginosa*. Clearly, there is a limited heterogeneity in this "species-specific" enzyme. In 1976, Matthew and Harris examined the β-lactamases in 49 strains of *P. aeruginosa* by isoelectric focusing (7). Although the methods for isoelectric focusing used by these authors differed from those used in the current study, the results were surprisingly similar, i.e., there was a limited number of distinct β-lactamase bands among different strains of *P. aeruginosa*. The pIs of these bands ranged from 9.4 to 7.3, with two pIs representing 89 to 91% of the enzymes in either study. Methodologic differences probably account for the variation of approximately 1 pH unit in the pIs for the more frequently encountered β-lactamases in the two studies. Preliminary runs with our procedure and sonic extracts of the strains tested by Matthew and Harris (7) showed good agreement in the pI for *E. cloacae* P99 (7.6). However, as the pI varied below 7.0 or above 7.8 the differences between the results generated by the two procedures grew larger. Thus, enzymes focusing at pIs 9.4, 9.2, and 8.4 in the current study are probably analogous to the pI 8.2, 8.0, and 7.5 enzymes of Matthew and Harris (7), respectively.

All but one of the major phenotypes for class I enzyme expression that had previously been reported for *P. aeruginosa* (2–6, 11, 14) were observed in this study. The absence of isolates producing little or no β-lactamase in this study was probably due to the bias toward β-lactam-resistant

TABLE 2. Relationship between class I enzyme expression and β-lactam resistance in *P. aeruginosa*[a]

Phenotype	No. of strains	% of strains resistant to:				
		Mezlocillin	Piperacillin	Carbenicillin	Ceftazidime	Imipenem
Low basal, inducible	36	31	8	33	8	6
Moderate basal, inducible	8	100	38	38	38	25
Moderate basal, constitutive	25	72	40	56	36	12
High basal, constitutive	14	100	100	100	100	14

[a] See Table 1 for enzyme levels.

strains in the collection. This bias also affected the relative prevalence of each of the four phenotypes that were observed. Thus, these data do not reflect the prevalence of each phenotype among clinical isolates of *P. aeruginosa*. Rather, they indicate that each phenotype previously associated with β-lactam resistance can be encountered in clinical isolates and is not merely an altered state of enzyme expression observed in laboratory-derived mutants.

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