Five Novel Plasmid-Determined β -Lactamases

ANTONE A. MEDEIROS,^{1*} MENASHI COHENFORD,¹ AND GEORGE A. JACOBY²

The Miriam Hospital, Providence, Rhode Island 02906,¹ and Massachusetts General Hospital, Boston, Massachusetts 02114²

Received 22 January 1985/Accepted 11 February 1985

Five novel plasmid-determined β -lactamases named TLE-1, OXA-4, OXA-5, OXA-6, and OXA-7 were detected in ampicillin-resistant isolates of *Escherichia coli* and carbenicillin-resistant strains of *Pseudomonas aeruginosa*. TLE-1 resembled TEM-1 in substrate profile and reactions with inhibitors but differed in isoelectric point (5.55) and enzyme banding pattern on flat-bed electrofocusing. OXA-4, OXA-5, OXA-6, and OXA-7 hydrolyzed oxacillin, methicillin, and cloxacillin readily but differed from OXA-1, OXA-2, and OXA-3 in substrate profiles, inhibitor reactions, and isoelectric points (7.5 to 7.8). OXA-4 and OXA-6 were unusual for members of the OXA group in their sensitivity to inhibition by cloxacillin. OXA-5 and OXA-7 had isoelectric points close to that of SHV-1, emphasizing the need in β -lactamase classification for studies in addition to isoelectric focusing. These five new enzymes bring the number of plasmid-determined β -lactamases known in gram-negative organisms to more than 20. The evolution of such enzymatic diversity remains to be explored.

In gram-negative bacteria, plasmid-determined β -lactamases can be classified into a variety of types (17, 19, 20) by several criteria, including substrate profile, reaction with inhibitors, molecular weight, and particularly isoelectric point. The enzymes termed OXA-1, OXA-2, and OXA-3, for example, are distinguished by ready hydrolysis of oxacillin, methicillin, and cloxacillin, sensitivity to inhibition by NaCl but resistance to inhibition by cloxacillin, and characteristic band patterns and distinct isoelectric points on isoelectric focusing in polyacrylamide gels. TEM-1, TEM-2, SHV-1, and HMS-1 β -lactamases, in contrast, have low activities against oxacillin and related isoxazolyl penicillins and are sensitive to inhibition by cloxacillin but not NaCl, whereas PSE-1, PSE-2, PSE-3, and PSE-4 β -lactamases readily hydrolyze carbenicillin and possess distinct and characteristic isoelectric points.

Matthew reviewed the distribution of these eleven plasmid-mediated β -lactamase types in 363 gram-negative isolates in 1979 (16). Implicit in such surveys is the stability of each β -lactamase variety in various host strains. Since the study by Matthew, further β -lactam-resistant gram-negative isolates have been examined. In this report we present evidence for five additional and novel plasmid-determined β -lactamases detected in strains of *Escherichia coli* and *Pseudomonas aeruginosa*. Four belong to the OXA group, and the fifth is a new TEM-type enzyme. These new plasmid-mediated β -lactamase types will be useful for studies of plasmid epidemiology and the evolution of resistance mechanisms.

MATERIALS AND METHODS

Bacterial strains. *E. coli* 7181, 7259, and 7604 were obtained from Jose Ximenez, Laborerapica-Bristol, São Paulo, Brazil. *P. aeruginosa* Ming, isolated in Chicago, and 76072601, isolated in London, were provided by George Miller, Shering Corp., Bloomfield, N.J. The recipient strains for plasmids included *E. coli* J53 *met pro*, J53 *Nal^r*, J53 *Rif^r*, W677 *lac leu thi thr*, and C *Ilv⁻ ilv res mod* and *P. aeruginosa* PA038 *leu Rif^r* (7, 24). *E. coli* TEM(R6K) producing TEM-1 β -lactamase and *E. coli* 1573E(R46) producing OXA-2

were provided by Margaret Matthew, Glaxo Pharmaceuticals Ltd., Greenford, United Kingdom. The reference strains used for isoelectric focusing have been previously described (29).

Plasmid characterization. Conjugative (*Tra⁺*) plasmids were detected by mating donor *E. coli* with *E. coli* J53 *Rif^r* or J53 *Nal^r* and donor *P. aeruginosa* with *P. aeruginosa* PA038 *Rif^r* by using nalidixic acid or rifampin for counterselection (21, 24). *Tra⁻* plasmid pMG204b was transferred by transformation (12) with plasmid DNA purified from *E. coli* 7604 by cesium chloride-ethidium bromide gradient centrifugation (4). Transconjugants or transformants were characterized by spot testing on plates containing antibiotics, HgCl₂, or phenylmercuric acetate (24). *Tra⁺* plasmids were tested for incompatibility (*Inc*) specificity by mating with recipients containing standard plasmids of known *Inc* groups (8, 24). Plasmid molecular weights were estimated by agarose gel electrophoresis (3) in comparison with standard plasmids of known size.

Reagents. Antibiotics were from the following sources: ampicillin and penicillin (E. R. Squibb and Sons, Princeton, N.J.); cephalothin, cephaloridine, cefamandole, and moxalactam (Eli Lilly & Co., Indianapolis, Ind.); clavulanate and carbenicillin (Beecham Laboratories, Bristol, Tenn.); cefotaxime (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.); cefoxitin (Merck Sharp & Dohme, West Point, Pa.); and nitrocefin (BBL Microbiology Systems, Cockeysville, Md.). Sephadex gels and standard proteins for molecular weight determination were obtained from Pharmacia, Inc., Piscataway, N.J., and Ampholines with a pH range of 3.5 to 10 were from LKB Produkter AB, Bromma, Sweden. All other reagents were of analytical grade.

β -Lactamase preparations. Sonic extracts were prepared as described previously (29) and dialyzed against deionized distilled water. Extracts with low β -lactamase activity were concentrated with a Micro-ProDiCon apparatus (Bio-Molecular Dynamics, Beaverton, Oreg.) by using membranes with a 10,000-molecular-weight cutoff. For the study of substrate and inhibition profiles, sonic extracts from the previous step were fractionated on a Sephadex G-100 column (2.5 by 90 cm), which was equilibrated and eluted with 50 mM phosphate buffer (pH 7.0) containing 0.02% sodium azide. Active

* Corresponding author.

TABLE 1. Properties of plasmids determining novel β -lactamases

Isolate	Source	β -Lactamase type	Plasmid	Inc group	Mol wt (10 ⁶)	Resistance phenotype ^a
<i>E. coli</i>						
7181	Brazil	OXA-7	pMG202	C	86	Ap Cm Gm Km Su Tm Hg
7259	Brazil	OXA-4	pMG203	C	85	Ap Cm Sm Sp Su Tp
7604	Brazil	TEM-1	pMG204a	C	86	Ap Cm Gm Km Su Tc Tm Tp Hg
		TLE-1	pMG204b		70	Ap Cm Km Sm Su Tra ⁻
<i>P. aeruginosa</i>						
Ming	Chicago	OXA-6	pMG39	P-11	<i>b</i>	Cb Gm Km Sm Su Tm
76072601	London	OXA-5	pMG54	P-1	54	Cb Gm Km Su Tc Tm Hg Pma

^a Ap, Ampicillin; Cb, carbenicillin; Cm, chloramphenicol; Gm, gentamicin; Hg, mercuric ion; Km, kanamycin; Pma, phenylmercuric acetate; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim; Tra⁻, transfer deficient.

^b Not seen by agarose gel electrophoresis.

fractions were combined, dialyzed against distilled water, and concentrated. As analyzed by isoelectric focusing, the partially purified enzymes contained little, if any, chromosomal β -lactamase.

β -Lactamase assays. Enzyme activity was assayed either by monitoring the rate of β -lactam hydrolysis under conditions of constant pH with an automated titrator system or by using the chromogenic substrate nitrocefin and following the change in optical density at the optimal wavelength of absorbance spectrophotometrically.

Alkalimetric assays were conducted by the method of Labia et al. (10) with the following modifications. The titrant was 0.02 N NaOH, and the reaction mixture contained 0.5 mM substrate and 3 mM NaCl. NaOH consumption with time was monitored with a pH Stat Radiometer comprising a pHM 82 pH meter, a TTT 80 titrator, and an ABU 80 autoburette (Radiometer, Copenhagen, Denmark). All readings were compared with control reaction mixtures to which no enzyme was added. Assays involving nitrocefin were carried out by a modification of the procedure of O'Callaghan (23). The reaction mixture contained 0.25 mM nitrocefin, 30 to 60 mU of enzyme, and 100 mM sodium phosphate buffer (pH 7.0) in a total volume of 3 ml. Incubation was at 37°C in a Hitachi model 100-60 double-beam spectrophotometer with absorbance monitored continuously at 486 nm. Experimental values were compared with controls in which either enzyme or inhibitor was omitted.

One unit of activity is defined as that amount of β -lactamase that will hydrolyze 1.0 μ mol of potassium penicillin G in 1 min at 37°C and pH 7.0. Protein was determined by the method of Lowry et al. (15) with bovine serum albumin as the standard.

Inhibition of β -lactamase activity. Enzymes were preincubated at 37°C for 5 min with each inhibitor at the indicated concentrations (see Table 3) and then assayed with nitrocefin as described above.

Analytical isoelectric focusing. Isoelectric focusing on polyacrylamide gels was performed by a modification of the procedure of Matthew et al. (17) on crude sonic extracts. Unless otherwise indicated, the acrylamide was at a concentration of 7.5%, and the current was applied at a constant power of 1 W with a maximum of 320 V for 15 h and 1 W with a maximum of 400 V for 1 h. Final pH readings of the gels were taken at 0.5-in. intervals with a Corning pH meter (Corning Glass Works, Corning, N.Y.) equipped with a surface electrode. Enzyme activity was located in the gels

with nitrocefin and photographed with a Polaroid MP-3 camera through a dark green filter.

Molecular weight determination. β -Lactamase molecular weight was estimated by the method of Andrews (2) by using gel filtration on a column of Sephadex G-100 (1.5 by 90 cm), which was equilibrated and eluted with 50 mM phosphate buffer (pH 7.0) containing 0.02% sodium azide. The column was calibrated with the following markers (molecular weight in parentheses): RNase (13,700), myoglobin (17,800), chymotrypsinogen (25,000), ovalbumin (43,000), and bovine serum albumin (67,000). Void volume was determined with dextran blue 2000.

RESULTS

Detection of novel plasmid-mediated β -lactamases. In the course of characterizing 196 ampicillin-resistant clinical isolates of *E. coli* by isoelectric focusing for the type of β -lactamase produced (22), three strains from Brazil were detected that produced novel enzyme types (Table 1). *E. coli* 7181 and 7259 transferred resistance to ampicillin and other antibiotics by conjugation to *E. coli* recipient J53 where testing demonstrated that the plasmids involved belonged to Inc group C. *E. coli* 7604, in contrast, produced both TEM-1 and a novel β -lactamase and by agarose gel electrophoresis contained five plasmid bands. Ampicillin resistance could be transmitted by conjugation to strain J53 from strain 7604, but the transferred plasmid pMG204a determined TEM-1. When plasmid DNA from strain 7604 was used to transform *E. coli* C Ilv⁻, ampicillin-resistant colonies were obtained that carried a transfer-deficient (Tra⁻) plasmid pMG204b, which determined the novel β -lactamase alone as well as resistance to other antibiotics.

The novel β -lactamases detected in *P. aeruginosa* were discovered among about 60 strains carrying carbenicillin resistance plasmids that have been analyzed by isoelectric focusing (24). Plasmid pMG54 belonged to Inc group P1, and pMG39 belonged to Inc group P11. Like some other members of Inc group P11, pMG39 could not be visualized by agarose gel electrophoresis and was not transmissible to *E. coli* by conjugation (24), but plasmid pMG54, like other Inc group P1 plasmids, had a broad host range.

Properties of novel β -lactamases. Figures 1 and 2 compare the isoelectric focusing patterns of β -lactamases produced by these plasmids with those of established types. The enzyme produced by pMG204b had a main band at a pI of 5.55, which was distinct from those of PSE-4 (pI, 5.3),

TEM-1 (pI, 5.4), TEM-2 (pI, 5.6), and PSE-1 (pI, 5.7) (Fig. 1). The other β -lactamases had more alkaline isoelectric points and focused in the region where the OXA enzymes are located but at points distinct from OXA-1, OXA-2, and OXA-3 prototypes: pMG203 enzyme, 7.5; pMG54 enzyme, 7.62; pMG202 enzyme, 7.65; pMG39 enzyme, 7.68 (Fig. 2).

Determination of activity profiles for various β -lactam antibiotics confirmed the similarity of TEM and the novel pMG204b β -lactamase, which consequently will be termed TLE-1 (TEM-like enzyme). Similarly, the substrate profiles of the enzymes produced by pMG203, pMG54, pMG39, and pMG202 established them as members of the OXA group with designations OXA-4, OXA-5, OXA-6, and OXA-7, respectively (Table 1). Table 2 compares the relative hydrolysis rates of the novel enzymes with those of TEM-1 and OXA-2. TEM-1 and TLE-1 had virtually identical hydrolytic activities. OXA-4, OXA-5, OXA-6, and OXA-7, like OXA-2, readily hydrolyzed oxacillin, methicillin, and cloxacillin. They could be distinguished from each other and from OXA-2 by their hydrolysis rates with various substrates. Compared with OXA-2, all four new OXA enzymes hydrolyzed cefotaxime more actively. OXA-4 and OXA-5 were the most active against moxalactam, and OXA-5 was the most active against cefoxitin.

The rate of penicillin hydrolysis by OXA-7 β -lactamase was nonlinear. The velocity of the reaction initially increased, became constant, and then decreased. Consequently, the activity of this enzyme was evaluated from the linear portion of the hydrolysis curve. The other novel OXA enzymes did not demonstrate this change in reaction rate, but OXA-1 exhibited a similar reaction profile (unpublished data).

Reactions with inhibitors also served to differentiate these enzymes. Table 3 shows that TLE-1, like TEM-1, was sensitive to inhibition by clavulanate and cloxacillin but resistant to inhibition by NaCl. Like OXA-2, the new OXA types were resistant to inhibition by clavulanate but were more variable in their response to the other inhibitors. In particular, OXA-6 was as sensitive to cloxacillin inhibition as were the TEM enzymes, whereas OXA-4, and to a lesser extent OXA-6, exhibited a biphasic reaction to inhibition by NaCl. NaCl did not inhibit the early phase of nitrocefin hydrolysis by either OXA-4 or OXA-6 but caused 67 and 40% inhibition, respectively, of the later phase.

FIG. 1. Isoelectric focusing gel comparing the band patterns of the novel β -lactamase produced by pMG204b (TEM-3) with those of well-established, plasmid-determined β -lactamases that have isoelectric points in the same pH range. A, PSE-4; B, TEM-1; C, TLE-1; D, TEM-2; E, PSE-1; F, LCR-1; G, PSE-2.

FIG. 2. Isoelectric focusing gel comparing the band patterns of the novel β -lactamases produced by pMG203 (OXA-4), pMG54 (OXA-5), pMG39 (OXA-6), and pMG202 (OXA-7) with those of well-established, plasmid-determined β -lactamases that have isoelectric points in the same pH range. A, OXA-1; B, OXA-4; C, SHV-1; D, OXA-5; E, OXA-7; F, OXA-6; G, OXA-2. An additional band representing the chromosomally determined β -lactamase of *E. coli* is seen in lanes A, D, and G (pI, 8.0).

Table 3 also shows molecular weight estimates for the novel β -lactamases. TLE-1 was close in size to TEM-1. OXA-4, OXA-5, and OXA-7 had similar sizes, but OXA-6, like OXA-2, was about twice as large.

DISCUSSION

It is difficult to know whether these novel β -lactamases have recently appeared, have been detected because unusual regions, such as Brazil, were sampled, or have been found because subtle differences in isoelectric focusing patterns and substrate profiles were appreciated. OXA-4, for example, resembled OXA-1 in substrate profile and molecular weight but was recognized by a 0.1 pH unit difference in isoelectric point; it also differed in reactions with inhibitors and in the linearity of its penicillin hydrolysis rate. Other plasmids that determine what we would classify as OXA-4 have been found in *P. aeruginosa* (A. Philippon, G. Paul, and G. A. Jacoby, Abstr. XIII Int. Congr. Microbiol. 1982, P42:1), and plasmid R753, previously classified as producing OXA-1 (18), makes OXA-4 by our criteria. Another plasmid producing OXA-5 has also been detected in a *P. aeruginosa* isolate from London, but the plasmid involved resembles pMG54 in resistance properties, Inc group specificity, and molecular weight and may be epidemiologically linked. OXA-5 (pI, 7.62) and OXA-7 (pI, 7.65) also have virtually the same isoelectric point as does SHV-1 β -lactamase (pI, 7.6). They could easily be confused with SHV-1 if distinctive accessory bands were not visualized on the gel or if substrate profiles and inhibition reactions were not performed. Indeed, the finding of three β -lactamases with virtually identical isoelectric points illustrates the need to carry out additional tests in addition to isoelectric focusing when classifying strains for the type of β -lactamase produced.

β -Lactamases TEM-1 and TEM-2 differ by a single amino acid residue that is responsible for their difference in isoelectric point (1, 27). TLE-1 resembles the TEM enzymes closely in substrate profile and inhibitor reactions. The difference in pI between TLE-1 (5.55) and TEM-1 (5.4) or TEM-2 (5.6) could have a similar basis, but the failure of a

TABLE 2. Substrate profiles of novel β -lactamases

β -Lactamase type	Activity ^a (mU/mg of protein)	Relative rate of hydrolysis ^b (%)											
		Penicillin	Ampicillin	Carbenicillin	Oxacillin	Methicillin	Cloxacillin	Cephalexin	Cephalexin	Cefamandole	Cefoxitin	Cefotaxime	Moxalactam
TEM-1	1,850	100	72	12	4	1	<0.2	60	13	20	<0.2	4	<0.2
TLE-1	1,400	100	67	13	4	5	6	52	15	24	2	6	<0.2
OXA-2	1,050	100	236	27	581	31	157	108	61	32	2	2	2
OXA-4	85	100	438	39	220	711	64	194	83	23	<0.2	63	7
OXA-5	38	100	188	40	210	109	258	89	175	35	10	49	8
OXA-6	30	100	596	46	1,048	585	301	149	24	82	<0.2	28	2
OXA-7	82	100	545	48	702	424	494	136	51	47	4	31	3

^a Activity of sonic extracts with penicillin G as the substrate before gel filtration.^b Expressed as hydrolysis rate relative to that of penicillin G (100%).

TEM-1 DNA probe to hybridize under stringent conditions with pMG204b (unpublished data) suggests a more distant evolutionary relationship. The seven OXA enzymes show greater variability in relative hydrolysis rates, have isoelectric points between 7.1 and 7.8, and demonstrate distinctive reactions with inhibitors, such that OXA-4 and OXA-6, unlike the others, are cloxacillin sensitive. Furthermore, OXA-6, like OXA-2 and OXA-3, which are dimers (5), has a molecular weight about twice that of other plasmid-determined enzymes. The sequences modified to produce these differences will be of interest.

These new β -lactamases should provide useful epidemiological markers for following the spread of β -lactam resistance. The OXA-1 and OXA-2 β -lactamases are determined by transposons in which β -lactamase production is linked to other resistance markers (9, 30). Several of the new OXA types are also determined by multiresistance transposons that differ in size but share associated resistances and restriction sites with the OXA-1 and OXA-2 units and belong to the same transposon family (unpublished data).

The five novel enzymes described here bring the number of plasmid-determined β -lactamases to more than 20. Since 1979 (16), the list includes ROB-1 (pI, 8.1; TEM-like in substrate profile [25], LCR-1 (pI, 6.5; also TEM-like in hydrolytic activity [26], CARB-3 (pI, 5.55; similar to PSE-1 and PSE-4 in activity, and plasmid location suspected but not established [11]), two other CARB- or PSE-like enzymes as yet unnamed (pI, 6.0 and 6.9 [28]), and CEP-2 (pI, 8.1; predominantly active against cephalosporins [13, 14]). Diversity is typical for other plasmid-mediated resistance mechanisms as well (6). The range of properties known for the plasmid-determined β -lactamases makes them attractive candidates for exploring how such diversity has evolved.

TABLE 3. Additional properties of novel β -lactamases

β -Lactamase type	Inhibition ^a by:			pI	Mol wt (10 ³)
	Cloxacillin (0.1 mM)	Clavulanate (20 μ M)	NaCl (100 mM)		
TEM-1	57	96	0	5.4	23.5
TLE-1	54	95	0	5.55	19.8
OXA-2	0	0	90	7.7	43.9
OXA-4	25	0	0 ^b	7.5	23
OXA-5	0	0	80	7.62	27
OXA-6	69	0	0 ^b	7.68	40
OXA-7	0	0	89	7.65	25.3

^a Expressed as 100 minus the percentage of activity against nitrocefin at the indicated concentration of inhibitor. Each number represents an average of duplicate measurements varying by no more than 10%.^b Inhibition reaction was biphasic. In the secondary phase of the reaction, the activity of OXA-4 was inhibited by 67%, whereas that of OXA-6 was inhibited by 40%.

ACKNOWLEDGMENTS

We thank Lorraine Sutton, Joe Bisson, Frank Prevost, and Elaine Papa for expert assistance.

This work was supported in part by Food and Drug Administration contract 223-79-7060, the Miriam Hospital Research Trust, and grant PCM-8110808 from the National Science Foundation.

LITERATURE CITED

- Ambler, R. P., and G. K. Scott. 1978. Partial amino acid sequence of penicillinase coded by *Escherichia coli* plasmid R6K. Proc. Natl. Acad. Sci. U.S.A. **75**:3732-3736.
- Andrews, P. 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. **91**:222-233.
- Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513-1523.
- Clewell, D. B., and D. R. Helinski. 1969. Supercoiled circular DNA-protein complex in *Escherichia coli*; purification and induced conversion to an open circular DNA form. Proc. Natl. Acad. Sci. U.S.A. **62**:1159-1166.
- Dale, J. W., and J. T. Smith. 1976. The dimeric nature of an R-factor mediated β -lactamase. Biochem. Biophys. Res. Commun. **68**:1000-1005.
- Foster, T. J. 1983. Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol. Rev. **47**:361-409.
- Hedges, R. W., and A. E. Jacob. 1974. Transposition of ampicillin resistance from RP4 to other replicons. Mol. Gen. Genet. **132**:31-40.
- Hedges, R. W., and G. A. Jacoby. 1980. Compatibility and molecular properties of plasmid Rms 149 in *Pseudomonas aeruginosa* and *Escherichia coli*. Plasmid **3**:1-6.
- Kratz, J., F. Schmidt, and B. Wiedemann. 1983. Transposition of a gene encoding OXA-2 β -lactamase. J. Gen. Microbiol. **129**:2951-2957.
- Labia, R., J. Andrillon, and F. Le Goffic. 1973. Computerized microacidimetric determination of β -lactamase Michaelis-Menten constants. FEBS Lett. **33**:42-44.
- Labia, R., M. Guionie, M. Barthélmy, and A. Philippon. 1981. Properties of three carbenicillin-hydrolyzing β -lactamases (CARB) from *Pseudomonas aeruginosa*: identification of a new enzyme. J. Antimicrob. Chemother. **7**:49-56.
- Lederberg, E. M., and S. N. Cohen. 1974. Transformation of *Salmonella typhimurium* by plasmid deoxyribonucleic acid. J. Bacteriol. **119**:1072-1074.
- Levesque, R., and P. H. Roy. 1982. Mapping of a plasmid pLQ3 from *Achromobacter* and cloning of its cephalosporinase gene in *Escherichia coli*. Gene **18**:69-75.
- Levesque, R., P. H. Roy, R. Letarte, and J.-C. Pechere. 1982. A plasmid-mediated cephalosporinase from *Achromobacter* species. J. Infect. Dis. **145**:753-761.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
- Matthew, M. 1979. Plasmid-mediated β -lactamases of gram-negative bacteria: properties and distribution. J. Antimicrob. Chemother.

- mother. 5:349-358.
17. Matthew, M., A. M. Harris, M. Marshall, and G. W. Ross. 1975. The use of analytical isoelectric focusing for detection and identification of β -lactamases. *J. Gen. Microbiol.* **88**:169-178.
 18. Matthew, M., and R. W. Hedges. 1976. Analytical isoelectric focusing of R factor-determined β -lactamases: correlation with plasmid compatibility. *J. Bacteriol.* **125**:713-718.
 19. Matthew, M., R. W. Hedges, and J. T. Smith. 1979. Types of β -lactamase determined by plasmids in gram-negative bacteria. *J. Bacteriol.* **138**:657-662.
 20. Medeiros, A. A. 1984. β -lactamases. *Br. Med. Bull.* **40**:18-25.
 21. Medeiros, A. A., R. W. Hedges, and G. A. Jacoby. 1982. Spread of a "*Pseudomonas*-specific" β -lactamase to plasmids of enterobacteria. *J. Bacteriol.* **149**:700-707.
 22. Medeiros, A. A., J. Ximenez, K. Blickstein-Goldworm, T. F. O'Brien, and J. Acar. 1980. β -Lactamases of ampicillin-resistant *Escherichia coli* from Brazil, France, and the United States, p. 761-762. In J. D. Nelson and C. Grassi (ed.), *Current chemotherapy and infectious disease*. American Society for Microbiology, Washington, D.C.
 23. O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler. 1972. Novel method for detection of β -lactamases by using a chromogenic cephalosporin substrate. *Antimicrob. Agents Chemother.* **1**:283-288.
 24. Philippon, A. M., G. C. Paul, and G. A. Jacoby. 1983. Properties of PSE-2 β -lactamase and genetic basis for its production in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **24**:362-369.
 25. Rubin, L. G., R. H. Yolken, A. A. Medeiros, and E. R. Moxon. 1981. Ampicillin treatment failure of apparently β -lactamase negative *Haemophilus influenzae* type b meningitis due to novel β -lactamase. *Lancet* **2**:1008-1010.
 26. Simpson, I. N., S. J. Plested, M. J. Budin-Jones, J. Lees, R. W. Hedges, and G. A. Jacoby. 1983. Characterization of a novel plasmid-mediated β -lactamase and its contribution to β -lactam resistance in *Pseudomonas aeruginosa*. *FEMS Microbiol. Lett.* **19**:23-27.
 27. Sutcliffe, J. G. 1978. Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. *Proc. Natl. Acad. Sci. U.S.A.* **75**:3737-3741.
 28. Takahashi, I., K. Tsukamoto, M. Harada, and T. Sawai. 1983. Carbenicillin-hydrolyzing penicillinases of *Proteus mirabilis* and the PSE-type penicillinases of *Pseudomonas aeruginosa*. *Microbiol. Immunol.* **27**:995-1004.
 29. Vecoli, C., F. E. Prevost, J. J. Ververis, A. A. Medeiros, and G. P. O'Leary, Jr. 1983. Comparison of polyacrylamide and agarose gel thin-layer isoelectric focusing for the characterization of β -lactamases. *Antimicrob. Agents Chemother.* **24**:186-189.
 30. Yamamoto, T., M. Tanaka, C. Nohara, Y. Fukunaga, and S. Yamagishi. 1981. Transposition of the oxacillin-hydrolyzing penicillinase gene. *J. Bacteriol.* **145**:808-813.