Novel Plasmid-Mediated β -Lactamase in Members of the Family *Enterobacteriaceae* from Ohio

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Epidemiologic studies of plasmid-mediated resistance at the Cleveland Veterans Administration Medical Center revealed that related plasmids had disseminated among members of the family *Enterobacteriaceae*. We studied the β -lactamases encoded by these plasmids in *Escherichia coli* C600 transformants or transconjugants. Substrate and inhibition profiles of the enzymes determined by two of these plasmids suggested an activity resembling TEM-1; however, isoelectric focusing revealed a pI of 7.0. These two plasmids were originally found in a *Serratia marcescens* (pDS076) and an *Enterobacter cloacae* (pDS075) strain isolated from the same sink in the medical intensive care unit and later, in an *Enterobacter cloacae* (pDS142 identical to pDS076) isolate colonizing a patient in the same unit. The plasmids also carried the aminoglycoside resistance determinant, 2'-aminoglycoside nucleotidyl transferase. A 2-kilobase *Ava*I restriction endonuclease digestion fragment of pDS075 known to carry the β -lactamase determinant was used as a molecular probe. This probe did not recognize sequences of any plasmid-mediated β -lactamase tested including the recently described determinants ROB-1, TLE-1, and OXA-4-7. A TEM-1 probe derived from the 0.7-kilobase *Pst*I-*Eco*RI fragment of pBR322 failed to recognize the new β -lactamase gene. Four additional *Enterobacter cloacae* and two *Enterobacter aerogenes* strains isolated in Columbus, Ohio, have been shown to produce a pI 7.0 β -lactamase and to carry plasmids recognized by the 2-kilobase probe. These data suggest dissemination of a novel plasmid-mediated β -lactamase among members of the family *Enterobacteriaceae* in Ohio and demonstrate the development and utility of a molecular probe for the new determinant. We suggest that the novel β -lactamase be named OHIO-1.

A number of plasmid-mediated β -lactamases have been described, with most occurring in members of the family *Enterobacteriaceae* or *Pseudomonas aeruginosa* (1, 5, 6, 8, 9, 16, 17, 24, 25; A. A. Medeiros and G. A. Jacoby, in S. W. Queener, S. F. Queener, and J. A. Webber, ed., *Beta-Lactam Antibiotics for Clinical Use*, in press). We have been examining gentamicin resistance plasmids of members of the family *Enterobacteriaceae* through epidemiologic studies of the spinal cord injury unit and the medical intensive care unit at the Cleveland Veterans Administration Medical Center (22). All such plasmids characterized so far carry a 2'-aminoglycoside nucleotidyl transferase and a β -lactamase determinant (D. M. Shlaes and D. Bobey, unpublished observations). To further characterize these plasmids, we studied their associated β -lactamases in *Escherichia coli* C600 transformants or transconjugants. Our results suggest that a novel β -lactamase determinant which we have designated OHIO-1 has disseminated among some of these plasmids.

(A preliminary report of this work was presented [D. M. Shlaes, A. Medeiros, C. Currie-McCumber, and C. V. Vartian, Program Abstr. 24th Intersci. Conf. Antimicrobial Agents Chemother. abstr. no. 993, 1984].)

MATERIALS AND METHODS

Bacterial strains used. Standard plasmids in *Escherichia coli* or *Pseudomonas aeruginosa* hosts were used for the following plasmid-mediated β -lactamases: TEM-1, TEM-2, HMS-1, SHV-1, OXA-1-3, and PSE-1-3 (1, 3, 4, 6, 8-10, 14,

20, 24, 25). PU21 PMG19 carried PSE-4 on the chromosome (9; G. Jacoby, personal communication). Plasmids bearing recently described novel β -lactamases were also studied, including OXA-4-7 and TLE-1 (17). *Haemophilus influenzae* carrying a plasmid encoding the ROB-1 β -lactamase was used (19).

Enterobacter cloacae 01020, 12101, 20229, and 20218 and *Enterobacter aerogenes* 01014 and 20220 were obtained during a survey of resistance to extended-spectrum cephalosporins carried out by the Schering Corp. For this survey, isolates from hospitals across the United States were solicited. Participants were asked to avoid duplication of submitted strains. All of these *Enterobacter* strains were isolated from University Hospitals in Columbus, Ohio, and all also produced 2'-aminoglycoside nucleotidyl transferase.

Plasmids pDS075 and pDS076 were isolated from environmental *Enterobacter cloacae* and *Serratia marcescens*, respectively, during an epidemiologic study of aminoglycoside-resistant gram-negative bacilli in an intensive care unit (22). *Escherichia coli* C600 was used as a plasmid host for conjugation, transformation, and cloning as previously described (11, 21).

β -Lactamase preparations. Sonic extracts were prepared as described previously (26).

β -Lactamase assays. Enzyme activity was assayed by monitoring the rate of β -lactam hydrolysis under conditions of constant pH with an automated titrator system. Assays were conducted according to the method of Labia et al. (10) with the following modifications. The titrant was 0.005 N NaOH and the reaction mixture contained 0.5 mM substrate and 85 mM NaCl. All readings were compared with control reaction mixtures where no enzyme was added.

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TABLE 1. Substrate profile of the OHIO-1 β -lactamase relative to that of the TEM-1 enzyme

β -Lactamase type	Relative rate of hydrolysis ^a								
	Ampicillin	Carbenicillin	Cloxacillin	Methicillin	Oxacillin	Cephalothin	Cephaloridine	Cefamandole	Cefoperazone
OHIO-1	140 $\pm$ 14	11 $\pm$ 2	<0.5	<0.5	<0.5	8 $\pm$ 1	79 $\pm$ 8	15 $\pm$ 2	35 $\pm$ 2
TEM-1	92 $\pm$ 3	10 $\pm$ 1	<0.5	<0.5	4.3 $\pm$ 1	15 $\pm$ 2	79 $\pm$ 7	44 $\pm$ 6	39 $\pm$ 3

^a Activity relative to that with penicillin G (set at 100). Each number represents the average $\pm$ standard deviation of three to six determinations.

A unit of activity is defined as that amount of β -lactamase which will hydrolyze 1.0 μ mol of potassium penicillin G in 1 min at 37°C and pH 7.0. Protein was determined by using the Bio-Rad assay procedure (Bio-Rad Chemicals, Richmond, Calif.).

Inhibition of β -lactamase activity. Enzymes were preincubated at 37°C for 5 min with each inhibitor at the indicated concentrations (Table 1) and then assayed with nitrocefin according to the method of O'Callaghan et al. (18). Absorbance was monitored continuously at 482 nm, utilizing a Hitachi model 100-60 double-beam spectrophotometer.

Analytical isoelectric focusing. Isoelectric focusing was performed on polyacrylamide gels by a modification of the method of Matthew et al. (13) as described previously (26).

Agarose gel electrophoresis of plasmid DNA. Plasmid DNA was extracted by using an alkaline lysis procedure (21). Lysates were subjected to electrophoresis through 0.7% agarose gels with 0.5 μ g of ethidium bromide added per ml, using an 89 mM Tris-borate buffer at 25 to 50 V overnight. Plasmid bands were visualized after restaining with 1 μ g of ethidium bromide per ml in water and destaining in water followed by UV transillumination. Polaroid photographs were taken with a no. 8 filter as previously described (21). Molecular weights were calculated from comparison of plasmid bands and control plasmids of known molecular weight from 4.1 to 93 kilobases (kb). Molecular weights of restriction digestion fragments were calculated by comparison with control fragments ranging from 0.1 to 23 kb.

Restriction endonuclease digestion and cloning. Plasmid DNA was extracted by using a scaled-up cleared lysate procedure and purified on CsCl-ethidium bromide density equilibrium gradients as previously described (21). Digestions were carried out with enzymes obtained from Bethesda Research Laboratories (Gaithersburg, Md.) according to the manufacturer's instructions. Cloning was carried out as described previously (11).

Construction of β -lactamase probes and Southern blotting. A 2-kb *Ava*I fragment of pDS075 known to contain the OHIO-1 determinant was used as a molecular probe. The 0.7-kb *Pst*I-*Eco*RI double-digestion fragment of pBR322 carrying the majority of the TEM-1 determinant was used as a probe for the TEM-1 gene (2, 24). Probe fragment DNA was isolated by electroelution of the appropriate digestion fragment from 0.7% agarose gels and purified over NACS-52 Prepac columns (Bethesda Research Laboratories) (11).

Southern blots were prepared from 0.7% agarose gels of cleared lysates or restriction endonuclease digests of purified plasmid preparations obtained from *Escherichia coli* C600 transformants or transconjugants (23). The gels were denatured with 0.2 N NaOH–0.6 M NaCl with gentle agitation at room temperature for 30 min. The gels were then washed in 0.025 M Na₂HPO₄/NaH₂PO₄ (pH 6.5) at room temperature for 1 h with three changes of buffer. Gene Screen membranes (New England Nuclear Corp., Boston, Mass.) were cut to the exact size of the gels and placed in the phosphate buffer

to soak for 15 to 20 min. Capillary blotting was performed as described by Southern (23). Prehybridization of the blots was carried out with 4.8 ml of double-distilled H₂O and 0.2 ml of sheared salmon sperm DNA (10 mg/ml). This solution was then heated at 100°C for 7 min and cooled on ice, and a solution containing 4 ml of 10 $\times$ PIPES (piperazine-*N,N'*-bis[2-ethanesulfonic acid]; Sigma Chemical Co., St. Louis, Mo.) buffer (1 $\times$ PIPES buffer = 0.01 M PIPES, 0.4 M NaCl, 1 mM EDTA, pH 6.4), 10 ml of formamide (final concentration, 50%), and 1 ml of 10% sodium dodecyl sulfate was added. Prehybridization was carried out at 37°C for 2 to 4 h. Probe DNA was prepared by using a 100-ng DNA fragment per hybridization. Nick translation was carried out with a commercial kit (Bethesda Research Laboratories) with a total reaction volume of 17.5 μ l. [³²P]CTP was obtained at a specific activity of >3,000 Ci/mmol from either New England Nuclear or Amersham Corp. (Arlington Heights, Ill.). [³²P]DNA was purified with spun columns (11). The probe was then denatured and added to the plastic bags with filters in prehybridization mix and incubated at 37°C overnight. After hybridization, the filters were washed as follows: three times for 20 min each at room temperature in 2 $\times$ SSC (1 $\times$ SSC = 0.15 M NaCl plus 0.015 M sodium citrate) plus 0.1% sodium dodecyl sulfate followed by once for 30 min at 65°C in 1 $\times$ SSC plus 0.1% sodium dodecyl sulfate followed by two times for 30 min each at 65°C in 0.2 $\times$ SSC plus sodium dodecyl sulfate. The filters were then air dried and developed for various periods of time, using Kodak XRP film and two intensifying screens at –70°C.

RESULTS

Characterization of pDS075 and pDS076. *Enterobacter cloacae* 075 was isolated from a sink during an epidemiologic survey of the Cleveland Veterans Administration Medical Center medical intensive care unit. The strain contained two plasmids of 60 and 12.3 kb. *Escherichia coli* C600 transformants were selected on gentamicin-containing agar, and all contained the 12.3-kb plasmid pDS075. These transformants were also shown to produce a β -lactamase by nitrocefin testing. The parent strain, 075, did not transfer gentamicin resistance by filter mating using *Escherichia coli* C600 resistant to nalidixic acid and rifampin as a recipient in matings performed at 37 or 30°C. Similar experiments performed with the transformant as the donor also yielded no transconjugants. This system detects mating frequencies as low as 10^{–7}. *S. marcescens* strain 076 was isolated at the same time from the same sink. It carried a single 60-kb plasmid which transferred gentamicin resistance at a frequency of about 10^{–7}, using the same filter-mating technique. These transconjugants also produced β -lactamase.

Characterization of plasmid-mediated β -lactamases. Isoelectric focusing of sonic extracts in polyacrylamide gels showed that the wild-type isolates of *Enterobacter cloacae* isolated from University Hospitals of Columbus, Ohio,

FIG. 1. Isoelectric focusing gel showing band patterns of β -lactamases from wild-type isolates of *Enterobacter cloacae* and an *Escherichia coli* C600 transformant containing the pDS075 plasmid producing OHIO-1 β -lactamase. (Lanes A and F) A control strain of *Escherichia coli* K-12 producing TEM-1 (pI 5.4), OXA-2 (pI 7.7), and chromosomal β -lactamase (pI 8.0). (Lanes B to D) *Enterobacter cloacae* clinical isolates 20229, 12101, and 01020 producing OHIO-1 (pI 7.0) and hyperproducing chromosomal β -lactamase (pI 8.0). (Lane E) *Escherichia coli* C600(pDS075) producing OHIO-1 β -lactamase (pI 7.0).

produced two β -lactamases which appeared as dense bands at pIs of 8.0 and 7.0 (Fig. 1). pDS075 in *Escherichia coli* C600 produced only the pI 7.0 enzyme. In addition to the main band of pI 7.0, a faint satellite was seen at pI 6.0.

The sonic extract prepared from *Escherichia coli* C600 carrying pDS075 had moderate β -lactamase activity, i.e., 9.0 μ mol of penicillin G hydrolyzed/min per mg of protein. The pDS075 β -lactamase had broad-spectrum activity against both penicillins and cephalosporins, similar to TEM-1. However, it hydrolyzed ampicillin relatively more rapidly than TEM-1 and cefamandole, oxacillin, and cephalothin less rapidly (Table 1). In comparison with TEM-1, the pDS075 enzyme was less susceptible to inhibition by aztreonam, moxalactam, and imipenem and more susceptible to inhibition by SCH 34343. Neither β -lactamase was inhibited by 100 mM NaCl (Table 2). The apparent molecular weight of the pI 7.0 β -lactamase, determined by high-performance

liquid chromatography, was 22,000. (Molecular weight was determined by M. Cohenford, using a Waters I-125 protein analysis column [1.25 by 60 cm] which was equilibrated and eluted with 50 mM sodium phosphate buffer, pH 7, containing 0.02% sodium azide. The column was standardized with proteins ranging in size from 13,700 to 67,000 daltons. Void volume was determined with dextran blue 2,000; Cohenford, personal communication.)

A 2-kb *Ava*I fragment of pDS075 contains the β -lactamase gene. Upon digestion with *Hind*III, pDS075 yielded two fragments of 4.2 and 8.1 kb. In vitro deletion of the 4.2-kb fragment left an 8.1-kb plasmid bearing both the 2'-aminoglycoside nucleotidyl transferase and β -lactamase genes. *Ava*I deletion of the 8.1-kb derivative yielded a 5-kb plasmid bearing only the β -lactamase gene. Digestion of the latter derivative with *Ava*I revealed two fragments of 2 and 3 kb, respectively. Shotgun cloning of these fragments into the *Ava*I sites of pMK20 revealed that the 2-kb fragment contained the β -lactamase gene. The 2-kb *Ava*I fragment derived from the 8.1-kb *Hind*III deletion derivative of pDS075 was used subsequently as a DNA probe for this β -lactamase gene.

The OHIO-1 determinant is genetically unrelated to other plasmid β -lactamase genes. For these experiments, the 2-kb *Ava*I fragment containing the novel β -lactamase gene was electroeluted from 0.7% agarose gels. Purified fragment was nick translated and used to probe Southern blots of cleared lysates and colony blots of bacterial strains containing known plasmid-determined β -lactamase genes. Duplicate agarose gels of cleared lysates of strains bearing plasmids mediating synthesis of β -lactamase enzymes were blotted onto filters. The filters were probed under stringent conditions with either the 2-kb fragment carrying the novel β -lactamase determinant or a pBR322 fragment bearing TEM-1 sequences. Selected blots are shown in Fig. 2. These data show that the TEM-1 probe does not recognize pDS075 bearing the OHIO-1 determinant but does hybridize with TEM-1 (pBR322)- and TEM-2 (RP1)-producing plasmids. Also, the 2-kb OHIO-1-bearing fragment hybridizes with itself, but not with TEM-1- or TEM-2-bearing plasmids. Similar blots were prepared with strains carrying plasmid-mediated β -lactamases OXA-1-7, PSE-1-3, ROB-1, TLE-1, SHV-1, and HMS-1. PU21 PMG19 bearing the chromosomal marker PSE-4 was also used. The 2-kb β -lactamase-bearing fragment did not hybridize with any of these blots (not shown). The TEM-1-bearing fragment showed only slight hybridization with the OXA-2 plasmid used (not shown), a result noted previously (2).

Novel β -lactamase determinants outside Cleveland Veterans Administration Medical Center. Four strains of *Enterobacter cloacae* and two of *Enterobacter aerogenes* produced pI 7.0 β -lactamases. All strains also determined 2'-aminoglycoside nucleotidyl transferase and *Enterobacter aerogenes* strain 20220 also determined TEM-1. These β -lactamases were detected during a nationwide survey of members of the

TABLE 2. Inhibition of the OHIO-1 β -lactamase relative to that of the TEM-1 enzyme

β -Lactamase type	Inhibition by ^a :							
	NaCl (100 mM)	Cloxacillin (13 mM)	Clavulanate (0.006 mM)	Sulbactam (0.075 mM)	Moxalactam (0.375 mM)	SCH 34343 (0.375 mM)	Imipenem (8.5 mM)	Aztreonam (1.15 mM)
OHIO-1	3	56 $\pm$ 6	84 $\pm$ 4	54 $\pm$ 7	31 $\pm$ 5	56 $\pm$ 4	57 $\pm$ 2	19 $\pm$ 5
TEM-1	4	63 $\pm$ 4	83 $\pm$ 1	54 $\pm$ 3	50 $\pm$ 3	37 $\pm$ 3	76 $\pm$ 4	75 $\pm$ 6

^a Expressed as 100 minus the percentage of activity against nitrocefin at the indicated concentrations of inhibitor. Each number represents an average $\pm$ 1 standard deviation of two to five measurements.

family *Enterobacteriaceae* resistant to extended-spectrum cephalosporins. The six strains that produced the pI 7.0 β -lactamase had been submitted by University Hospitals of Columbus, Ohio. Cleared lysates of these strains were electrophoresed through 0.7% agarose gels and Southern blots were prepared. The 2-kb pDS075 and 0.7-kb pBR322 TEM-1 probes were used. Figure 3 shows that the 57-kb plasmid in *Enterobacter aerogenes* 20220 shares homology with the 2-kb β -lactamase gene probe. In addition, the same strain carried a 4.1-kb plasmid sharing homology with the TEM-1 gene probe. In this strain, the TEM-1 probe did not hybridize to the 57-kb plasmid and the 2-kb OHIO-1-bearing fragment did not hybridize to the 4.1-kb plasmid. The other five *Enterobacter* strains all carried plasmids that hybridized with the 2-kb probe (not shown). The 0.7-kb TEM-1 probe did not hybridize to any DNA in these five strains (not shown). These data suggest that this novel β -lactamase determinant is not limited to the Cleveland Veterans Administration Medical Center. Our choice of the name "OHIO-1" is based on our current understanding of the geographic distribution of this gene and on its lack of genetic similarity to other plasmid β -lactamase genes, rather than on its substrate profile, which resembles TEM-like enzymes.

DISCUSSION

Over 20 plasmid-mediated β -lactamases have been described prior to this report (1, 5, 6, 8, 9, 16, 17, 24, 25; Medeiros and Jacoby, in press). Seven of these enzymes are TEM-like in that they have a substrate profile characterized by more rapid hydrolysis of penicillin, ampicillin, and cephaloridine than oxacillin and carbenicillin and are inhibited by cloxacillin but not NaCl (16). They range in molecular weight from 19,800 to 44,000 and in pI from 5.4 to 8.1. OHIO-1 has properties similar to those of this group of β -lactamases but can readily be distinguished from the other TEM-like enzymes by isoelectric focusing. However, it

FIG. 2. Southern hybridization of TEM-1 and OHIO-1 gene probes to plasmids mediating TEM-1, TEM-2, or OHIO-1 β -lactamase. Agarose (0.7%) gel electrophoresis of cleared lysates of plasmid-bearing strains was performed and blotted to filters. The plasmids used were pBR322 (TEM-1), RP1 (TEM-2), and pDS075 (OHIO-1) in *Escherichia coli* C600. For each panel, the left lane shows the results of Southern hybridization when the OHIO-1 probe is used under stringent conditions. The right lane shows the results of Southern hybridization with the TEM-1 gene probe under the same conditions.

FIG. 3. Southern hybridization of *Enterobacter aerogenes* 20220 producing TEM-1 and OHIO-1 β -lactamases. The left lane is a blot of an agarose gel electrophoresis of a cleared lysate of strain 20220 probed with the 2-kb OHIO-1 gene probe from pDS075. The right lane is a similar blot from a separate experiment probed with the pBR322 0.7-kb TEM-1 gene probe. The molecular masses of the relevant plasmid bands are indicated. l.f., Linear fragments.

differs by only 0.1 pH unit from PSE-3 (pI 6.9). Consequently, identification based solely on pI may be misleading.

In addition, our work indicates the structural gene for OHIO-1 is different enough from other β -lactamase genes to allow differentiation by Southern hybridization under stringent conditions. Although the OHIO-1 β -lactamase resembles TEM-1 in its substrate profile and inhibition profile, a probe containing the structural gene for OHIO-1 did not hybridize to TEM-containing plasmids and a small TEM-1 gene probe did not hybridize with OHIO-1-containing plasmids, indicating a more distant evolutionary relationship. The OHIO-1 gene probe we used also did not hybridize to plasmids encoding a wide variety of other β -lactamases.

We believe that the OHIO-1 determinant is not uncommon and appears to be present in approximately 5% of multiply resistant *Serratia* spp. isolated at the Cleveland Veterans Administration clinical microbiology laboratory (Shlaes and Medeiros, unpublished observations). It is also of interest that the gene occurred on both self-transferrable (e.g., pDS076) and nonconjugative (e.g., pDS075) plasmids, and it has always been associated with gentamicin resistance mediated by 2'-aminoglycoside nucleotidyl transferase even in strains that were not isolated selecting for gentamicin resistance. So far, these plasmids have been found disseminated among *S. marcescens*, *S. liquefaciens*, *Enterobacter cloacae*, and *Enterobacter aerogenes*. This new enzyme provides an additional epidemiologic marker for following spread of antibiotic resistance.

In summary, we have described a novel plasmid-mediated β -lactamase disseminated among *Serratia* spp. and *Enterobacter* spp. from two hospitals in Ohio. The enzyme has a broad-spectrum substrate profile resembling TEM-1. However, the new enzyme, called OHIO-1, can be distinguished from other plasmid-mediated β -lactamases, including

TEM-1 and several recently described novel enzymes (16), on the basis of pI, substrate, and inhibition profiling and by Southern hybridization with gene probes.

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LITERATURE CITED

- Bobrowski, M. M., M. Matthew, P. T. Barth, N. Datta, N. J. Grinter, A. E. Jacob, P. Kontomichalou, J. W. Dale, and J. T. Smith. 1976. Plasmid-determined β -lactamase indistinguishable from the chromosomal β -lactamase of *Escherichia coli*. *J. Bacteriol.* **125**:149–157.
- Cooksey, R. C., N. E. Clark, and C. Thornsberry. 1985. A gene probe for TEM type β -lactamases. *Antimicrob. Agents Chemother.* **28**:154–156.
- Dale, J. W., and J. T. Smith. 1974. R-factor-mediated β -lactamases that hydrolyze oxacillin: evidence for two distinct groups. *J. Bacteriol.* **119**:3515–3516.
- Egawa, R., T. Sawai, and S. Mitsuhashi. 1967. Drug resistance of enteric bacteria. XII. Unique substrate specificity of penicillinase produced by R-factor. *Jpn. J. Microbiol.* **11**:173–178.
- Eliasson, I., and C. Kamme. 1985. Characterization of the plasmid-mediated β -lactamase in *Branhamella catarrhalis*. *J. Antimicrob. Chemother.* **15**:139–149.
- Furth, A. 1975. Purification and properties of a constitutive beta-lactamase from *Pseudomonas aeruginosa* strain Dagleish. *Biochim. Biophys. Acta* **377**:431–443.
- Hedges, R. W., N. Datta, P. Kontomichalou, and J. T. Smith. 1974. Molecular specificities of R-factor-determined beta-lactamases: correlation with plasmid compatibility. *J. Bacteriol.* **117**:56–62.
- Hedges, R. W., A. A. Medeiros, M. Cohenford, and G. A. Jacoby. 1985. Genetic and biochemical properties of AER-1, a novel carbenicillin-hydrolyzing β -lactamase from *Aeromonas hydrophila*. *Antimicrob. Agents Chemother.* **27**:479–484.
- Jacoby, G. A., and M. Matthew. 1979. The distribution of β -lactamase genes on plasmids found in *Pseudomonas*. *Plasmid* **2**:41–47.
- Labia, R., J. Andrillon, and F. Le Goffic. 1973. Computerized microacidometric determination of beta-lactamase Michaelis-Menten constants. *FEBS Lett.* **33**:42–44.
- Maniatis, T., E. F. Fritsch, and J. Sambrook (ed.). 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Matthew, M. 1978. Properties of the beta-lactamase specified by the pseudomonas plasmid R151. *FEMS Microbiol. Lett.* **4**:241–244.
- Matthew, M., A. M. Harris, M. Marshall, and G. W. Ross. 1975. The use of analytical isoelectric focusing for detection and identification of beta-lactamases. *J. Gen. Microbiol.* **88**:169–178.
- Matthew, M., and R. B. Sykes. 1977. Properties of the beta-lactamase specified by the *Pseudomonas* plasmid RPL11. *J. Bacteriol.* **132**:341–345.
- Matthew, M. R., R. W. Hedges, and J. T. Smith. 1979. Types of β -lactamase determined by plasmids in gram-negative bacteria. *J. Bacteriol.* **138**:657–662.
- Medeiros, A. A. 1984. β -Lactamases. *Br. Med. Bull.* **40**:18–25.
- Medeiros, A. A., M. Cohenford, and G. A. Jacoby. 1985. Five novel plasmid-determined β -lactamases. *Antimicrob. Agents Chemother.* **27**:715–719.
- O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler. 1972. Novel method for detection of beta-lactamase by using a chromogenic cephalosporin substrate. *Antimicrob. Agents Chemother.* **1**:283–288.
- Rubin, L. G., A. A. Medeiros, R. H. Yolken, and E. R. Moxon. 1981. Ampicillin treatment failure of apparently beta-lactamase negative *Hemophilus influenzae* type b meningitis due to novel beta-lactamase. *Lancet* **ii**:1008–1010.
- Sawada, Y., S. Yaginuman, M. Tai, S. Iyobe, and S. Mitsuhashi. 1974. Resistance of beta-lactam antibiotics in *Pseudomonas aeruginosa*, p. 391–397. In S. Mitsuhashi and H. Hashimoto (ed.), *Microbial drug resistance*. University of Tokyo Press, Tokyo.
- Shlaes, D. M., and C. A. Currie. 1983. Gentamicin resistance R factors on a spinal cord injury unit. *J. Clin. Microbiol.* **18**:236–241.
- Shlaes, D. M., C. Vartian, and C. A. Currie. 1983. Variability in DNA sequence of closely related nosocomial gentamicin-resistance plasmids. *J. Infect. Dis.* **148**:1013–1018.
- Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503–517.
- Sutcliffe, J. G. 1978. Nucleotide sequence of the ampicillin resistance gene of *E. coli* plasmid pBR322. *Proc. Natl. Acad. Sci. USA* **75**:3737–3741.
- 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.
- 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 beta-lactamase. *Antimicrob. Agents Chemother.* **2**:186–189.