JOURNAL OF BACTERIOLOGY, Feb. 1982, p. 700–707
0021-9193/82/020700-08$02.00/0

Vol. 149, No. 2

Spread of a "*Pseudomonas*-Specific" β-Lactamase to Plasmids of Enterobacteria

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Eleven isolates including *Escherichia coli*, *Salmonella enteritidis*, and *Shigella sonnei*, obtained in Brazil, Hong Kong, Indonesia, Thailand, and the United States, were found to produce β-lactamase of the PSE-1 type, which was previously considered to be *Pseudomonas* specific. The enterobacterial strains produced a β-lactamase with the same isoelectric point, immunological reactions, and substrate profile as those of the prototype PSE-1 enzyme determined by *Pseudomonas* plasmid RPL11. The producer strains were resistant to multiple antibiotics, and all contained plasmids, ranging in size from 37×10^6 to 130×10^6, that belonged to at least six incompatibility groups. Plasmids of IncH2 and IncFI*me* were shown to contain 8×10^6-molecular-weight transposons Tn*1401* and Tn*1402* that encoded PSE-1 β-lactamase production, resistance to streptomycin and spectinomycin via AAD(3″), and resistance to sulfonamide. PSE-1 β-lactamase was not *Pseudomonas* specific and appeared to have spread among plasmids found in enterobacteria by transposition.

The β-lactamases of gram-negative bacteria may be determined either by plasmid or chromosomal genes (34). Of the former, 11 types have been identified, based mainly on extensive studies of strains of gram-negative bacteria isolated in Europe, Japan, and North America (21). Among these 11 types, 4 were found only in strains of *Pseudomonas aeruginosa* and were termed PSE for *Pseudomonas*-specific enzymes (10). The *Pseudomonas* plasmids specifying PSE β-lactamases either lack the ability to transfer to enterobacteria by conjugation or are transfer deficient (Tra^-) (15).

The genes responsible for PSE β-lactamase production can, however, be transmitted to *Escherichia coli* by mobilization or recombinant plasmid formation (10, 14). In an *E. coli* host, PSE β-lactamase genes function efficiently and confer high levels of resistance to ampicillin, carbenicillin, and other β-lactam antibiotics.

One of the PSE enzymes, PSE-1 has previously been found only in *P. aeruginosa* and is determined by high-molecular-weight plasmids of the IncP-2 group that fail to transfer to *E. coli* or other enterobacteria by conjugation (12, 31). In this paper, we report the finding of PSE-1 β-lactamase production in 11 naturally occurring isolates or enteric bacteria, including six strains of *E. coli*, four strains of *Salmonella*, and one strain of *Shigella*; the isolates were obtained from the United States, Brazil, Thailand, and the Far East. In nine strains, PSE-1 production was definitely determined by plasmids that belonged to at least six different incompatibility (Inc) groups. The production of PSE-1 by such a variety of plasmid types implied that PSE-1 might be determined by a transposable genetic element. Transposons of about 8×10^6 molecular weight that determine PSE-1 β-lactamase and resistance to streptomycin, spectinomycin, and sulfonamide were detected on two plasmids belonging to different Inc groups.

MATERIALS AND METHODS

Bacterial strains. Clinical isolates of enterobacteria were all known to be resistant to ampicillin. *E. coli* (371 strains) was isolated in hospitals in six countries: United States (Miriam Hospital, Providence, R.I.; Brigham and Women's Hospital, Boston, Mass.); France (Hôpital St. Joseph, Paris); Indonesia (Central General Hospital, Bandung, Java); Thailand (Ramathibodi Hospital, Bangkok); South Africa (Baragwanath Hospital, Johannesburg); and Brazil (hospitals and private laboratories in 14 cities). All of the *Salmonella* isolates were from the United States. Human isolates (113 strains) came from the Massachusetts State Laboratory, Boston, and animal isolates (150 strains) came from the U.S. Department of Agriculture Veterinary Services Laboratory, Ames, Iowa. A single strain of *Shigella sonnei* was isolated in Canada from a patient who acquired it in Brazil (35). Recent clinical isolates from Providence or Boston represented other genera.

E. coli K-12 F^- derivatives J53-2 (*met pro rif*) and J62-2 (*his lac pro trp rif*) (3) and *P. aeruginosa* PAO FP^- derivative PU21 (*ilv leu rifA strA*) (12) were used as recipients in mating experiments.

Bacteriophage. Phage PR4 was used to test for the presence of IncP group plasmids and to select Tra⁻ IncP plasmid derivatives (33).

Plasmids. Reference plasmids included R6K [Ap-(TEM-1) Sm IncX] (17); pUZ8 (Km Tc Hg IncP) (10); RPL11 [Cb(PSE-1) Cm Gm Sm Su Tc Hg Pma Ter IncP-2] (19); R388 (Su Tp IncW) (4); R751 (Tp IncP) (16); R751-RPL11 [Cb(PSE-1) Cm Gm Sm Su Tc Tp Hg IncP], prepared as described in Jacoby et al. (14); and *Flac* (*lac* IncFI) (11).

Plasmid transfer and incompatibility testing. Techniques for plasmid transfer and incompatibility testing have been described previously (3, 5, 10, 12).

Selection of antibiotic-susceptible segregants. Spontaneous antibiotic-susceptible segregants were obtained by replica plating.

Agarose gel electrophoresis. Plasmid purification the size determination were by agarose gel electrophoresis, performed by several techniques (2, 5, 7, 25).

β-lactamase preparation. Sonic extracts were prepared as described previously (24). We dialyzed and concentrated preparations with low β-lactamase activity with a Micro-ProDiCon apparatus (Bio-Molecular Dynamics, Beaverton, Oreg.), using membranes with a 10,000-molecular-weight cutoff.

β-lactamase assay. Assays of β-lactamase activity were performed by a modification (28) of the iodometric method (26). Inhibition of β-lactamase by cloxacillin were tested spectrophotometrically (24).

Preparative isoelectric focusing. In strains producing two β-lactamases, a single-step separation of the enzyme activities was attempted by isoelectric focusing in a flat bed of granulated gel and subsequent elution of the enzymes from gel fractions showing discrete bands. The gel slurry was prepared by adding 4 g of prewashed Sephadex G-75 to a solution containing 4.5 ml of ampholyte pH 4 to 6 (40%, wt/vol), 0.5 ml of ampholyte pH 3.5 to 10, 10 ml of sonic extract, and 85 ml of distilled water. Electrofocusing was carried out for 14 h at a constant power setting of 8 W, maximal 1,200 V, using an LKB Multiphor apparatus (LKB-Produkter AB, Bromma, Sweden).

Analytical isoelectric focusing. Isoelectric focusing was performed on polyacrylamide gels as described by Matthew et al. (22), except that acrylamide was used at a concentration of 7.5% and the current was applied at a constant power of 1 W, maximal 320 V, for 15 h and 1 W, maximal 400 V, for 1 h, using an LKB 2103 power supply.

AAD(3″) assay. Nucleotidytransferase activity was assayed by the phosphocellulose paper-binding assay, using [^{14}C]ATP as the cofactor (8).

RESULTS

Frequency of PSE-1 β-lactamase production. Over 600 ampicillin-resistant strains of *E. coli* and *Salmonella* isolated between 1973 and 1980 were examined by isoelectric focusing to determine the type of β-lactamase produced. Among 371 isolates of *E. coli*, 4 from Brazil and 1 each from Indonesia and Thailand produced PSE-1 (Table 1). Among 263 isolates of *Salmonella* from human and animal sources, PSE-1 β-lactamase was produced by three strains belonging to *Salmonella enteritidis* serotypes: *Salmonella heidelberg*, *Salmonella newport*, and *Salmonella typhimurium*. These strains were all human isolates. Selected hospital isolates of other enteric species from the United States were also examined. PSE-1 β-lactamase production was not determined by 68 *Klebsiella*, 7 *Proteus mirabilis*, and 3 *Serratia* strains.

In addition to these clinical isolates, two plasmids originating in enterobacteria were found to produce PSE-1. One plasmid came from a strain of *Salmonella johannesburg* isolated in Hong Kong, and the other originated in a strain of *Shigella sonnei* isolated in Canada in 1978 but acquired in Brazil (35).

Of the 11 PSE-1–producing strains, 6 produced PSE-1 alone and 5 produced both PSE-1 and TEM-1 β-lactamase. By agarose gel electrophoresis, plasmids of various sizes were detected in every PSE-1–producing strain. In mating experiments, PSE-1 production was shown to be plasmid determined in nine strains, and two isolates, although resistant to multiple antibiotics, failed to transfer β-lactam antibiotic resistance.

Characterization of β-lactamases. Figure 1 shows examples of the isoelectric focusing patterns of isolates producing PSE-1 β-lactamase either alone or in combination with TEM-1. PSE-1 β-lactamase focused as a main band at pI ~5.7, with two accessory bands sometimes visible around pI 5.6 and 5.3. In contrast, the main band of TEM-1 β-lactamase appeared at pI 5.4, with several accessory bands above and below. Differential isoelectric focusing (1) confirmed that the mobility in polyacrylamide gels of PSE-1 produced by *E. coli* was identical to that of the prototype PSE-1 enzyme mediated by *Pseudomonas* plasmid RPL11.

Table 2 shows the substrate profiles of the β-lactamases from natural isolates of *E. coli* and *Salmonella* and from *E. coli* J53-2 and J62-2 transconjugants containing plasmids from these isolates (the plasmids are described more fully below and in Table 3). Substrate profiles for the β-lactamases produced by control strains of *E. coli* producing PSE-1 or TEM-1 are also shown in Table 2. A high rate of carbenicillin hydrolysis and a low rate of cephaloridine hydrolysis distinguished PSE-1 from TEM-1 β-lactamase. Transconjugants containing plasmids from *E. coli* strains 7535 and Ind22 produced β-lactamases with profiles similar to those of the β-lactamases of the original isolates and that of the β-lactamase of *E. coli* carrying the PSE-1 gene from RPL11. The activity of TEM-1 but not PSE-1 β-lactamase was inhibited by 10^{-4} M cloxacillin (21) (Table 2).

E. coli strains 7010 and 7047 and *Salmonella heidelberg* strain 1707, all three of which produced both TEM-1 and PSE-1 β-lactamase as

TABLE 1. Frequency of PSE-1 β-lactamase among clinical isolates of ampicillin-resistant *E. coli* and *Salmonella* from different countries

Isolate	Origin	No. tested	No. producing PSE-1 β-lactamase
Escherichia coli	United States	160	0
	France	21	0
	Brazil	98	4
	Indonesia	21	1
	Thailand	29	1
	South Africa	42	0
Salmonella heidelberg	United States	23	1
Salmonella newport	United States	8	1
Salmonella typhimurium	United States	94	1
Salmonella, other serotypes	United States	138	0

determined by isoelectric focusing, hydrolyzed carbenicillin and cephaloridine at rates intermediate to those of TEM-1 and PSE-1. Partial separation of PSE-1 from TEM-1 β-lactamase was achieved in these strains by elution of enzyme activity from preparative isoelectric gels. The gel-purified activity resembled PSE-1 in substrate profile, particularly in its low activity with cephaloridine, but had an intermediate degree of cloxacillin inhibition, presumably reflecting incomplete separation from TEM-1.

The β-lactamase activity of *E. coli* 7535 was inhibited by an antiserum that inhibits PSE-1 β-lactamase from *Pseudomonas* spp. but not TEM-1 β-lactamase (G. Paul, personal communication).

Properties of plasmids determining PSE-1. Table 3 details the properties of plasmids found in the PSE-1–producing strains. *E. coli* strains 7010 and 7237 contained plasmids of 112 × 10^6 and 86 × 10^6 molecular weight, respectively, but failed to transfer carbenicillin resistance or other antibiotic resistances. *E. coli* strain 7047 also failed to transfer antibiotic resistance directly, but plasmid pMG210 was detected by mobilization with *Flac*. *Salmonella newport* strain 80-E1572 also failed to transfer antibiotic resistance directly, but plasmid pMG218 was obtained by mobilization with plasmid R751. *Salmonella typhimurium* strain 81-E138 failed to transfer resistance at 37°C but was an efficient donor when matings were performed at 30°C. The other strains readily transferred resistance at 37°C. After transfer to recipient *E. coli*, five of the plasmids produced PSE-1 alone and four produced both PSE-1 and TEM-1. *Salmonella heidelberg* strain 1707, in addition to containing plasmid pMG215 encoding PSE-1 and TEM-1, also contained plasmid pMG214 encoding only TEM-1.

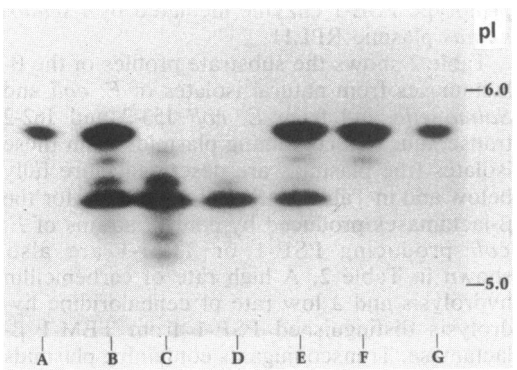

FIG. 1. Isoelectric focusing gel demonstrating band patterns of β-lactamases from *P. aeruginosa* PU21 (RPL11) (PSE-1 β-lactamase prototype) (lane A), *Salmonella heidelberg* 1707 (lane B), *E. coli* J53-2 (R6K) (TEM-1 β-lactamase prototype) (lane C), *E. coli* J53-2 (pMG214) (lane D), *E. coli* J53-2 (pMG215) (lane E), *E. coli* J62-2 (pMG215S) (a kanamycin-susceptible segregant that also lost TEM-1 β-lactamase production) (lane F), and *P. aeruginosa* PU21 (RPL11) (lane G). β-Lactamase activity was detected with the chromogenic substrate nitrocefin (22).

The PSE-1–producing plasmids all determined resistance to streptomycin, spectinomycin, and sulfonamide. As would be expected from this pattern of aminoglycoside-aminocyclitol resistance (6), they determined AAD(3″) activity. Resistance to other antibiotics and mercuric ion was more variable. By agarose gel electrophoresis, we determined that the plasmids ranged in molecular weight from 37 × 10^6 to 130 × 10^6. At least six Inc groups were represented, including FI*me*, FII, C, H2, M, and X. Of these Inc groups, only plasmids of IncC are known to be transmissible to *P. aeruginosa* by conjugation (13). Not all IncC plasmids have this broad host range, but IncC plasmid pMG213 was transferable to *P. aeruginosa* by mating.

The structure of plasmids present in PSE-1– and TEM-1–producing *E. coli* strains 7010 and 7047 was examined by preparing ampicillin-, kanamycin-, or tetracycline-susceptible segregants. The segregants contained plasmids small-

TABLE 2. Substrate profiles of β-lactamases produced by clinical isolates and transconjugants

Strain	β-Lactamase type(s)	Activity relative to that with penicillin G[a]										Inhibition by 10^{-4} M cloxacillin (%)
		Amp	Carb	Clox	Meth	Oxa	Ceph	Lori	Lex	Fox	Man	
E. coli 7535	PSE-1	112	107	2	2	9	6	19	<1	<1	13	0
J53-2(pMG211)	PSE-1	132	93	1	1	7	1	11	<1	—[b]	—	—
E. coli 7237	PSE-1	113	99	2	<1	7	2	15	<1	<1	10	0
E. coli Ind22	PSE-1	110	95	2	<1	7	2	29	<1	<1	19	0
J53-2(pMG212)	PSE-1	—	114	—	—	8	—	16	—	—	—	—
E. coli 7010	PSE-1, TEM-1	—	47	—	—	—	—	46	—	—	—	36
7010 gel[c]	PSE-1	—	106	—	—	9	—	19	—	—	—	—
E. coli 7047	PSE-1, TEM-1	—	65	—	—	—	—	67	—	—	—	—
7047 gel[c]	PSE-1	—	93	—	—	9	—	27	—	—	—	—
S. heidelberg 1707	PSE-1, TEM-1	—	33	—	—	7	—	98	—	—	—	—
1707 gel[c]	PSE-1	—	94	—	—	4	—	20	—	—	—	43
J62-2(pMG215S)[d]	PSE-1	—	81	—	—	7	—	13	—	—	—	0
J53-2(pMG214)	TEM-1	—	14	—	—	7	—	72	—	—	—	—
J53-2(pMG216)	PSE-1, TEM-1	—	57	—	—	9	—	78	—	—	—	—
J53-2(pMG216) gel[c]	PSE-1	—	91	—	—	6	—	21	—	—	—	37
J53-2(R751-RPL11)	PSE-1	111	108	2	<1	9	5	19	<1	2	14	0
J53-2(R6K)	TEM-1	113	11	1	<1	10	11	128	1	<1	56	82

[a] Activity relative to that with penicillin G set at 100. With penicillin G as the substrate, β-lactamase activity ranged from 9 to 38 μmol/h per mg of protein. Amp, Ampicillin; Carb, carbenicillin; Clox, cloxacillin; Meth, methicillin; Oxa, oxacillin; Ceph, cephalothin; Lori, cephaloridine; Lex, cephalexin; Fox, cefoxitin; Man, cefamandole.

[b] —, Not done.

[c] The enzyme was partially purified by preparative isoelectric focusing.

[d] pMG215S is a kanamycin-susceptible segregant of pMG215 that is also deficient in TEM-1 β-lactamase production.

er than those present in the parent strains. From segregants of strain 7010 that had lost more than one resistance determinant, a deletion map of plasmid pMG209 was determined (Fig. 2). None of the deletions separated the *amp* (PSE-1), *str*, and *sul* genes. The order of these three resistance genes was determined to be next to the *amp* (TEM-1) gene. In confirmation of this order, segregants from strain 7047 could be obtained that carried *amp* (TEM-1) as the only resistance marker on a 105 × 10^6-molecular-weight deletion plasmid.

Transposition of the PSE-1 gene from pMG217 and pMG209. The finding that PSE-1 is produced by plasmids belonging to at least six different incompatibility groups in enterobacteria suggests that the gene for PSE-1 production may be carried by a transposable genetic element. Transposition was studied with plasmids pMG217 and pMG209.

As is typical of IncH2 plasmids, pMG217 transferred efficiently at low temperature but not at 37°. A kanamycin-susceptible segregant of J53-2 (pMG217) was selected, and plasmid pUZ8 (Tc Km Hg IncP; molecular weight, 34 × 10^6) was introduced. When the strain containing both plasmids was tested for transfer of ampicillin resistance to strain J62-2 at 37°C, a transfer rate greater than 10^{-5} per donor was observed. Of 80 transconjugants, 4 were resistant to ampicillin, kanamycin, streptomycin, sulfonamide, tetracycline, and mercuric ion but susceptible to chloramphenicol and hence lacked at least part of pMG217. One of these transconjugants was chosen for study. It conferred susceptibility to phage PR4; produced PSE-1, as determined by isoelectric focusing; and contained a single plasmid species with a molecular weight of about 42 × 10^6. In conjugation, all resistance markers cotransferred whatever was used for selection, and in compatibility tests, all resistances were lost upon entry of IncP plasmid R751. These properties are consistent with the transposition to pUZ8 of a genetic segment of pMG217 carrying the PSE-1 gene and resistance to streptomycin and sulfonamide. This transposon will be hereafter termed Tn*1401*. Tn*1401* could be transposed from a Tra^- derivative of pUZ8::Tn*1401* to IncW plasmid R388.

Plasmid pMG209 is a Tra^- FI*me* plasmid determining both PSE-1 and TEM-1 production in *E. coli* strain 7010. A segregant that had lost kanamycin and trimethoprim resistance was prepared, and pUZ8 was introduced. This double-

TABLE 3. Properties of plasmids detected in PSE-1–producing strains

Isolate	Source	Strain β-lactamase type (s)	Plasmid	Inc group	Mol wt (10^6)	Plasmid β-lactamase type(s)	Resistance phenotype[a]
Escherichia coli							
7010	Brazil	PSE-1, TEM-1	pMG209	FI*me*	112	PSE-1, TEM-1	Ap Cm Km Sm Sp Su Tc Tp Tra^-
7047	Brazil	PSE-1, TEM-1	pMG210		130	PSE-1, TEM-1	Ap Cm Km Sm Sp Su Tc Tp Hg Tra^-
7237	Brazil	PSE-1	Tra^-		86		
7535	Brazil	PSE-1	pMG211	FI*me*	79	PSE-1	Ap Cm Sm Sp Su Tc
Ind22	Indonesia	PSE-1	pMG212[b]	FII	69	PSE-1	Ap Cm Sm Sp Su Tc
1-99	Thailand	PSE-1, TEM-1	pMG213	C	93	PSE-1, TEM-1	Ap Sm Sp Su Tc Tp
Salmonella heidelberg							
1707	United States	PSE-1, TEM-1	pMG214		67	TEM-1	Ap Sm
			pMG215	FI*me*	99	PSE-1, TEM-1	Ap Cm Km Sm Sp Su Tc
Salmonella johannesburg	Hong Kong	PSE-1, TEM-1	pMG216	FI*me*	118	PSE-1, TEM-1	Ap Cm Km Sm Sp Su Hg
Salmonella newport							
80-E1572	United States	PSE-1	pMG218	X	37	PSE-1	Ap Cm Km Sm Sp Su Tc Hg
Salmonella typhimurium							
81-E138	United States	PSE-1	pMG217	H2	100	PSE-1	Ap Cm Km Sm Sp Su Tc Hg
Shigella sonnei	Brazil	PSE-1	pDT201	M	74	PSE-1	Ap Cm Sm Sp Su Tc

[a] Ap, ampicillin; Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline; Tp, trimethoprim; Hg, mercuric ion; Tra^-, transfer deficient.

[b] This plasmid has also been identified as pIN22 by S. Levy.

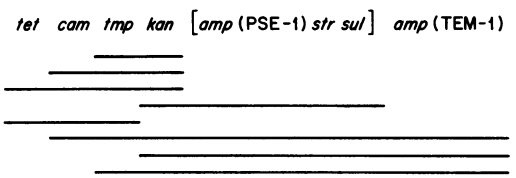

FIG. 2. Deletion map of plasmid pMG209. The genotypic designations for resistance are as follows: *amp*, ampicillin; *cam*, chloramphenicol; *kan*, kanamycin; *str*, streptomycin; *sul*, sulfonamide; *tet*, tetracycline; *tmp*, trimethoprim.

plasmid-containing strain was mated with strain J62-2, selecting either for ampicillin- or streptomycin-resistant transconjugants by using rifampin for counterselection. Of 460 colonies selected as streptomycin resistant, one transconjugant was susceptible to chloramphenicol and hence lacked at least part of pMG209 but had all of the resistances of pUZ8 plus resistance to ampicillin, streptomycin, and sulfonamide. These resistances regularly cotransferred and were eliminated by entry of IncP plasmid R751. This strain carried a single plasmid species with a molecular weight of about 42×10^6 and produced only PSE-1 β-lactamase, again apparently determined by an 8×10^6 molecular weight transposon that also determined streptomycin and sulfonamide resistance. This transposon will be hereafter termed Tn*1402*.

The properties of ampicillin-resistant transconjugants demonstrated that the TEM-1 gene of plasmid pMG209 was also determined by a transposon. Of 369 ampicillin-resistant colonies, 20 were chloramphenicol susceptible and 19 of these carried only ampicillin resistance in addition to the markers of pUZ8. These strains contained a plasmid with a molecular weight of about 36×10^6 and produced TEM-1 exclusively, as though pUZ8 had acquired a 3×10^6 molecular weight transposon of the Tn*A* class (9). The 20th ampicillin-resistant, chloramphenicol-susceptible transconjugant was resistant to streptomycin and sulfonamide and had the kanamycin and tetracycline resistances of pUZ8 but lacked resistance to mercuric ion. These resistances were transferred together in conjugation and were eliminated together by the entry of R751. The strain contained a single plasmid with molecular weight of about 46×10^6 and produced both PSE-1 and TEM-1. In this instance, pUZ8 appeared to have acquired both a Tn*A*-like transposon and Tn*1402*, with insertion of one or both transposons into the gene for mercuric ion reistrance.

DISCUSSION

Although PSE-1 β-lactamase production was first discovered in plasmid-containing strains of *P. aeruginosa* (23, 30), the finding that the PSE-1 gene was efficiently expressed if incorporated in a broad-host-range plasmid and transferred to *E. coli* (10) implies that no barrier to the establishment of this gene on plasmids of enterobacteria exists. Previous surveys of the types of β-lactamase produced by over 200 ampicillin-resistant enteric organisms isolated from urinary tract infections in England (32) or by 185 ampicillin-resistant R plasmids from *E. coli*, *Klebsiella*, *P. mirabilis*, *Salmonella*, *Serratia*, and *Shigella* isolates from a variety of geographical sources (21) failed to detect PSE-1 production. However, in 1977, Sawada et al. described a single *E. coli* isolate from Japan that produces a β-lactamase with properties consistent with PSE-1, although analytical isoelectric focusing was not performed in parallel with a prototype PSE-1 enzyme (29). Our finding that 9 of 634 *E. coli* and *Salmonella* isolates produced PSE-1 β-lactamase could reflect the recent acquisition of this gene by enteric plasmids but may merely result from our sampling of South American and Far Eastern strains not studied by previous investigators.

More intriguing than the documentation that PSE-1 β-lactamase could be produced by enterobacteria was the finding that the responsible plasmids belonged to at least six incompatibility groups. This promiscuity is a strong indication that the spread of the PSE-1 gene has been effected, at least in part, by transposition. We have shown that two plasmids of distinct incompatibility groups, detected in strains of different genera and isolated in different hemispheres, carried transposons with similar molecular weights and resistance properties. In addition to genes for PSE-1 production and sulfonamide resistance, transposons Tn*1401* and *1402* determined streptomycin and spectinomycin resistance by production of AAD(3″). It is interesting that Tn*4* also determines AAD(3″) sulfonamide resistance and β-lactamase (TEM-1) production (18) and that transposons encoding oxacillin-hydrolyzing β-lactamase genes also carry resistance to streptomycin and sulfonamide (36).

The PSE-1–producing plasmids described here all determined streptomycin, spectinomycin, and sulfonamide resistance, as well as resistance to other antibiotics. Also determining these resistances are IncP-2 *Pseudomonas* plasmids RPL11 and Rms139, which have been previously found to produce PSE-1 (15). Preliminary studies indicate that the PSE-1 gene of plasmid RPL11 is transposable, along with resistance to streptomycin, sulfonamide, and chloramphenicol (unpublished data); thus, there is presumptive evidence that transposons are responsible for PSE-1 β-lactamase production by plasmids in a variety of bacteria.

Although rare in other genera, PSE-1 production is common in carbenicillin-resistant *P. aeruginosa* strains (27, 30) but is often not transmissible or even associated with the presence of plasmids (A. Philippon and G. A. Jacoby, unpublished data). In such strains, a PSE-1–determining transposon may be located on the chromosome. A broad-host-range plasmid could have carried a PSE-1 transposon from either a plasmid or a chromosomal location in a *Pseudomonas* strain to enterobacteria. Indeed, one of the plasmids we detected, pMG213, like certain other members of Inc group C, was readily transmissible between *E. coli* and *P. aeruginosa* and could act as such a vector.

Our findings indicate that the designation of this β-lactamase as PSE-1 is no longer appropriate, since it is not *Pseudomonas* specific. Two other terms have been used to describe this enzyme: penicillinase type IV (30) and CARB-2 (20). Whatever name is finally chosen, one based on its distinctive enzymological properties, i.e., the relatively rapid hydrolysis of carbenicillin, seems more suitable than the present designation, with its implication of species specificity.

ACKNOWLEDGMENTS

We thank J. Acar, B. Blackburn, M. Holmes, M. R. Jacobs, P. Jayanetra, S. B. Levy, B. E. Murray. T. F. O'Brien, D. T. Taylor, and J. Ximenez for providing strains; E. J. Threlfall for confirming our assignment of plasmids to the Fl*me* group; G. Paul for performing inhibition studies with β-lactamase antisera; J. W. Bisson, L. Coyle, and L. Sutton for expert technical assistance; and J. Feiner and M. C. Sousa for secretarial assistance.

This study was supported in part by contract no. 223-79-7060 from the U.S. Food and Drug Administration and by grant PCM-7912052 from the National Science Foundation.

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