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Novel Plasmid-Mediated β-Lactamase (TEM-10) Conferring Selective Resistance to Ceftazidime and Aztreonam in Clinical Isolates of *Klebsiella pneumoniae*

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Two clinical isolates of *Klebsiella pneumoniae* from seriously ill patients in Chicago, Ill., have been identified as resistant to ceftazidime and aztreonam but susceptible to other cephalosporins. This unusual antibiogram was shown to be due to a novel plasmid-mediated β-lactamase which readily hydrolyzed ceftazidime and aztreonam in addition to penicillins such as piperacillin and carbenicillin. This enzyme and its attendant resistance were transferred to *Escherichia coli* by conjugation on a 50-kilobase plasmid. Isoelectric focusing revealed a single β-lactamase band with a molecular weight of 29,000 and an isoelectric point of 5.57 in the resistant isolates and transconjugants. The β-lactamase inhibitors clavulanic acid and sulbactam restored β-lactam susceptibility in the resistant isolates. Fifty percent inhibitory concentrations of clavulanic acid and sulbactam were 4.4 and 940 nM, respectively. DNA hybridization studies indicated that this enzyme, designated TEM-10, is related to well-established TEM-type β-lactamases. However, the TEM-10 enzyme was inhibited by *p*-chloromercuribenzoate, in contrast to TEM-2 β-lactamase. On the basis of substrate and inhibition profiles, the TEM-10 enzyme could be easily discriminated from TEM-5 and RHH-1 β-lactamases.

Extended-spectrum β-lactam antibiotics are very active in vitro against most enteric bacteria, in large part due to their stability in the presence of common plasmid-mediated and chromosomal β-lactamases (8). Emergence of resistance and associated treatment failure have complicated β-lactam therapy of infections due to organisms harboring inducible chromosomal β-lactamases, especially *Enterobacter* spp., *Serratia* spp., and *Pseudomonas aeruginosa* (25, 27). This resistance has been attributed to slow hydrolysis mediated by β-lactamase (33) or diminished penetration of these agents across the bacterial outer membrane (3, 21, 26) or both.

Plasmid-mediated β-lactamases conferring resistance to the newer cephalosporins were first detected in gram-negative bacilli in Europe in 1983 (15). Resistant organisms harboring a variety of these enzymes carried on transferable plasmids have been isolated with increasing frequency in Europe over the past several years (5, 7, 11, 13, 14, 23, 29, 31, 34). Two well-described β-lactamases in this class from France include a cefotaxime-hydrolyzing enzyme denoted CTX-1, or TEM-3, and a ceftazidime-hydrolyzing enzyme designated CAZ-1, or TEM-5 (14, 23, 30). Another ceftazidime-hydrolyzing enzyme, RHH-1, was identified in England in 1987 (31). The occurrence of a similar enzyme in the United States has only recently been reported in a preliminary communication from Boston (G. A. Jacoby, A. A Medeiros, T. F. O'Brien, M. E. Pinto, and H. Jiang, Letter, N. Engl. J. Med. **319:**723–724, 1988).

In this report, we describe a novel plasmid-mediated β-lactamase detected in 1988 in clinical isolates of *Klebsiella pneumoniae* from seriously ill patients in Chicago, Ill. This enzyme conferred resistance to ceftazidime and aztreonam as well as to penicillins and was readily transferable to *Escherichia coli* by conjugation. The presence of this resistance determinant on a transferable plasmid raises the concern of its rapid and widespread dissemination among gram-negative bacilli.

(This work was presented in part at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, Calif. [J. P. Quinn, D. Miyashiro, B. Edlin, R. Flamm, and K. Bush, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 484, 1988].)

MATERIALS AND METHODS

Bacterial strains. Both clinical isolates of *K. pneumoniae* were identified by standard biochemical techniques. A nalidixic acid-resistant mutant of *E. coli* C600 was used as a recipient in conjugation studies (6). We thank D. Sirot for supplying *E. coli* CF604 harboring TEM-5 and A. Harris for supplying *K. pneumoniae* 2633E harboring RHH-1.

Typing. Serotyping of the *Klebsiella* isolates was performed in the laboratory of Peter B. Smith, Centers for Disease Control, Atlanta, Ga. (12). Allozyme typing (28) of the *Klebsiella* isolates was performed by Robert Selander, Pennsylvania State University, College Station.

Antibiotics and reagents. Antibiotics were supplied as follows: amoxicillin, carbenicillin, clavulanic acid, and ticarcillin, Beecham Laboratories, Bristol, Tenn.; ampicillin, aztreonam, benzylpenicillin, and cloxacillin, Squibb Institute for Medical Research, Princeton, N.J.; cefazolin, cephaloridine, and cephalothin, Eli Lilly & Co., Indianapolis, Ind.; cefoperazone and sulbactam, Pfizer Inc., New York, N.Y.; cefoxitin and imipenem, Merck Sharp & Dohme, Rahway, N.J.; ceftazidime and cefuroxime, Glaxo Group Research Ltd., Research Triangle Park, N.C.; piperacillin, Lederle Laboratories, Caroline, P.R.; cefotaxime, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.; ceftriaxone, Hoffmann-La Roche Inc., Nutley, N.J.; and nalidixic acid, Winthrop Laboratories, Div. Sterling Drug Inc., New York, N.Y. Sigma Chemical Co., St. Louis, Mo., was the supplier for EDTA, formamide, *p*-chloromercuribenzoate (pCMB), 6-aminohexanoic acid-activated Sepharaose 4B,

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m-aminophenylboronic acid, triethanolamine hydrochloride, and boric acid.

Antibiotic susceptibility testing. MICs for all isolates were determined by an agar dilution technique (36).

Isoelectric focusing. Isoelectric focusing was conducted by the method of Matthew et al. (20), using a Hoefer Isobox with prepared LKB Ampholine PAG plates (pH range, 4.0 to 6.5). Gels were stained with the chromogenic cephalosporin nitrocefin.

Conjugation. Conjugation studies were conducted by standard techniques (6). Transconjugants were selected on agar containing ceftazidime at 10 μg/ml and nalidixic acid at either 15 or 25 μg/ml.

β-Lactamase purification. TEM-10 β-lactamase was purified to >90% homogeneity as follows. *K. pneumoniae* KC2 was grown in tryptic soy broth with harvest during log phase. Cells were sonicated, and the clarified supernatant was eluted from Sephadex G75 (2). The fractions containing β-lactamase activity were pooled, dialyzed overnight against 20 mM triethanolamine–0.5 M NaCl, pH 7.0, and eluted from a type B aminophenylboronic acid-agarose column (4) equilibrated with the same buffer used for dialysis. Protein peaks with minimal β-lactamase activity eluted initially. TEM-10 β-lactamase was obtained immediately after the buffer was changed to 0.5 M borate–0.5 M NaCl, pH 7.0. Active fractions were pooled and dialyzed overnight against 0.05 M phosphate buffer, pH 7.0, with three buffer changes. This preparation was stored at 4°C in the presence of 0.02% sodium azide and used for determinations of kinetic constants.

TEM-5 from *E. coli* CF604 and RHH-1 β-lactamase from *K. pneumoniae* 2633E were purified in identical procedures to that used for the TEM-10 enzyme. All three enzymes exhibited similar behaviors when eluted from the boronic acid column.

TEM-2 β-lactamase was purified to >95% homogeneity by using a combination of Sephadex and DEAE chromatographic procedures (2).

Molecular weights of purified proteins were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (17).

β-Lactamase assays. Hydrolysis of β-lactam antibiotics was monitored spectrophotometrically at 25°C in 0.1 M phosphate buffer, pH 7.0, using a Gilford 250 or 2600 spectrophotometer. Spectral parameters for cephaloridine, cefoperazone, cefotaxime, ceftazidime, and aztreonam were described previously (2). Wavelengths used for other assays include the following: 240 nm for benzylpenicillin; 255 nm for ceftriaxone; 232 nm for ampicillin, carbenicillin, and piperacillin; 260 nm for cloxacillin and cephalothin; 265 nm for cefuroxime; 295 nm for imipenem; and 261 nm for cefoxitin. Kinetic parameters were determined in at least duplicate experiments, using four kinetic analyses: direct linear, Lineweaver-Burk, Hanes-Woolf, and Eadie-Hofstee, provided on the program ENZPACK (Elsevier-Biosoft). K_m and V_{max} values were reported as means, with relative standard deviations usually ≤10% for the four analyses.

Inhibition studies with EDTA and pCMB were performed by incubating enzyme and buffer or 200 μM inhibitor for 5 min at 25°C before the addition of 2.0 mM cephaloridine. Final assay concentrations were 100 μM inhibitor and 1.0 mM cephaloridine.

Inhibition by clavulanic acid and sulbactam was examined by incubating enzyme and buffer or various concentrations of inhibitor (1.0 to 2,000 nM) for 5.0 min at 25°C before the addition of 1.0 mM cephaloridine. Fifty percent inhibitory concentrations were calculated by determining the concentration of inhibitor necessary to diminish the activity of the control enzyme by 50%.

Plasmid DNA. The plasmid DNA of all isolates was detected by the rapid alkaline lysis technique of Portnoy et al. (24). Total DNA was isolated as described previously (10). Samples were subjected to electrophoresis through 0.7% agarose at 12 V/cm. Plasmid size was determined by comparison with plasmid molecular weight standards. Bacterial restriction endonuclease digests (New England BioLabs, Inc., Beverly, Mass.) were performed as described before (19). Samples were subjected to electrophoresis through 1% agarose at 12 V/cm.

DNA hybridization studies. A DNA probe for TEM β-lactamase sequences was kindly provided by R. Levesque, Quebec, Canada. This probe is a 424-base-pair *Bgl*I-plus-*Hinc*II fragment which is internal to the β-lactamase gene of pBR322 (18). Probe DNA was labeled, using a random primer method (Pharmacia LKB Biotechnology Inc.), with ^{32}P in vitro (9) and hybridized under stringent conditions to DNA immobilized on nitrocellulose (19). Stringent conditions were hybridization in 5× SSC (1× SSC is 3 M sodium chloride plus 0.3 M sodium citrate)–50% formamide at 42°C overnight followed by two washes in 2× SSC–0.1% sodium dodecyl sulfate for 5 min at room temperature and then two washes in 0.1× SSC–0.1% sodium dodecyl sulfate at 50°C for a total of 30 min (35).

RESULTS

Case histories. Patient 1 was a 27-year-old woman admitted to an outlying hospital for evaluation and treatment of a subarachnoid hemorrhage. She received mechanical ventilation, intravenous dexamethasone, and a single 2-g dose of intravenous cefotaxime. The next day she was transferred to the University of Chicago Hospitals. Cerebral angiography demonstrated an aneurysm of the posterior communicating artery. A lumbar drain was placed. Intravenous nafcillin, 2 g every 6 h, was given. Six days later she complained of neck stiffness. Physical examination revealed nuchal rigidity. She was afebrile and her mental status was intact. Cerebrospinal fluid obtained via the lumbar drain revealed 121 erythrocytes per ml; 200 leukocytes per ml with 60% neutrophils; glucose, 10 mg/dl; and protein, 70 mg/dl. The cerebrospinal fluid Gram stain revealed gram-negative bacilli and the culture grew *K. pneumoniae*, resistant to ceftazidime and aztreonam but susceptible to other cephalosporins. Ceftriaxone, 2 g intravenously every 12 h, was administered for 6 days. On the eighth hospital day, she was taken to the operating room and the aneurysm was clipped. Postoperative cultures of cerebrospinal fluid were sterile, and she made an uneventful recovery.

Patient 2 was a 57-year-old diabetic women admitted to the University of Chicago Hospitals for treatment of myonecrosis of the right leg. Operative debridement and disarticulation of the hip were performed. Her postoperative course was prolonged and complicated. Nosocomial bacteremic pneumonia due to *P. aeruginosa* was treated with a 3-week course of ceftazidime and tobramycin. Persistent fever of unknown origin after resolution of pneumonia led to repeated courses of empiric treatment with broad-spectrum β-lactams, including 4 weeks of ceftazidime and 2 weeks of aztreonam. After 3 months in the hospital, she developed evidence of acute peritonitis and septic shock. Empiric ceftazidime and metronidazole were given, and she underwent an abdominal laparotomy, in which several segments of

TABLE 1. β-Lactam MICs for the clinical *Klebsiella* isolates (KC1 and KC2), the recipient *E. coli* strain (C600), and transconjugants (*X*)

Isolate	MIC (μg/ml)															
	Cefazolin	Cefoxitin	Cefuroxime	Cefotaxime	Ceftriaxone	Imipenem	Ceftazidime	Ceftazidime plus clavulanic acid	Aztreonam	Aztreonam plus clavulanic acid	Piperacillin	Piperacillin plus clavulanic acid	Ampicillin	Ampicillin plus clavulanic acid	Ticarcillin	Ticarcillin plus clavulanic acid
C600	2	4	4	0.063	0.063	<0.5	<0.5	<0.5	<0.5	<0.5	2	2	4	4	4	4
KCl	4	4	4	0.5	1.0	<0.5	64	<0.5	32	<0.5	256	2	>256	8	>256	32
KCl *X*	8	8	16	1.0	2.0	<0.5	64	<0.5	32	<0.5	256	1	>256	16	>256	32
KC2	4	2	4	0.25	0.5	<0.5	64	<0.5	32	<0.5	128	2	>256	8	>256	16
KC2 *X*	2	8	ND[a]	0.25	0.25	<0.5	32	<0.5	8	<0.5	64	2	ND	ND	ND	ND

[a] ND, Not determined.

ischemic small and large bowel were resected. She had a progressive downhill course and died 24 h after surgery. Multiple blood cultures taken at the time of her death revealed a broadly susceptible isolate of *E. coli* and a *K. pneumoniae* isolate which was resistant to ceftazidime and aztreonam but susceptible to other cephalosporins.

Retrospective chart review did not reveal any obvious epidemiological link between these two patients. They were cared for by separate surgical services. However, they were housed on separate wards in the same building and the isolates were detected 1 week apart in January 1988. No additional cases of infection due to organisms with this unique antibiogram have been detected in our institution as of this writing.

Bacterial isolates. Both clinical isolates were identified as *K. pneumoniae* by standard biochemical criteria. Serotyping revealed that both isolates belonged to serogroup 71, an uncommon serogroup (P. B. Smith, personal communication). Allozyme typing showed that 16 metabolic enzymes in the two isolates were indistinguishable, strongly suggesting that the organisms are genetically related (R. Selander, personal communication).

Antimicrobial susceptibility. Both *K. pneumoniae* isolates were resistant to ceftazidime, aztreonam, and penicillins, including piperacillin, but were susceptible to imipenem and most cephalosporins, including cefotaxime (Table 1). The *E. coli* transconjugants exhibited a similar antibiogram. It is noteworthy that the MICs of cefotaxime and ceftriaxone for *E. coli* transconjugants are higher than those for *E. coli* C600. However, the magnitude of the increase for these agents is lower than that observed for ceftazidime, aztreonam, and piperacillin. The β-lactamase inhibitors clavulanic acid (Table 1) and sulbactam (data not shown) restored susceptibility to ceftazidime, aztreonam, piperacillin, ampicillin, and ticarcillin.

Physical properties of β-lactamases. Both *Klebsiella* isolates (Fig. 1, lanes 3 and 6) produced a single β-lactamase band (here denoted TEM-10) with a pI of approximately 5.57. This was distinct from that of the common TEM-2 enzyme (pI 5.6) (lane 2) but was very similar to those of RHH-1 (lane 4) and TEM-5 (lane 5). The *E. coli* transconjugants produced a single β-lactamase band with the same pI as the *K. pneumoniae* isolates (data not shown).

All four enzymes eluted from Sephadex G75 at the same elution volume, suggesting similar molecular sizes. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the molecular weight of purified TEM-10 was shown to be approximately 29,000, the same as that of TEM-2 β-lactamase.

β-Lactamase kinetic properties. Purified TEM-10 β-lactamase rapidly hydrolyzed piperacillin and ceftazidime (Table 2); aztreonam was hydrolyzed more slowly. Hydrolysis efficiencies (V_{max}/K_m) for penicillins, including carbenicillin, were higher than those for cephalosporins. Cefoxitin and imipenem were notably resistant to hydrolysis.

Major differences in substrate profiles were evident when the activity of TEM-10 β-lactamase was compared with those of the TEM-2 and TEM-5 enzymes (Table 2). Both carbenicillin and cloxacillin were hydrolyzed at faster rates with the TEM-10 enzyme than with the TEM-2 or TEM-5 β-lactamase. Most notable in the comparison of both the TEM-5 and TEM-10 enzymes with the standard TEM-2 β-lactamase was the increased hydrolysis of antibiotics carrying oxime side chains, such as cefuroxime, ceftazidime, cefotaxime, ceftriaxone, and aztreonam.

The TEM-10 hydrolytic activity with β-lactam antibiotics carrying aminothiazoleoxime side chains was also different from the profiles for the ceftazidime-hydrolyzing enzymes TEM-5 and RHH-1 (Table 3). Ceftazidime was hydrolyzed 42 times faster than cefotaxime with the TEM-10 enzyme but only 3.4 times faster with the TEM-5 enzyme and 2.9 times faster with the RHH-1 β-lactamase. These relative activities

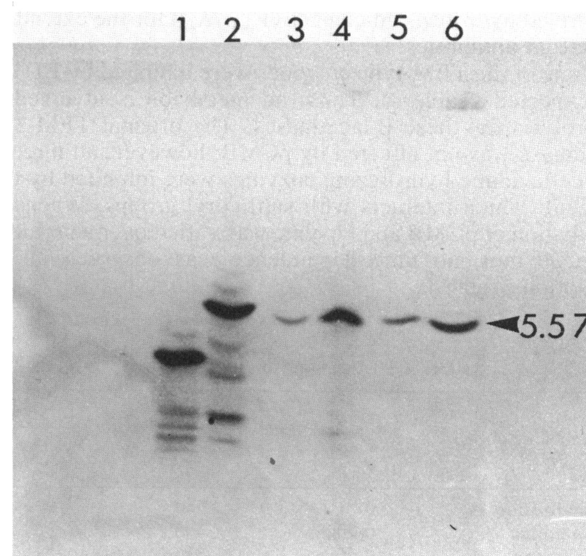

FIG. 1. Isoelectric focusing gel of β-lactamases from sonic extracts: TEM-1 and TEM-2 (lanes 1 and 2); TEM-10 (lanes 3 and 6); RHH-1 (lane 4); TEM-5 (lane 5). The pI of TEM-10 is indicated.

TABLE 2. Kinetic properties of purified TEM β-lactamases[a]

Antibiotic	Relative V_{max}		TEM-10[b]		
	TEM-2[c]	TEM-5[d]	Relative V_{max}	K_m (μM)	Relative V_{max}/K_m
Cephaloridine	100	100	100	68	100
Benzylpenicillin	80	33	130	4.8	1,800
Ampicillin	80	16	170	17	680
Carbenicillin	4.8	8.9	47	5.4	600
Cloxacillin	3.0	≤7	21	46	31
Piperacillin	89	17	130	8.4	1,000
Cephalothin	7.5	16	24	120	14
Cefoperazone	9.3	3.4	2.2	19	7.9
Cefuroxime	0.91	50	15	170	6
Ceftriaxone	0.077	18	1.1	9.7	8.3
Cefotaxime	0.078	9.7	2.1	43	3.3
Ceftazidime	0.002	33	90	170	36
Aztreonam	0.17	15	13	38	23
Imipenem	0.001	0.29	≤0.02	ND[e]	ND
Cefoxitin	0.004	—[f]	≤0.05	ND	ND

[a] Relative V_{max} values were obtained by normalizing V_{max} for each antibiotic to V_{max} for cephaloridine for each enzyme.
[b] Specific activity was 0.0090 μmol of cephaloridine hydrolyzed at V_{max} per min per μg of protein.
[c] Specific activity was 2.48 μmol of cephaloridine hydrolyzed at V_{max} per min per μg of protein.
[d] Specific activity was 0.049 μmol of cephaloridine hydrolyzed at V_{max} per min per μg of protein.
[e] ND, Rates were too slow to determine K_m.
[f] —, No data.

are similar to values reported from other laboratories for TEM-5 (23) and RHH-1 (31). TEM-5 and TEM-10 exhibited lower K_m values than the TEM-2 enzyme for all antibiotics studied. K_m values for the RHH-1 β-lactamase with cefotaxime and aztreonam were at least an order of magnitude higher than those observed for the TEM-10 enzyme, indicating more efficient binding of these antibiotics to the novel TEM-10 β-lactamase.

Specific activities of the purified extended-spectrum β-lactamases were at least 50 times lower than that observed for the TEM-2 enzyme (Tables 2 and 3). However, these novel enzymes bound most β-lactams better, resulting in improved hydrolysis efficiences (V_{max}/K_m) for the extended-spectrum antibiotics.

None of the TEM-type enzymes were inhibited by EDTA, as expected (Table 4). Thus, no metal ion is involved in hydrolysis for these β-lactamases. The original TEM-2 β-lactamase was not affected by pCMB; however, all three of the ceftazidime-hydrolyzing enzymes were inhibited by this reagent, which interacts with sulfhydryl groups. When the incubation of pCMB and enzyme was varied over a period of 1 to 30 min, no time dependence was observed in the inhibition profiles.

TABLE 4. Inhibition profiles of TEM-type β-lactamases

β-Lactamase	% Control activity				50% Inhibitory concn (nM)	
	No inhibitor	100 μM EDTA	100 μM pCMB[a]			
			Initial rate	Final rate	Clavulanic acid	Sulbactam
TEM-2	100	112		86[b]	130	1,600
RHH-1	100	108	66	39	ND[c]	ND
TEM-5	100	110	77	40	12	120
TEM-10	100	102	50	20	4.4	940

[a] Biphasic rates were observed, with a faster initial rate occurring for approximately 5 to 7 min followed by a slower, steady-state rate.
[b] Only a single rate was observed.
[c] ND, Not determined.

Clavulanic acid and sulbactam were stronger inhibitors for both the TEM-5 and TEM-10 enzymes, as compared with the TEM-2 β-lactamase (Table 4). However, the TEM-5 β-lactamase was inhibited by sulbactam 13 times better than the TEM-2 enzyme, whereas the TEM-10 enzyme was inhibited only 1.7 times better. Clavulanate was a better inhibitor than sulbactam for all enzymes.

Plasmid DNA. Plasmid DNA of both *K. pneumoniae* isolates and their corresponding transconjugants was analyzed by agarose electrophoresis (Fig. 2). Both isolates and transconjugants contain a 50-kilobase plasmid, pJPQ100. When plasmid preparations of the two transconjugants were digested by bacterial restriction endonucleases (*Eco*RI, *Hin*dIII; *Eco*RI and *Hin*dIII double enzyme digest), the same restriction fragment patterns were observed (data not shown).

Occasionally, along with pJPQ100, a smaller plasmid form was seen in *E. coli* transconjugants of KC1 and a larger plasmid form was seen in *E. coli* transconjugants of KC2; however, only pJPQ100 was always detected in association with multiple resistance.

DNA hybridization. A probe derived from TEM-1 was hybridized to dot blots under high-stringency conditions. The probe demonstrated homology to the TEM-1-containing pBR322 and both *K. pneumoniae* strains KC1 and KC2 and their corresponding *E. coli* transconjugants (Fig. 3). This confirmed the presence of TEM-type β-lactamase sequences in the above strains. The probe showed no homology to DNA from *E. coli* C600, the negative control.

DISCUSSION

The recent appearance of plasmid-mediated resistance to extended-spectrum β-lactams is clinically important for several reasons. First, these drugs are widely used in the treatment of serious gram-negative bacillary infections, and

TABLE 3. β-Lactamase-catalyzed hydrolysis of β-lactam antibiotics containing aminothiazoleoxime side chains[a]

Antibiotic	TEM-2		RHH-1[b]		TEM-5		TEM-10	
	Relative V_{max}	K_m (μM)	Relative V_{max}	K_m (μM)	Relative V_{max}	K_m (μM)	Relative V_{max}	K_m (μM)
Cephaloridine	100	640	100	118	100	71	100	72
Ceftazidime	0.0008	480	52	78	33	275	88	167
Cefotaxime	0.06	510	18	450	9.7	47	2.1	43
Aztreonam	0.17	2,900	60	490	13	270	14	37

[a] Rates were determined with highly purified preparations of each β-lactamase.
[b] Specific activity was 0.025 μmol of cephaloridine hydrolyzed at V_{max} per min per μg of protein.

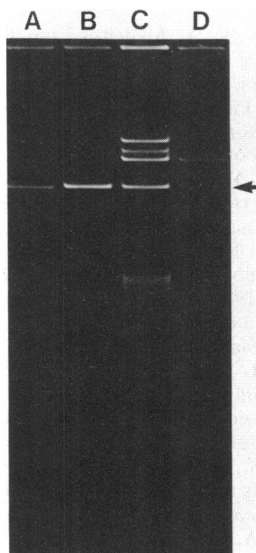

FIG. 2. Agarose gel electrophoresis of lysates of *K. pneumoniae* isolates and their corresponding *E. coli* C600 transconjugants. Plasmid DNA was fractionated on a 0.7% agarose gel at 12 V/cm. A, *K. pneumoniae* KC1; B, *E. coli* C600 transconjugant of KC1; C, *K. pneumoniae* KC2; D, *E. coli* C600 transconjugant of KC2. Plasmid pJPQ100 (50 kilobases) is indicated by the arrow.

the spread of this resistance determinant on a transferable plasmid may limit their future utility. Second, there is clearly potential for nosocomial spread of these resistant organisms, as exemplified by the two patients described in this report. Serotyping, alloenzyme typing, and antibiogram strongly suggest that the two *K. pneumoniae* isolates are genetically related. The difference in plasmid profiles emphasizes that the results of several typing systems may be needed for epidemiological evaluation. Third, many clinical microbiology laboratories use "class" susceptibility testing for broad-spectrum cephalosporins. In some institutions, for example, ceftazidime and aztreonam susceptibility data for enteric bacteria are not reported to clinicians when the organisms tested are susceptible to ceftriaxone. This practice is clearly inappropriate in light of this report and others (5, 7, 11, 13–15, 23, 29, 31, 34) describing selective resistance to various drugs in this class mediated by novel β-lactamases.

On the basis of the evidence presented, TEM-10 is a novel TEM-derived β-lactamase that confers resistance to ceftazidime and aztreonam, antibiotics with the same acidic aminothiazole side chain. Comparisons were made with other β-lactamases responsible for similar decreased susceptibilities. Of those enzymes that have been well characterized, only MJ-2, TEM-5, and RHH-1 have isoelectric points that are close to that of TEM-2, used as a marker β-lactamase band in most studies (5, 7, 13, 30, 31). The MJ-2 β-lactamase has been reported to be a pure penicillinase with little capability of hydrolyzing cloxacillin or cephalosporins and thus was not considered to be similar to TEM-10. The TEM-10 enzyme can be distinguished by its high preferential hydrolytic activity against ceftazidime as compared with cefotaxime. This activity is unique among those β-lactamases that hydrolyze extended-spectrum β-lactam antibiotics, including the TEM-3 (23), TEM-5, TEM-7 (11), RHH-1, CAZ-2 (5), SHV-2 (16), and SHV-3 (13) enzymes.

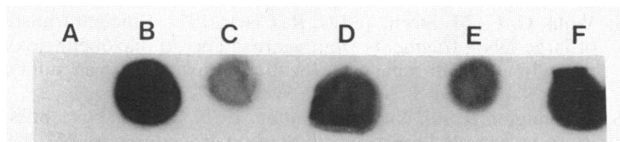

FIG. 3. Analysis of DNA by dot blot hybridization. Total DNA for *E. coli* C600 or plasmid DNA was transferred to a nitrocellulose filter and hybridized to an in vitro ^{32}P-labeled 424-base-pair *Bgl*I-plus-*Hin*cII internal TEM-1 fragment. A, *E. coli* C600 negative control; B, pBR322 positive control; C, *K. pneumoniae* KC1; D, *E. coli* C600 transconjugant of *K. pneumoniae* KC1; E, *K. pneumoniae* KC2; F, *E. coli* C600 transconjugant of *K. pneumoniae* KC2.

Inhibition profiles for these ceftazidime-hydrolyzing enzymes are also characteristic. Although the TEM-5 and TEM-10 β-lactamases both had high affinities for clavulanic acid, TEM-10 was less affected by sulbactam than the TEM-5 enzyme. Although all of the newer enzymes were capable of being inhibited by pCMB, the original TEM-2 β-lactamase was not inactivated by this amino acid modifier. These results suggest that a common amino acid substitution pattern may prevail within this family of biochemically related enzymes.

DNA hybridization data suggest that this enzyme is derived from a TEM-type enzyme. These enzymes are very common among enteric bacteria. For example, approximately 30% of *E. coli* strains in the United States are now resistant to ampicillin, primarily due to plasmid-mediated β-lactamases, the most common of which are the TEM-1 and TEM-2 enzymes (22). TEM-1 and TEM-2 are closely related, differing by only a single amino acid substitution (1, 32). The nucleotide sequence of the gene coding for TEM-3, a newly described β-lactamase which hydrolyzes several of the broad-spectrum cephalosporins, was determined recently (30). Only two amino acid differences were detected between TEM-3 and the well-established TEM-2 enzyme. Similarly, the TEM-5 enzyme, which hydrolyzes ceftazidime, has been shown by DNA hybridization studies to be related to the original TEM enzymes (23). TEM-3 and TEM-5 are among a family of at least seven related TEM β-lactamases (30). To determine the relationship of TEM-10 to these enzymes, the gene coding for TEM-10 β-lactamase is now being sequenced.

Since these new resistance genes are readily derived from older ones which are already widely distributed among enteric bacteria, one might anticipate that this may soon be an increasingly common and important problem in the United States, analogous to the current situation in Europe. Although cephamycins and imipenem are resistant to degradation by these enzymes, these agents in turn may fail clinically due to destruction by unrelated enzymes or diminished penetration across the bacterial outer membrane (26). β-Lactamase inhibitors appear to be highly effective in preventing β-lactam hydrolysis mediated by these new enzymes and may prove useful in this regard in the future.

The history of antibiotic use is replete with examples of plasmid-mediated resistance following the introduction of new agents. Extended-spectrum β-lactams have proven no exception in this regard. The judicious use of these agents may prove useful in limiting the rapidity of spread of this resistance by limiting selective pressure.

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