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Outbreak of Ceftazidime Resistance Caused by Extended-Spectrum β-Lactamases at a Massachusetts Chronic-Care Facility

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During a 4-month period in late 1988, we isolated ceftazidime-resistant strains of *Klebsiella pneumoniae* and other members of the family *Enterobacteriaceae* from 29 patients at a chronic-care facility in Massachusetts. Ceftazidime resistance resulted from two distinct extended-spectrum β-lactamases of the TEM type which efficiently hydrolyzed the cephalosporin: YOU-1 with a pI of 5.57 and YOU-2 with a pI of 5.2. Genes encoding these enzymes were present on different but closely related high-molecular-weight, multiple antibiotic resistance plasmids of the HI2 incompatibility group and were transferable by conjugation in vitro. Agarose gel electrophoresis of extracts from clinical isolates indicated that this outbreak arose from plasmid transmission among different strains of the family *Enterobacteriaceae* rather than from dissemination of a single resistant isolate. Isolation rates of ceftazidime-resistant organisms transiently decreased after use of this drug was restricted, but resistant isolates continued to be recovered 7 months after empiric use of ceftazidime ceased.

The introduction of expanded-spectrum cephalosporins has facilitated safe and effective treatment of serious infections caused by enteric gram-negative bacilli. Unfortunately, increasing use of these agents has been associated with emergence of resistant bacterial strains. This resistance can be mediated by diminished outer membrane permeability, hyperproduction of chromosomal β-lactamase, acquisition of plasmid-mediated β-lactamases, or combinations of these mechanisms (1, 9, 17). The extended-spectrum cephalosporin ceftazidime has gained wide popularity from its substantial activity against most members of the family *Enterobacteriaceae* as well as *Pseudomonas aeruginosa*. Although ceftazidime is remarkably resistant to the activities of most β-lactamases, its use has recently been associated with the isolation of strains which elaborate plasmid-mediated enzymes with a high degree of activity against this agent (4, 13). Originally described in Europe and cultured primarily from patients hospitalized in intensive care units, occasional strains have recently been encountered in the United States as well (8, 16, 19, 27). We describe the hospital-wide dissemination of *Enterobacteriaceae* with plasmid-mediated ceftazidime resistance in a Massachusetts chronic-care facility and demonstrate that resistance in these isolates resulted from production of novel extended-spectrum β-lactamases. To the best of our knowledge, this represents the initial description of widespread dissemination of such strains within a hospital in the United States.

MATERIALS AND METHODS

Nosocomial isolates. Patients with ceftazidime-resistant organisms were identified by review of culture records in the clinical microbiology laboratory of Youville Hospital, a 305-bed chronic-care and rehabilitation facility in Cambridge, Mass. Identification and susceptibility testing were carried out by the View 20/20 System (Beckman Instruments, Palo Alto, Calif.) at Youville. Available isolates were reidentified by using the API Rapid E System (API Analytab Products, Plainview, N.Y.). Isolates that were either resistant (MIC, ≥ 32 μg/ml) or moderately resistant (MIC, 16 to 32 μg/ml) to ceftazidime were identified and saved, except during a 5-week period during which isolates were not available. When the isolates were available, organisms were included in the study if ceftazidime susceptibility was restored by the β-lactamase inhibitor sulbactam (3). If the isolate was not available, the patient was included only if, in addition to resistance to ceftazidime, resistance to chloramphenicol, gentamicin, and tetracycline was present, since this pattern was common to all but one of the available isolates and cotransferred with ceftazidime resistance.

Criteria for infection. Clinical data were obtained by review of patient records, including clinical and laboratory results pertinent to the diagnosis of infection (peripheral leukocyte count, urinalysis, chest X ray), prior antibiotic treatment, and clinical and microbiologic responses to treatment. Infection was defined as either isolation of a ceftazidime-resistant organism from a normally sterile body site or isolation from a nonsterile body site, in the absence of other possible pathogens, and two of the following three clinical criteria: (i) fever, (ii) leukocyte count of ≥10,000/mm^3, or (iii) evidence of local inflammation by physical or laboratory examination (i.e., infiltrate on chest radiography, leukocytes present on microscopic fluid analysis). Colonization was defined as isolation of an organism from a nonsterile body site in the absence of at least two of the signs of infection given above.

Among the patients judged to be infected, clinical response was defined as resolution of the signs of infection given above with appropriate therapy. Microbiologic response in patients for whom follow-up culture data were available was defined as the absence of the offending pathogen.

Susceptibility testing. Susceptibilities of clinical isolates were tested by using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.) containing a range of antimicrobial agent concentrations in serial twofold dilutions

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TABLE 1. Location and culture date of ceftazidime-resistant members of the family *Enterobacteriaceae*

Ward	No. of patients with resistant isolates	Species	Mo of isolation (8/1988–1/1989)
West 2	2	*K. pneumoniae* *E. cloacae*	8, 12
Main 3	1	*K. pneumoniae*	9
Main 4	3	*K. pneumoniae*	8, 11, 12
Main 5	3	*E. cloacae*, *E. coli*, *K. pneumoniae*, *Serratia* spp.	9, 9, 10
South 2	3	*K. pneumoniae* *E. agglomerans*	8, 9, 1
South 3	2	*K. pneumoniae* *E. cloacae*	10, 1
South 4	1	*K. pneumoniae*	11
East 1	3	*K. pneumoniae*	10, 11, 11
East 2	2	*K. pneumoniae*, *C. diversus*	9, 11
East 3	1	*K. pneumoniae*	10
East 4	1	*K. pneumoniae*	11
East 5	2	*K. pneumoniae*	9, 1

(26). Standard dilutions of overnight broth cultures were inoculated onto antibiotic-containing plates by using a 32-prong inoculating device (Craft Machine, Inc., Chester, Pa.) to yield a final inoculum of approximately 10^4 CFU per spot. The MIC was defined as the lowest concentration of antibiotic which inhibited visible growth after 20 h of incubation at 35°C.

Disk susceptibility testing was performed by using antibiotic-impregnated disks (Difco Laboratories, Detroit, Mich.).

Antimicrobial agents. Antimicrobial agents were obtained as follows: cefsulodin, Abbott Laboratories, North Chicago, Ill.; potassium clavulanate, Beecham Laboratories, Bristol, Tenn.; gentamicin, Elkin-Sinn, Cherry Hill, N.J.; cefuroxime, Glaxo Pharmaceuticals, Research Triangle Park, N.C.; cefotaxime and cefpirome, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.; ceftriaxone, Hoffmann-La Roche Inc., Nutley, N.J.; tazobactam, Lederle Laboratories, Pearl River, N.Y.; ceftazidime, cephalothin, and moxalactam, Eli Lilly, Inc., Indianapolis, Ind.; cefoxitin and imipenem, Merck Sharp & Dohme, West Point, Pa.; ciprofloxacin, Miles Pharmaceuticals, West Haven, Conn.; cefoperazone and sulbactam, Pfizer, Inc., New York, N.Y.; ceftibuten, Schering Corp., Bloomfield, N.J.; chloramphenicol; cloxacillin, tetracycline, and rifampin, Sigma Chemical Co., St. Louis, Mo.; cefotetan, Stuart Pharmaceuticals, Wilmington, Del.; and aztreonam, E. R. Squibb & Sons, Princeton, N.J.

Conjugation experiments. Tests for transfer of resistance were carried out by using as recipient *Escherichia coli* K-12 strain J53-2 (*met pro* Rif^r) (5). Liquid matings were performed in L broth containing 0.2% glucose incubated overnight at 37°C or by a filter paper mating technique with incubation at both 30 and 37°C overnight as described previously (15). Transconjugants were selected on glucose minimal medium (15) agar plates containing 10 μg of ceftazidime per ml, 30 μg of methionine per ml, 50 μg of proline per ml, and 100 μg of rifampin per ml for counterselection.

β-Lactamase characterization. Bacterial extracts were prepared by sonication, and β-lactamase was identified with the chromogenic substrate nitrocefin after analytical isoelectric focusing on a polyacrylamide gel (24), except that the gel contained 1.7 ml of Ampholine at pH 3.5 to 10.0 and 1.5 ml of Ampholine at pH 4.0 to 6.0.

An inhibitor profile was determined by a microdilution plate assay in which a standard amount of β-lactamase was reacted with nitrocefin in the presence of various inhibitors and competing substrates (11). Results are expressed in terms of maximal percent inhibition, which occurred at 2 min or, occasionally, at 5 min. When the maximal inhibition exceeded 25%, the rate of decrease of inhibition (slope) was also calculated from the linear portion of the inhibition curve as follows: (maximum percent inhibition − percent inhibition at 10 min)/10. Comparison enzymes included TEM-1, TEM-2, TEM-101 (6), TEM-5 (13), TEM-6 (2), TEM-7 (6), TEM-9 (21), TEM-10 (16), and CAZ-2 (4). The specific strains and plasmids used have been described in detail elsewhere (11). Each assay was repeated three to seven times.

DNA techniques. Plasmids were isolated by alkaline lysis techniques (7, 22), separated by electrophoresis on 0.7% agarose (Sigma), and visualized with UV light after treatment with ethidium bromide.

Restriction digestion was carried out with the restriction endonucleases *Hin*dIII and *Eco*RI (Boehringer-Mannheim, Indianapolis, Ind.) according to the specifications of the manufacturer.

Plasmid DNA was transferred to nitrocellulose filters by the method of Southern (20). A 424-bp *Bgl*I-*Hin*cII fragment of plasmid pBR322, internal to the TEM-1 β-lactamase gene (10), was labeled with digoxigenin as described by the manufacturer (Boehringer-Mannheim). Hybridization was carried out under conditions of high stringency with detection of label by enzyme-linked immunoassay.

RESULTS

Ceftazidime was introduced at Youville Hospital in December 1985 and quickly came into wide use for empiric

TABLE 2. Inhibition profiles of TEM-derived β-lactamases

β-Lactamase source	pI	Clavulanate		Sulbactam		Tazobactam		Cefoxitin		Cefotetan		Moxalactam		Cefotaxime		Ceftazidime	
		Avg ± SE[a]	SL[b]	Avg ± SE	SL	Avg ± SE	SL	Avg ± SE	SL	Avg ± SE	SL	Avg ± SE	SL	Avg ± SE	SL	Avg ± SE	SL
YOU-1	5.57	83 ± 2	<0.3	85 ± 2	<0.3	88 ± 1	<0.3	84 ± 1	<0.3	89 ± 1	<0.3	72 ± 4	<0.3	86 ± 1	0.5	45 ± 5	1.7
TEM-5	5.57	33 ± 3	0.6	39 ± 2	0.9	16 ± 4	<0.3	87 ± 3	<0.3	85 ± 2	<0.3	70 ± 3	0.4	75 ± 2	1.5	6 ± 1	
TEM-10	5.57	73 ± 3	<0.3	77 ± 2	0.3	69 ± 1	0.4	81 ± 4	<0.3	81 ± 2	<0.3	76 ± 3	<0.3	79 ± 4	0.3	38 ± 0	1.4
CAZ-2	6.0	64 ± 2	0.4	83 ± 1	0.3	70 ± 1	0.5	78 ± 1	<0.3	87 ± 2	<0.3	75 ± 2	<0.3	83 ± 1	0.7	38 ± 0	2.2
YOU-2	5.2	90 ± 1	<0.3	95 ± 2	<0.3	94 ± 1	<0.3	84 ± 4	<0.3	83 ± 3	<0.3	56 ± 5	<0.3	66 ± 4	2.7	0	
TEM-101	5.2	82 ± 5	0.5	93 ± 0	<0.3	93 ± 2	0.4	86 ± 3	<0.3	80 ± 4	<0.3	47 ± 5	<0.3	66 ± 7	1.4	4 ± 2	

[a] Average of maximum percent inhibition ± standard error.
[b] SL, slope, given when the maximum inhibition was >25%.

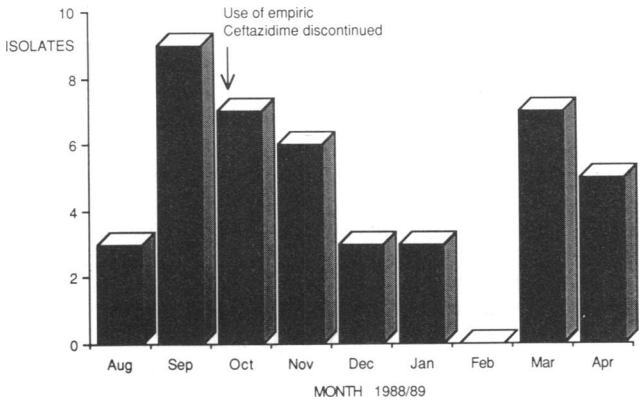

FIG. 1. Number of ceftazidime-resistant enterobacteria isolated at Youville Hospital from the start of the outbreak through April 1989. The date on which empiric ceftazidime use was discontinued (1 October 1989) is indicated.

therapy. Ceftazidime-resistant members of the family *Enterobacteriaceae* were first noted in December 1987 and occurred sporadically until it was recognized as a serious problem in August 1988.

Clinical evaluation. Between August 1988 and January 1989, we identified 29 ceftazidime-resistant *Enterobacteriaceae* from 25 patients. Among these strains were 22 *Klebsiella pneumoniae*; 3 *Enterobacter cloacae*; and 1 each of *Enterobacter agglomerans*, *Citrobacter diversus*, *Escherichia coli*, and *Serratia* species. Of the 15 isolates available for confirmatory identification, 13 were *K. pneumoniae* and 2 were *E. cloacae*.

Resistant organisms were isolated from sputum (n = 11), urine (n = 8), wound (n = 7), conjunctiva (n = 1), blood (n = 1), and peritoneal dialysate (n = 1). Of the 29 isolates, 16 were cultured in conjunction with significant signs of clinical infection (eight cases of pneumonia, five urinary tract infections, one wound infection, one peritoneal dialysis-associated case of peritonitis, and one case of septicemia). The remaining 13 isolates were judged to be colonizing only. Of 14 infected patients treated with agents that were active against the isolates, all had a favorable clinical response. Antibiotics used to treat these infections included amikacin (n = 4), cefotaxime (n = 4), trimethoprim-sulfamethoxazole (n = 3), and ciprofloxacin (n = 3). The offending organism was absent from subsequent cultures in eight of eight cases for which follow-up cultures were available.

Review of patient location revealed no geographical clustering of cases (Table 1). Each ward had at least one patient from whom a ceftazidime-resistant isolate was cultured, and no ward had more than three patients from whom a ceftazidime resistant isolate was cultured. There was some evidence of temporal clustering within a given ward. In ward Main 5, for example, there were three isolates in three patients in a 24-day period. Two patients developed sequential infections with different ceftazidime-resistant species. One of these patients developed a ceftazidime-resistant *E. cloacae* pneumonia on 13 October 1988 which was successfully treated with trimethoprim-sulfamethoxazole. On 31 October 1988, she developed septicemia with ceftazidime-resistant *E. coli*, which was successfully treated with cefotaxime. She then developed recurrent pneumonia on 14 November 1988 with a ceftazidime-resistant *Serratia* species cultured from her sputum.

In 15 of 29 (53%) cases, patients had received ceftazidime in the month prior to the isolation of resistant organisms. Ten of 16 (63%) episodes of infection also occurred in patients with prior exposure to ceftazidime. An additional 11 patients had been exposed to some β-lactam in the month prior to isolation of a resistant organism, although in 6 of these patients the β-lactam was cefotaxime, to which the organisms appeared to be susceptible.

Empiric ceftazidime use was discontinued at Youville Hospital on 1 October 1988. Although there has been a decline in the number of ceftazidime-resistant isolates since then, they have not been eradicated (Fig. 1).

Laboratory studies. Analysis of MIC data revealed two distinct patterns of β-lactam susceptibility. One strain demonstrating each pattern was selected for further study. *K. pneumoniae* 5934, which was cultured from the urine of a patient whose primary illness was pneumonia, was inhibited by 32 μg of ceftazidime per ml, while *K. pneumoniae* 5657, which was cultured from the sputum of a patient with pneumonia, was inhibited only by 256 μg of ceftazidime per ml. Both strains were inhibited by 0.5 μg of ceftazidime per ml in combination with 10 μg of the β-lactamase inhibitor sulbactam per ml.

Conjugation experiments. *K. pneumoniae* 5934 and 5657 transferred ceftazidime resistance to *E. coli* J53-2 at frequencies of 5×10^{-5} and 1×10^{-3}, respectively, yielding transconjugants termed J53-2(pMG224) and J53-2(pMG225). In addition to β-lactam resistance, both pMG224 and pMG225 determined resistance to chloramphenicol, gentamicin, kanamycin, streptomycin, sulfonamide, tetracycline, trimethoprim, mercuric chloride, and potassium tellurite. Transfer of both plasmids was much more efficient at 30°C than it was at 43°C. The properties of tellurite resistance and

TABLE 2—*Continued*

Aztreonam		Ceftriaxone		Cefsulodin		Cefpirome		Ceftibuten (Avg ± SE)	Cefuroxime (Avg ± SE)	Cefoperazone		Imipenem		Cloxacillin	
Avg ± SE	SL	Avg ± SE	SL	Avg ± SE	SL	Avg ± SE	SL			Avg ± SE	SL	Avg ± SE	SL	Avg ± SE	SL
74 ± 1	1.6	75 ± 3	1.4	35 ± 4	2.7	48 ± 2	2	25 ± 2	4 ± 4	90 ± 1	0.2	82 ± 5	<0.3	90 ± 1	<0.3
30 ± 4	2.9	56 ± 2	3.9	78 ± 3	1.3	8 ± 2		14 ± 5	10 ± 2	72 ± 2	1.6	92 ± 2	<0.3	92 ± 2	0.5
78 ± 2	0.5	73 ± 2	0.7	63 ± 3	0.9	26 ± 4	1.4	24 ± 3	16 ± 3	72 ± 5	0.6	84 ± 0	<0.3	84 ± 4	>0.3
69 ± 2	1.6	71 ± 1	1.6	23 ± 2		56 ± 2	2.5	22 ± 5	3 ± 1	84 ± 1	0.7	87 ± 1	<0.3	89 ± 2	<0.3
20 ± 2		33 ± 4	2.1	32 ± 2	2.1	33 ± 1	2.5	10 ± 1	8 ± 2	85 ± 3	1.5	90 ± 4	<0.3	95 ± 2	<0.3
17 ± 9		35 ± 11	2.2	31 ± 7	2.0	33 ± 6	2.1	16 ± 8	9 ± 5	81 ± 3	1.5	95 ± 2	<0.3	94 ± 1	<0.3

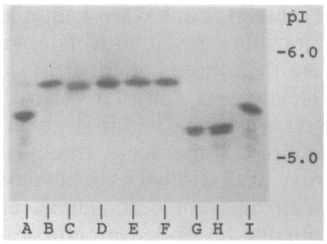

FIG. 2. Analytical isoelectric focusing of β-lactamases encoded by pMG224 (YOU-2) and pMG225 (YOU-1) compared with known extended-spectrum enzymes with isoelectric points in the same pH range. Lanes: A, TEM-1 (reference standard); B, YOU-1; C, TEM-9 (RHH-1); D, TEM-5 (CAZ-1); E, TEM-10; F, YOU-1; G, TEM-101; H, YOU-2; I, TEM-1.

thermosensitive transfer suggest that pMG224 and pMG225 belong to incompatibility group HI2 (23).

The two available *E. cloacae* strains contained similar transmissible ceftazidime resistance plasmids encoding the same multiresistance pattern as that encoded by pMG224 or pMG225.

β-Lactamase characterization. The β-lactamase encoded by pMG224, designated YOU-2, had a pI of 5.2, while that produced by pMG225, termed YOU-1, had a pI of 5.57 (Fig. 2). The results in Table 2 show that YOU-2 was indistinguishable both in pI and reactions with inhibitors from TEM-101, an enzyme derived in the laboratory from TEM-1 by selection on ceftazidime plates (6). YOU-1 focused at a pI higher than that of TEM-9 but similar to that of TEM-5 and TEM-10 (Fig. 2). However, YOU-1 differed from TEM-5 in that it was poorly inhibited by cefsulodin and was highly inhibited by cefpirome. Additionally, YOU-1 presented a relatively fast decrease in inhibition by aztreonam, cefpirome, and ceftriaxone compared with TEM-10. Indeed, YOU-1 more closely resembled CAZ-2 than TEM-5 or TEM-10 in its inhibition profile but could readily be distinguished from it by isoelectric focusing (Fig. 3).

The spectra of resistance conferred by YOU-1, YOU-2, and several previously described ceftazidime hydrolyzing enzymes are given in Table 3. Each β-lactamase enhanced resistance to ceftazidime and aztreonam and, to a lesser degree, to cefotaxime and ceftriaxone, unlike TEM-1 or TEM-2, which could not effectively hydrolyze these substrates. YOU-1 provided a higher level of β-lactam resistance than that provided by YOU-2. The majority of the ceftazidime-resistant isolates available for study (11 of 15 isolates) followed the pattern of *K. pneumoniae* 5657 and *E. coli* J53-2(pMG225).

Plasmid analysis. Ceftazidime resistance could be transferred to *E. coli* J53-2 from 9 of 15 isolates. In each case, resistance to at least chloramphenicol, gentamicin, and tetracycline transferred along with ceftazidime resistance. Transconjugants contained a plasmid of at least 180 kb, but in those derived from strain 5934, the responsible plasmid was slightly smaller.

The clinical isolates themselves showed a variety of plasmid patterns, supporting the impression that the outbreak was not caused by dissemination of a single organism throughout the hospital (Fig. 4). One isolate (strain 5624) lacked the 180-kb plasmid seen in other strains and was susceptible to chloramphenicol and tetracycline but, nonetheless, elaborated a β-lactamase with a pI of 5.57 like that encoded by pMG225.

Restriction digestion of plasmids in transconjugants revealed identical patterns in eight of nine isolates, with only pMG224 (derived from strain 5934) showing a different pattern. Figure 5 shows a comparison of pMG225, which represented the most common pattern and encoded YOU-1 β-lactamase, and pMG224, which encoded YOU-2 β-lactamase. Many bands of identical size were seen in the digests of the two plasmids, but there were at least two bands that were unique to pMG224 and one that was unique to pMG225.

With an internal fragment of the TEM-1 β-lactamase gene used as a probe, hybridization to both plasmids under stringent conditions was demonstrated, indicating that both

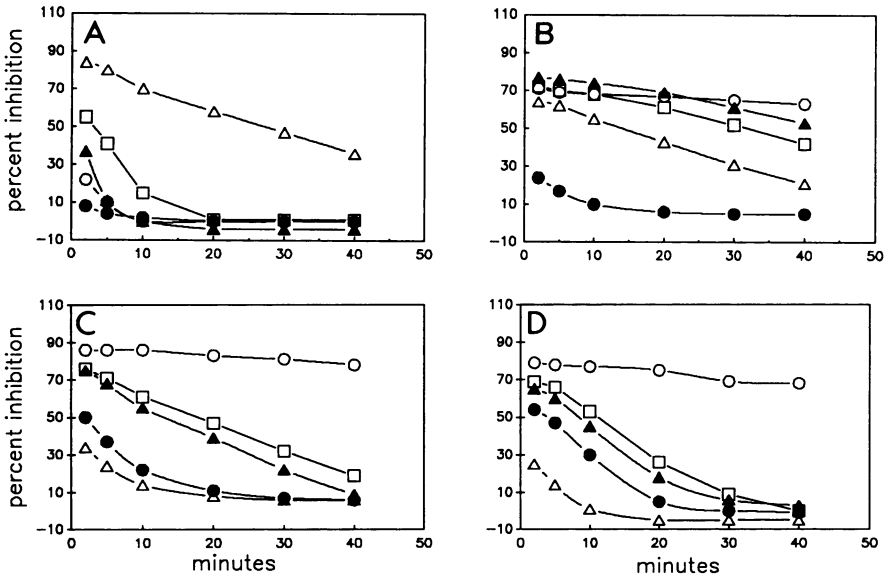

FIG. 3. Inhibition profile of TEM-5 (A), TEM-10 (B), YOU-1 (C), and CAZ-2 (D). Symbols: ▲, aztreonam; ●, cefpirome; △, cefsulodin; □, ceftriaxone; ○, tazobactam.

TABLE 3. β-Lactam MICs for strains producing extended-spectrum β-lactamases

J53-2 derivative	β-Lactamase	pI	MIC (μg/ml)						
			Ceftazidime	Ceftazidime + sulbactam[a]	Aztreonam	Cefotaxime	Ceftriaxone	Cefoxitin	Imipenem
R^{-}[b]			0.5	0.5	1	0.25	0.12	16	0.25
R1	TEM-1	5.4	0.12	≤0.25	1	0.12	0.12	4	0.5
RP4	TEM-2	5.6	0.25	0.5	1	0.12	0.12	4	0.25
pCFF14	TEM-5	5.57	256	0.5	32	4	16	4	0.5
pMG226[c]	TEM-6	5.9	512	0.5	32	2	2	16	0.25
pIF100	TEM-7	5.41	32	0.5	8	0.5	0.5	8	0.25
pMG228[d]	TEM-9	5.5	512	0.5	64	2	4	16	0.25
pMG224	YOU-2	5.2	64	0.5	8	0.5	0.25	16	0.25
pMG225	YOU-1	5.57	256	0.5	32	1	1	8	0.5

[a] Sulbactam was used at 10 μg/ml.
[b] R^{-}, Plasmid free.
[c] Plasmid not named in original report (2). It originated in *E. coli* HB 251.
[d] Plasmid not named in original report (21). It was supplied in *K. pneumoniae* 2633E.

YOU-1 and YOU-2 are encoded by genes that are closely related to the TEM-1 gene. However, after restriction digestion of plasmids pMG224 and pMG225, the hybridizing fragments differed in size (Fig. 5), lending further support to the concept that the enzymes are encoded by related but distinct plasmids. Included as a control in Fig. 5 is a digest of plasmid pMG223, which was isolated from a patient at the Massachusetts General Hospital in February 1988 (8). This plasmid also belongs to the HI2 incompatibility group and encodes both TEM-1 and an extended-spectrum enzyme with a pI of 5.57 and with an inhibition profile indistinguishable from that of TEM-10, accounting for hybridization to two separate restriction fragments with the TEM-1 probe.

DISCUSSION

Ceftazidime has gained widespread use by virtue of its substantial activity against a broad spectrum of gram-negative pathogens, including *P. aeruginosa*. Recent reports, however, have described members of the family *Enterobacteriaceae* with β-lactamases that have a high degree of activity against this agent (14). These isolates have generally occurred sporadically, especially in intensive care wards, and have been reported outside the United States. We described what we believe is the first reported hospital-wide outbreak in the United States of colonization and infection with isolates of *Enterobacteriaceae* that are highly resistant to ceftazidime.

This outbreak demonstrates the ease with which *Enterobacteriaceae* can acquire and spread resistance determinants when they are subjected to antibiotic pressure. The development of resistance appears to have followed at least two courses with the production of two separate enzymes that differ in their pIs and in the level of resistance to β-lactams. The plasmids encoding these β-lactamases, however, are related in that they have similar restriction digests, equivalent resistances to non-β-lactam antibiotics, and identical incompatibility specificities. Furthermore, both novel β-lactamase genes are related to TEM-1 by DNA hybridization,

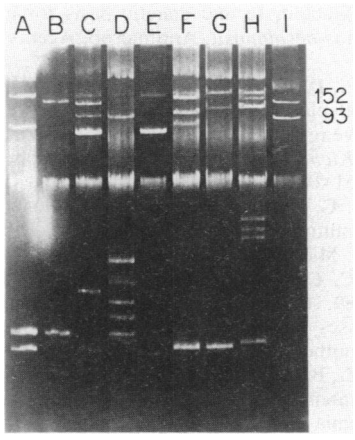

FIG. 4. Agarose gel electrophoresis of plasmids in selected clinical isolates. Lanes: A, *K. pneumoniae* 5657 containing pMG225; B, *K. pneumoniae* 5934 containing pMG224; C through H, other ceftazidime-resistant *K. pneumoniae* strains, except for lane D, which is *E. cloacae*; I, plasmid size standards with the molecular size (in kilobase pairs) indicated in the right margin.

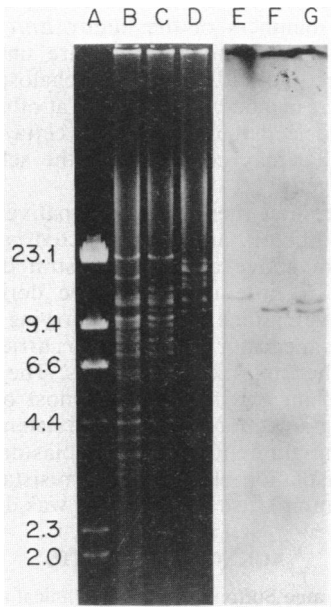

FIG. 5. Agarose gel electrophoresis of *Hin*dIII-digested DNA. Lanes: A, phage lambda molecular size standard; B, pMG225; C, pMG224; D, pMG223, a plasmid producing two TEM-related β-lactamases; E through G, Southern blot of lanes B through D, respectively, hybridized with the TEM-1 probe. Fragment size (in kilobases) is indicated in the left margin.

suggesting that the enzymes arose from a single parent carrying an ancestral TEM gene, either through separate or sequential mutational events.

Nosocomial transmission of resistant organisms was suggested by analysis of patterns of spread and plasmid analysis which demonstrated the presence of the same 180-kb plasmid in organisms derived from different patients. However, the wide variety of plasmid patterns seen, along with the identification of the resistance characteristics in five distinct genera of bacteria, argues that the primary mode of transmission in this outbreak was via the dissemination of resistance plasmids between different organisms.

So many extended-spectrum β-lactamases have been described that isoelectric focusing is becoming insufficient for their differentiation (14). Inhibitor profiling should prove useful, but oligonucleotide typing (C. Mabilat, I. Guilvout, and P. Courvalin, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 667, 1989) or nucleotide sequencing may soon become obligatory for classifying these enzymes. Several naturally occurring ceftazidime-hydrolyzing enzymes with pIs of between 5.2 and 5.3 have been described, including CAZ-3 (18), TEM-E2 (12), and others (19, 27). The relationship of these enzymes to each other, laboratory mutants like TEM-101 (6), or YOU-2 with pI of 5.2 is not yet known. Several extended-spectrum β-lactamases have also been reported in the pI range of between 5.5 and 5.6, including TEM-5 (13), TEM-10 (16), and CAZ-lo (25). YOU-1 (pI 5.57) differed from TEM-5 and TEM-10 in its reactions with inhibitors. Nucleotide sequencing is in progress to define its relationship to other extended-spectrum β-lactamases.

The frequency with which patients were exposed to ceftazidime prior to the isolation of resistant organisms suggested that the outbreak was related to selective pressure from ceftazidime use. The prolonged lengths of patient stays at the chronic-care facility may also have facilitated emergence of organisms that were repeatedly selected for ceftazidime resistance. Most of the previously reported ceftazidime-resistant members of the family *Enterobacteriaceae* have been isolated from intensive-care units where prolonged exposure to broad-spectrum cephalosporins was also seen. Study of resistance development at other chronic-care hospitals will be needed to differentiate ceftazidime use from other factors that may contribute to the selection of such resistant pathogens.

It is fortunate that therapeutic alternatives existed in all cases in this outbreak, and each infected patient who was administered an active agent demonstrated a clinical response. There is little comfort to be derived from this success, however, since plasmids carrying the genes for these extended-spectrum enzymes also carried resistance to a variety of other antimicrobial agents. The activity of the extended-spectrum enzymes against most other β-lactams provides strong selection for their persistence, despite removal of the initial offending agent, a bias demonstrated by the continued isolation of ceftazidime-resistant organisms 7 months after empiric use of this agent was discontinued.

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