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Extended-Spectrum β-Lactamase-Producing *Klebsiella pneumoniae* Strains Causing Nosocomial Outbreaks of Infection in the United Kingdom

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Representative isolates from 10 distinct extended-spectrum β-lactamase-producing strains of *Klebsiella pneumoniae* that caused hospital outbreaks in the United Kingdom from 1991 to 1994 were examined for relationships between their enzymes and plasmids. The β-lactamases were identified by a combination of isoelectric focusing and gene sequencing. SHV-2 β-lactamase was produced by isolates from four outbreaks, SHV-5 was involved in three, and SHV-4, TEM-15, and TEM-26 were involved in one outbreak each. All of the extended-spectrum β-lactamases were encoded by self-transmissible plasmids, with sizes ranging from about 70 to 160 kb. No similarities between the restriction digest patterns of the extended-spectrum β-lactamase-encoding plasmids were detected, except to some extent between those that produced TEM-15 and TEM-26. Thus, outbreaks of hospital infection with these organisms in the United Kingdom from 1991 to 1994 involved distinct organisms and resistance plasmids and appeared to be unrelated.

Members of the family *Enterobacteriaceae* expressing extended-spectrum β-lactamases conferring resistance to ceftazidime and other cephalosporins and derived from TEM-1 or SHV-1 enzymes (15) have become an increasing problem during the past two decades. During the 1980s, occurrences were rare and most reports involved single isolates (32), although there were a few outbreaks, most notably of TEM-3-producing *Klebsiella pneumoniae* in France (38). However, in the 1990s there have been many outbreak reports, most frequently of single strains of *Klebsiella* spp., including SHV-5-producing *Klebsiella* strains, in Australia (25), Germany (3), Austria (33), Great Britain (10), Italy (31), and the United States (27, 41). In addition, an SHV-5-encoding plasmid has spread among several strains of *Klebsiella* in Greece (21) and Austria, where three outbreaks caused by distinguishable klebsiellae carrying the same plasmid were reported (33). Other outbreaks have included SHV-3-producing *Klebsiella* in Great Britain (17), *Klebsiella* and other *Enterobacteriaceae* producing the related β-lactamases TEM-10B, TEM-12B, and TEM-26B in Great Britain (13), *Klebsiella* producing an extended-spectrum β-lactamase that was not identified but that spread between two hospitals in Great Britain (9), SHV-3- and SHV-4-producing klebsiellae in France (2, 4, 7, 28), TEM-26-producing *K. pneumoniae* in the United States (27, 40), and TEM-10-plus-TEM-12-producing *K. pneumoniae* in the United States (6). Thus, no single extended-spectrum β-lactamase has predominated. However, interhospital spread of extended-spectrum β-lactamase-producing *K. pneumoniae* has been demonstrated in the United States (24).

Since 1991, outbreaks of infection or colonization with distinct strains of cephalosporin-resistant *K. pneumoniae* have occurred in a number of hospitals in the United Kingdom, in addition to those already reported (9, 10, 13, 17). The epidemiology of a number of these outbreaks is to be described by other workers (18a).

Organisms, outbreaks, and susceptibility. In the present study, representative isolates of 10 distinct strains of cephalosporin-resistant *K. pneumoniae* producing hospital outbreaks in the United Kingdom between 1991 and 1994 were investigated to determine the relationships between their extended-spectrum β-lactamases and the plasmids encoding them. These organisms had been submitted to the Central Public Health Laboratory by hospitals who had epidemiological evidence of clinical outbreaks. All the outbreaks had been characterized by capsular serotyping and either bacteriophage typing or DNA fingerprinting by pulsed-field gel electrophoresis in the Laboratory of Hospital Infection. The isolates chosen for study were representative of the outbreaks and were distinct from each other by the typing methods used (18a).

MICs were determined by broth or agar dilution in Iso-Sensitest broth or agar (Oxoid, Basingstoke, United Kingdom) as described previously (17, 19, 35). National Committee for Clinical Laboratory Standards criteria were used to categorize strains as susceptible, intermediately resistant, or resistant (26). β-Lactamases were characterized by isoelectric focusing as described previously (22). Plasmids were extracted by use of either the method of Kado and Liu (18) or the alkaline lysis method of Birnboim and Doly (5). Extracted plasmids were digested with the restriction enzymes *Eco*RI, *Cla*I, *Bam*HI, and *Hin*dIII (Life Technologies, Paisley, United Kingdom), and the resulting fragments were separated by agarose gel electrophoresis. Each clinical isolate was mated with the recipient strain, *Escherichia coli* K-12 J62.1 (nalidixic acid resistant), or a rifampin-resistant mutant of it, in broth as described previ-

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ously (22). Transconjugants were selected on medium containing ceftazidime (4 μg/ml) plus nalidixic acid (100 μg/ml) or rifampin (200 μg/ml). Transconjugants were assessed for plasmid content and their antibiotic susceptibility.

Isolates from 10 distinct hospital outbreaks of infection or colonization with extended-spectrum β-lactamase-producing klebsiellas in the United Kingdom that occurred during the period 1991 to 1994 were investigated. Representative isolates (chosen on the basis of their typical antibiogram and serotype), which were all *K. pneumoniae*, were designated D1 to D10 and are listed in Table 1. The strains were considerably more resistant to ceftazidime, cefotaxime, and cefepime than were strains producing non-extended-spectrum β-lactamases. Clavulanic acid (2 μg/ml) substantially reduced the MICs of ceftazidime for all strains. Although the MICs of cefoxitin for some of the strains were higher than those usually found for *Klebsiella*, none was very high, and all strains were susceptible to carbapenems. The β-lactam resistance patterns of the transconjugants were similar to those of the corresponding donors, although sometimes with a lower degree of resistance (Table 1). Only one strain (D2) was susceptible to all the aminoglycosides tested (Table 2), but most strains were susceptible to amikacin. The strains were mostly susceptible to ciprofloxacin and trimethoprim but resistant to chloramphenicol and tetracycline.

Single-plasmid transconjugants were obtained from each of the clinical strains, and their plasmids were extracted and compared by restriction endonuclease digestion with *Eco*RI, *Cla*I, *Bam*HI, or *Hin*dIII. The plasmids in the transconjugants were large, 70 to 170 kb (Table 1). With each of these enzymes, the fragment patterns appeared distinct from the patterns for transconjugants obtained from donors D1 and D5. With *Eco*RI digestion, a number of common bands were noted for these two transconjugants (Fig. 1). However, greater diversity was noted with *Cla*I and *Hin*dIII digestion. Neither plasmid appeared to be digested by *Bam*HI.

DNA sequences of β-lactamases. PCR was used to amplify parts of SHV or TEM gene sequences in whole-cell DNA preparations as described previously (22). DNA preparations were made from the klebsiellae for detection of TEM gene sequences but from the transconjugants for detection of SHV gene sequences because of the chromosomal SHV-1 gene usually present in *K. pneumoniae*. An automated laser fluorescent DNA sequencer (Pharmacia Biotech, St. Albans, Hertfordshire, United Kingdom) was used to sequence the TEM PCR product, which had been labelled by the quick annealing method, as described previously (39). A similar procedure was used for SHV PCR products except that cycle sequencing was performed on 1 μg of purified PCR product obtained from transconjugants and with the reagents supplied in a Thermo Sequenase fluorescently labelled primer cycle sequencing kit (Amersham International, Buckinghamshire, United Kingdom). Reactions were performed as described in the manufacturer's instructions, with 2 pmol of fluorescently labelled primer OS-1 (22) or a primer we have designated SHV-i (5′-CCAGATCGGCGAACAACGTCACC-3′; bases 447 to 468 of the SHV structural gene) and the following cycling conditions: 25 cycles of 30 s at 60°C and 30 s at 98°C.

Thermostable DNA polymerase from *Thermus brockianus* (DynaZyme), 10× polymerase buffer, and magnesium chloride were supplied by Flowgen (Lichfield, Staffordshire, United Kingdom). Nucleotides were obtained from Sigma (Poole, Dorset, United Kingdom). Sterile distilled water was molecular biology grade (Bio-Rad, Hemel Hempstead, Hertfordshire, United Kingdom). Synthetic oligonucleotide primers, AutoRead sequencing kits, and automated laser fluorescence-grade

TABLE 1. MICs of β-lactam antibiotics and sizes of β-lactamase-encoding plasmids for *Klebsiella* outbreak strains and their *E. coli* transconjugants

Strain	MICs (μg/ml) of[a]:											β-Lactamase-encoding plasmids (kb)
	Ampicillin	Amox + Clav (2)	Amox + Clav (2:1)	Pip	Pip + Taz (4)	Temocillin	Ceftaz	Ceftaz + Clav (2)	Cefotaxime	Cefepime	Cefoxitin	Imipenem	Meropenem	
D1	>4,096	16	16	512	4	8	>64	1	1	4	2	0.25	0.06	78
D1 transconjugant	4,096	16					>64	1	1		4			
D2	>4,096	32	16	512	128	16	32	1	16	16	32	0.06	0.06	70
D2 transconjugant	2,048	4					2	0.25	2		4			
D3	4,096	8	4	128	4	8	64	0.5	16	2	16	0.06	0.06	130
D3 transconjugant	4,096	4					64	0.25	16		4			
D4	>4,096	64	8	512	128	8	>64	0.5	64	8	16	0.06	0.03	150
D4 transconjugant	>4,096	16					16	0.5	32		8			
D5	4,096	8	8	256	2	4	16	0.5	8	1	4	0.06	0.03	85
D5 transconjugant	2,048	8					16	0.5	16		8			
D6	>4,096	8	8	256	32	4	>64	0.5	8	2	4	0.06	0.06	125
D6 transconjugant	4,096	8					>64	0.5	16		16			
D7	>4,096	256	32	>512	128	8	>64	1	16	1	8	0.06	0.03	125
D7 transconjugant	1,024	4					16	0.25	2		4			
D8	4,096	8	8	128	4	4	8	0.5	4	0.5	8	0.06	0.03	150
D8 transconjugant	>4,096	16					8	0.5	16		4			
D9	>4,096	64	32	512	128	16	64	2	32	8	16	0.25	0.06	160
D9 transconjugant	>4,096	16					32	1	32		4			
D10	1,024	4	4	64	2	≤2	32	0.25	2	0.5	2	0.12	0.03	150
D10 transconjugant	512	4					32	0.25	1		4			

[a] Antibiotic abbreviations: Amox, amoxicillin; Clav, clavulanic acid; Pip, piperacillin; Taz, tazobactam; Ceftaz, ceftazidime.

TABLE 2. MICs of aminoglycosides and other antibiotics for *Klebsiella* outbreak strains

Strain	MICs (μg/ml) of:								
	Gentamicin	Netilmicin	Tobramycin	Amikacin	Streptomycin	Ciprofloxacin	Chloramphenicol	Tetracycline	Trimethoprim
D1	128	16	16	2	1	≤0.25	8	>64	0.5
D2	0.5	≤0.5	1	2	2	0.5	>128	8	0.5
D3	4	64	32	16	8	>8	16	4	>8
D4	32	16	128	32	4	1	>128	16	2
D5	1	64	32	16	1	1	32	>64	4
D6	128	16	16	≤1	1	≤0.25	128	8	>8
D7	128	128	64	32	256	>8	>128	>64	>8
D8	64	≤0.5	32	≤1	16	1	128	>64	4
D9	64	8	8	≤1	64	4	>128	>64	>8
D10	32	16	128	64	2	≤0.25	4	2	0.5

urea were supplied by Pharmacia Biotech. Hydrolink Long Ranger gel was obtained from Hoefer (Newcastle-under-Lyme, Staffordshire, United Kingdom). A Thermo Sequenase fluorescently labelled primer cycle sequencing kit with 7-deaza-dGTP was purchased from Amersham International. All other reagents were ANALAR grade obtained from BDH (Lutterworth, Leicestershire, United Kingdom).

The properties of the β-lactamases found in the outbreak strains are summarized in Table 3. Apart from strain D7, which produced TEM-1 in addition to an extended-spectrum β-lactamase, the strains possessed only one transferable β-lactamase. Two strains (D1 and D5) produced TEM-group extended-spectrum β-lactamases (TEM-26 and TEM-15, respectively). The other eight strains produced SHV-group extended-spectrum β-lactamases (SHV-2 by four, SHV-5 by three, and SHV-4 by one).

In this study, we investigated the extended-spectrum β-lactamases and the plasmids encoding them in a series of distinct outbreak strains of *K. pneumoniae* isolated in the United Kingdom between 1991 and 1994. SHV-group extended-spectrum β-lactamases were more common than TEM-group enzymes. The identification of the former was straightforward, but the nomenclature of one of the TEM enzymes requires some comment. The amino acid changes detected in the enzyme from strain D5 were identical to those given by Jacoby and Medeiros (15) and Knox (20) for TEM-15 on the basis of oligotyping (23). However, this enzyme has been omitted from the most recently published list of TEM enzymes produced by Bush and Jacoby (8), presumably because it had not at that time been fully sequenced. However, it has been reinstated in their list, which is accessible on the World Wide Web (at http://www.lahey.org/studies/webt.htm), as an enzyme with changes of glutamic acid to lysine at position 104 and of glycine to serine at position 238. We found some variability in the codon corresponding to the amino acid at position 240 in SHV β-lactamases, with glutamic acid encoded by GAG or GAA and lysine encoded by AAA or AAG (Table 3); such variability has been reported previously (29).

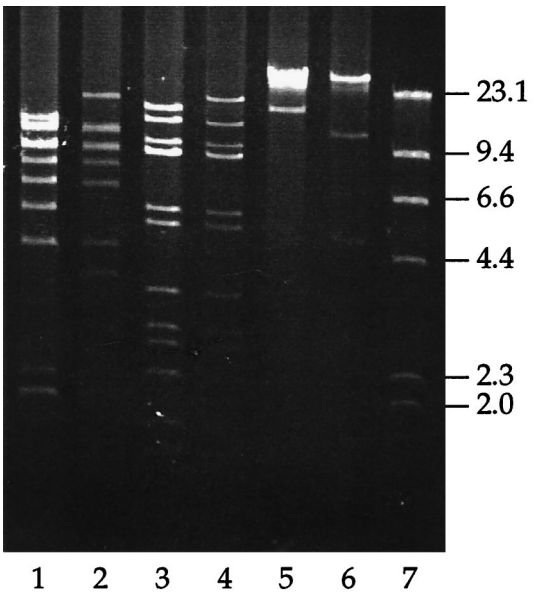

FIG. 1. Agarose gel electrophoresis of restriction digests of plasmids from the transconjugants of strains D1 and D5. Lane 1, D1 digested with *Cla*I; lane 2, D5 digested with *Cla*I; lane 3, D1 digested with *Eco*RI; lane 4, D5 digested with *Eco*RI; lane 5, D1 digested with *Hin*dIII; lane 6, D5 digested with *Hin*dIII; lane 7, molecular weight markers (with weights indicated, in thousands, to the right of the gel).

Since most strains of *K. pneumoniae* synthesize a chromosomally encoded β-lactamase with an isoelectric point of 7.6 that is closely related to SHV-1 (11, 22), surveys of β-lactamases in this species that do not take the location of the gene encoding the enzyme into account are impossible to interpret. Consequently, there is little information on the frequencies of plasmid-encoded β-lactamases in this organism. Although extended-spectrum SHV β-lactamases appear to have evolved from SHV-1, it is not known whether their genes are derived from the chromosomal gene and have subsequently moved on to plasmids or whether mutation of plasmid genes has occurred.

Although TEM-1 and TEM-2 can be transposon encoded (34) as can plasmid-encoded SHV-1 (30), extended-spectrum β-lactamases have generally not been found on transposons (16). However, genes for TEM-12 (12) and TEM-16 (37) have been reported to be located on transposons. It is not known whether the β-lactamases from the outbreak strains reported in this paper are transposon encoded, but the apparent lack of relatedness of the plasmids suggests that they are not, except perhaps for the TEM-group enzymes.

On the basis of National Committee for Clinical Laboratory Standards criteria (26), eight of the strains were susceptible to cefotaxime and one had intermediate resistance (Table 1), although all the strains were less susceptible than typical klebsiellae that do not produce extended-spectrum β-lactamases. However, extended-spectrum β-lactamase producers can readily mutate to hyperproduction of the enzyme and higher degrees of resistance (42), so we believe that therapy of infections caused by such organisms would not be appropriate. Similarly, although most of the strains were susceptible or had

TABLE 3. Isoelectric points and deduced amino acid sequence changes for β-lactamases from *Klebsiella* outbreak strains

Organism	pI	PCR results for:		Amino acid (codon)[a,b] at position:															Enzyme[a]
		TEM	SHV	39	42	69	104	153	164	165	182	205	237	238	240	244	265	275	276	
E. coli	5.4			Q	A	M	E (GAG)	H	R (CGT)	W	M	Q	A	G (GGT)	E	R	T	R	N	TEM-1[c]
Klebsiella D5	6.0 + 7.6	+					K (AAG)							S (AGT)						TEM-15
D5 transconjugant	6.0																			
Klebsiella D1	5.5 + 7.6	+					K (AAG)		S (AGT)											TEM-26
D1 transconjugant	5.5																			
E. coli	7.6			Q	G	M	D	R	R	W	T	R (CGG)	A	G (GGC)	E (GAG)	R	L	R	N	SHV-1[c]
Klebsiella D2	7.6													S (AGC)	E (GAA)					SHV-2
D2 transconjugant	7.6		+																	
Klebsiella D4	7.6													S (AGC)	E (GAG)					SHV-2
D4 transconjugant	7.6		+																	
Klebsiella D8	7.6													S (AGC)	E (GAG)					SHV-2
D8 transconjugant	7.6		+																	
Klebsiella D9	7.6 + 5.4	−												S (AGC)	E (GAG)					SHV-2
D9 transconjugant	7.6		+																	
Klebsiella D3	7.75											L (CTG)		S (AGC)	K (AAA)					SHV-4
D3 transconjugant	7.75		+																	
Klebsiella D6	7.6 + 8.2													S (AGC)	K (AAG)					SHV-5
D6 transconjugant	8.2		+																	
Klebsiella D7	5.4 + 7.6 + 8.2	+												S (AGC)	K (AAG)					TEM-1 + SHV-5
D7 transconjugant	5.4 + 8.2		+																	
Klebsiella D10	7.6 + 8.2													S (AGC)	K (AAG)					SHV-5
D10 transconjugant	8.2		+																	

[a] Sequence and enzyme data apply to the *Klebsiella*-transconjugant pair as a whole.
[b] Amino acid abbreviations: A, alanine; D, aspartic acid; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan. Nucleotide abbreviations: A, adenosine; C, cytosine; G, guanosine; T, thymidine. The consensus numbering scheme for group 2 (molecular class A β-lactamases) (1) is used.
[c] Data from Jacoby and Medeiros (15), Huletsky et al. (14), Knox (20), and Bush and Jacoby (8).

intermediate resistance to amoxicillin-clavulanic acid, mutation to hyperproduction may result in resistance to this and other β-lactam–β-lactamase inhibitor combinations.

All of the strains were multiply drug resistant. As has been reported previously for extended-spectrum β-lactamase producers (16), they were usually resistant to the aminoglycosides, with only one strain, D2, being susceptible to all four aminoglycosides tested. Five of the 10 strains were fully amikacin susceptible, a proportion that is slightly higher than the 9 of 15 reported by Jacoby and Sutton (16). Resistance to streptomycin was uncommon; this contrasts with the situation 20 years ago, when 86% of gentamicin-resistant enterobacteria were also resistant to streptomycin (36), and presumably reflects the reduced selective pressure resulting in loss of genes encoding streptomycin resistance from plasmids. The carbapenems (imipenem and meropenem) were the only agents tested that were active against all the strains, but seven strains were ciprofloxacin susceptible. However, greater use of this and other quinolones may lead to selection of resistant mutants.

In conclusion, hospital outbreaks of *K. pneumoniae* producing extended-spectrum β-lactamases in the United Kingdom between 1991 and 1994 have been caused by distinct single strains. Two outbreak strains produced TEM enzymes (TEM-15 and TEM-26), three produced SHV-2, three produced SHV-5, and one produced SHV-4. These enzymes were encoded on large transferable plasmids that appeared to be distinct from each other. The organisms were variably multiply resistant to other antimicrobial agents and were usually resistant to gentamicin and sometimes to other aminoglycosides.

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