JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1996, p. 358–363 Vol. 34, No. 2
0095-1137/96/$04.00+0

Hospital Outbreak of *Klebsiella pneumoniae* Resistant to Broad-Spectrum Cephalosporins and β-Lactam–β-Lactamase Inhibitor Combinations by Hyperproduction of SHV-5 β-Lactamase

G. L. FRENCH,* K. P. SHANNON, AND N. SIMMONS

Department of Microbiology, UMDS and Guy's and St. Thomas's Hospital Trust, London SE1 9RT, United Kingdom

Received 30 May 1995/Returned for modification 4 October 1995/Accepted 2 November 1995

An aminoglycoside- and ceftazidime-resistant strain of *Klebsiella pneumoniae* K2 producing the extended-spectrum β-lactamase SHV-5 infected or colonized 14 pediatric patients at Guy's Hospital. The patients were mostly neonates recovering from cardiac surgery for congenital defects. The organism was also isolated from a nurse and from the father of one of the children. Four patients had septicemia, and two septicemic neonates with postoperative renal failure died. Aminoglycoside and cephalosporin resistance transferred to *Escherichia coli* in vitro on a 160-kb plasmid, and a similar resistant *E. coli* strain was isolated from the stools of one of the affected children. The epidemic organism colonized the bowel and skin and was probably transmitted via staff hands. Five wards were involved because of extensive patient movements. The outbreak was controlled by patient isolation and attention to handwashing. All of the isolates of the outbreak strain were identical by phage typing, ribotyping, plasmid profiling, and biochemical and serological testing, but they varied in their production of SHV-5. Some isolates produced normal amounts of SHV-5 and were susceptible to β-lactam–β-lactamase inhibitor combinations. Others, including the single isolate of multiresistant *E. coli*, produced up to five times as much enzyme as "normal" isolates. This hyperproduction resulted in increased resistance to several penicillins and cephalosporins and to the β-lactam–β-lactamase inhibitor combinations amoxicillin-clavulanic acid, ampicillin-sulbactam, piperacillin-tazobactam, and ceftazidime-clavulanic acid. The hyperproduction of SHV-5 by *K. pneumoniae* and *E. coli* seen in this outbreak suggests that β-lactam–β-lactamase inhibitor combinations may be unreliable for the treatment of organisms producing extended-spectrum β-lactamases.

Klebsiella pneumoniae is naturally resistant to ampicillin and amoxicillin, usually by the production of SHV-1 β-lactamase encoded on the chromosome or a transferable plasmid (36, 48). Because of this natural resistance, *K. pneumoniae* often colonizes the skin and mucosa of hospitalized patients treated with ampicillin or similar drugs, and it may go on to produce invasive infection and septicemia. Most colonized patients are asymptomatic, but they may act as sources of cross-infection for others, with the outbreak strain usually being transferred on staff hands (9). During the 1970s and early 1980s, there were frequent reports of hospital epidemics of gentamicin-resistant *K. pneumoniae*. These organisms caused significant morbidity and mortality in compromised patients such as neonates, and they often transferred their resistances to other species of enterobacteria during the outbreaks (10, 11, 18, 20, 29, 35, 43, 54).

Following the introduction of the extended- and broad-spectrum cephalosporins and β-lactam–β-lactamase inhibitor combinations (which are resistant to the action of SHV-1 β-lactamase), outbreaks of gentamicin-resistant klebsiellas became much less common. Recently, however, strains of *K. pneumoniae* (and *K. pneumoniae* subsp. *ozaenae*) that are resistant to extended- and broad-spectrum cephalosporins and can spread within hospitals have appeared. Initially such organisms were isolated only sporadically or in small clusters (25, 45, 50), but recently much larger outbreaks have been reported (2, 4, 12, 23, 31, 34). Cephalosporin resistance is mediated by the production of extended-spectrum β-lactamases. These enzymes are usually the result of mutations of SHV-1 or of TEM-1 or TEM-2, the plasmid-mediated β-lactamases commonly found in *Escherichia coli* (22). Genes encoding these enzymes are located on transferable plasmids that often carry other resistance factors, including resistance to aminoglycosides.

These new multiresistant klebsiellas produce outbreaks similar to those of the 1970s, and they can pass aminoglycoside and cephalosporin resistances to other species (7, 34, 49). Nevertheless, most strains produce β-lactamases that remain susceptible to inhibition by clavulanic acid and other commercially available β-lactamase inhibitors. Thus, although these strains are resistant to cephalosporins, they are usually susceptible to amoxicillin-clavulanic acid and other β-lactam–β-lactamase inhibitor combinations (21).

E. coli is frequently resistant to ampicillin and amoxicillin by the production of TEM-1 β-lactamase, but resistant strains are usually susceptible to β-lactam–β-lactamase inhibitor combinations. Recently, however, there have been increasing reports of ampicillin- and amoxicillin-resistant strains of *E. coli* that are also resistant to amoxicillin-clavulanic acid. This is often due to the production of excessive amounts of TEM-1 β-lactamase that swamp the activity of clavulanic acid (15, 28, 40, 47). Such strains are said to be hyperproducers of TEM-1. We report here a hospital outbreak of aminoglycoside-resistant *K. pneumoniae* resistant to broad-spectrum cephalosporins and to amoxicillin-clavulanic acid and other available β-lactam–β-lactamase inhibitor combinations by hyperproduction of the ex-

* Corresponding author. Mailing address: Department of Microbiology, UMDS, St. Thomas's Campus, London, SE1 7EH, United Kingdom. Phone: (44) 171-928 9292, ext. 2597. Fax: (44) 171-928 0730.

TABLE 1. Patients and sites of isolation[a]

Patient	Age	Site(s)	Diagnosis
1	3 yr	Catheter, blood	CRF
2	8 mo	Line tip	CHD
3	2 mo	Urine	CHD
4	9 mo	CVL tip	CHD
5	16 days	Sputum, blood	CHD
6	18 days	Wound, blood	CHD
7	28 days	PDF, blood	CHD, RF
8	5 yr	Sputum	Trauma
9	8 yr	PDF	CRF
10	5 mo	Stool (carrier)	CHD
11	1 mo	Stool (carrier)	CHD
12	4 mo	Stool (carrier)	CHD
13	1 yr	Wound	RF
14	13 yr	Wound	CRF

[a] Abbreviations: CRF, chronical renal failure; CHD, congenital heart disease; RF, renal failure; CVL, central venous line; PDF, peritoneal dialysis fluid.

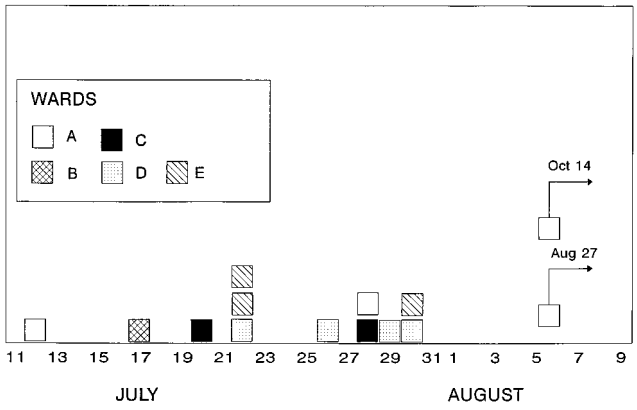

FIG. 1. Outbreak pattern. Each box represents a single patient and shows the time and ward of the isolation of the first *K. pneumoniae* isolate.

tended-spectrum β-lactamase SHV-5. These resistances transferred to *E. coli* both in vitro and in vivo.

MATERIALS AND METHODS

Organism identification and typing. Isolates of *K. pneumoniae* were identified by their reactions in standard biochemical tests. Serotyping, phage typing, and ribotyping were kindly performed in the Laboratory of Hospital Infection, Central Public Health Laboratory, Colindale, London, United Kingdom. Serotyping was performed by countercurrent immunoelectrophoresis with 77 antisera against capsular K antigens (37) and confirmed by capsule swelling with specific K antiserum. Isolates were phage typed by the scheme of Gaston et al. (17). Ribotyping was performed as described by Garaizar et al. (16).

Determination of MICs. MICs were determined by the agar dilution method on Oxoid IsoSensitest agar (CM471) with an inoculum of about 10^4 organisms per spot as described previously (24). The MICs of some antibiotics were determined by the broth dilution method either in Sensititre trays with an inoculum of 10^6 organisms per ml (19) or in microtiter trays with the Dynatech MIC2000 System with an inoculum of 10^4 organisms per 100 μl as described previously (46).

β-Lactamase studies. β-Lactamase activity was measured by monitoring the rate of hydrolysis of nitrocefin by ultrasonically disintegrated cell suspensions standardized for protein content as described previously (47). Isoelectric focusing was performed in Agarose-IEF gels (Pharmacia) containing Pharmalyte (pH range, 3 to 10; Pharmacia). Gels were run for 4 h at a maximum potential difference of 500 V and stained with nitrocefin (500 μg/ml). Preparations of TEM-1, SHV-1, and SHV-5 were included as standards in each gel.

Plasmid studies. Transfer of resistance to *E. coli* K-12 J62-1 was detected by broth culture mating as described previously (47), with nalidixic acid (50 μg/ml) and ceftazidime (4 μg/ml) as the selective antibiotics. Plasmid DNA was prepared by the alkaline lysis method and separated on agarose gels as described previously (47), except for the addition of a hexadecyltrimethyl ammonium bromide step to precipitate polysaccharide (55). Preparations of plasmid DNA from *E. coli* NCTC 50192, which contains four plasmids with molecular sizes of 7, 36.2, 63.8, and 148.5 kb, were used as standards.

RESULTS

Description of the outbreak. Fourteen patients on five pediatric wards were affected; the wards included the cardiac, renal, and intensive care units. The outbreak strain of multiply resistant *K. pneumoniae* was first isolated on 13 July 1992 from the blood of a 3-year-old child with congenital nephrotic syndrome who had been transferred from another hospital 1 month previously. The patient was treated with ciprofloxacin and imipenem and made a good recovery after an infected Haemocath was removed. Between 18 and 23 July, five more children aged between 16 days and 8 months were affected (Table 1 and Fig. 1). They were all recovering from surgery for congenital defects; four of them had congenital heart disease, and two had septicemia. There were three more cases between 27 and 29 July: one patient was a 28-day-old child with renal failure following cardiac surgery for congenital heart disease who had isolates from blood and peritoneal dialysis fluid, and the other two were older children (5 and 8 years), suffering from a head injury and chronic renal failure, respectively, who yielded the outbreak strain from sputum and dialysis fluid. More than 100 fecal screens were performed on all patients, staff, and relatives of patients on the five affected pediatric wards. Multiply resistant klebsiellas were isolated by culturing fecal samples in a nutrient broth containing 20 μg of gentamicin per ml. Three infants with congenital heart disease were found to be asymptomatic stool carriers between 30 and 31 July. Although the outbreak seemed to have ended, the *K. pneumoniae* strain was found in a wound swab from a 1-year-old child with renal failure on 27 August and in a wound of a 13-year-old patient with renal failure (patient 14) on 14 October. Stool screens were repeated in September and October. An *E. coli* strain also resistant to gentamicin and ceftazidime was isolated from a stool screen of patient 14 on 3 September. No further cases were seen.

As shown in Fig. 1, five pediatric wards were involved in the outbreak. At first a common source was suspected, but extensive environmental screening yielded only one isolate from a sink drain on ward C. However, some of the patients had been cared for on several of the affected wards, including the intensive care units used for postoperative support and renal dialysis, and the outbreak could be explained by patient-to-patient spread (Fig. 2 and 3).

Three affected children died. One (patient 11, who had transposition of great vessels) was a stool carrier only, and death was unrelated to *Klebsiella* infection. Patient 6 was born at another hospital on 6 July with truncus arteriosus and was admitted to Guy's Hospital the same day. This patient went into renal failure, and a peritoneal dialysis catheter was inserted on 10 July; cardiac surgery was performed on 17 July, and he died on 23 July, when for the first time wound and blood cultures were positive for *K. pneumoniae*. Patient 7 was born at Guy's Hospital on 2 July with transposition of great vessels. Cardiac surgery was performed on 10 July, and a peritoneal catheter was inserted on 25 July for renal failure; blood and peritoneal dialysis fluid cultures yielded klebsiellas on 28 July, and he died the same day, having received one dose of ciprofloxacin.

Five of the patients, including three who were asymptomatic, were stool carriers of the outbreak strain. Only two patients were screened for skin carriage, and both were positive. Staff and relatives were also screened: one staff nurse on the pedi-

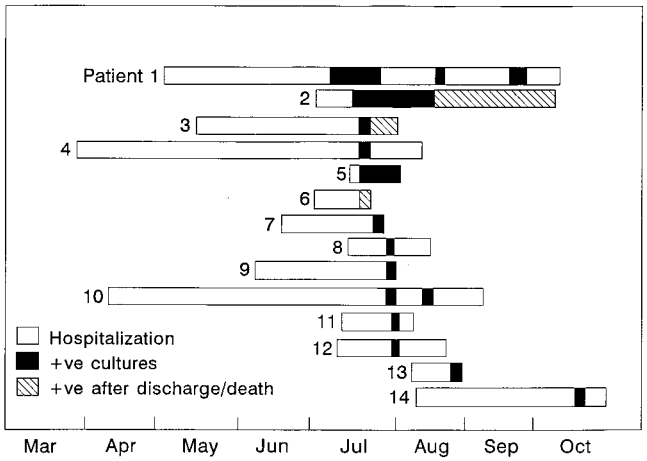

FIG. 2. Hospital stays and isolations of the epidemic strain. The bars represent the hospital stay of each patient and show the times of positive cultures for the outbreak strain of *K. pneumoniae*. +ve, positive.

atric intensive care ward and one parent (the father of patient 7) were stool carriers.

The outbreak was controlled by restricting admissions, removing the staff carrier from work, isolating infected and colonized patients, and instituting strict handwashing protocols. In addition, the affected wards were cleaned, and the contaminated sinks on ward C were replaced.

Microbiology. The outbreak strain was a *K. pneumoniae* capsular type K2 strain that was resistant to gentamicin and ceftazidime. All isolates tested reacted with the same two bacteriophages at RTD (routine test dilution)×100 and were indistinguishable by ribotyping. They all possessed two large plasmids of approximately 160 and 140 kb and three small ones of about 6, 3, and ≤1 kb. The multiresistant isolate of *E. coli* from patient 14 contained a 160-kb plasmid and one of about 125 kb.

The *Klebsiella* isolates were resistant to most β-lactam antibiotics but retained susceptibility to cefoxitin and imipenem (Table 2). They produced a β-lactamase that was indistinguishable from SHV-5 by isoelectric focusing. However, despite the fact that there was apparently a single outbreak strain, there was an approximately fivefold variation in β-lactamase activities, ranging from 169 to 1,138 mg of nitrocefin hydrolyzed per min per mg of protein (Fig. 4). Isolates with high-level β-lactamase activity showed higher degrees of resistance to sensitive β-lactams and were resistant to commercially available combinations of β-lactams with the β-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam (Fig. 4 and Table 2). The isolates were usually susceptible in vitro to the combination of ceftazidime and a fixed concentration of clavulanic acid (2 μg/ml); however, isolates that produced large amounts of SHV-5 showed reduced susceptibility, with ceftazidime MICs of 2 μg/ml. With larger inocula (10^6 organisms per spot), these high-level producers had ceftazidime MICs of 4 μg/ml in the presence of clavulanic acid, which constitutes resistance by the criteria of the British Society for Antimicrobial Chemotherapy (56) although not by those of the National Committee for Clinical Laboratory Standards (33). The isolates were also resistant to the aminoglycosides gentamicin, tobramycin, netilmicin, and amikacin but were susceptible to ciprofloxacin, tetracycline, and, usually, chloramphenicol. The *E. coli* isolate from patient 14 had a similar resistance pattern, was a high-level β-lactamase producer (897 mmol of nitrocefin hydrolyzed per min per mg of protein), and had an ampicillin MIC of 8,192 μg/ml and an amoxicillin-clavulanic acid MIC of 16 μg of amoxicillin per ml.

The β-lactam and aminoglycoside resistances and SHV-5 synthesis were transferable to *E. coli* K-12 by conjugation from the *Klebsiella* isolates and from the multiresistant *E. coli* isolate. The transconjugants had a single plasmid of approximately 160 kb.

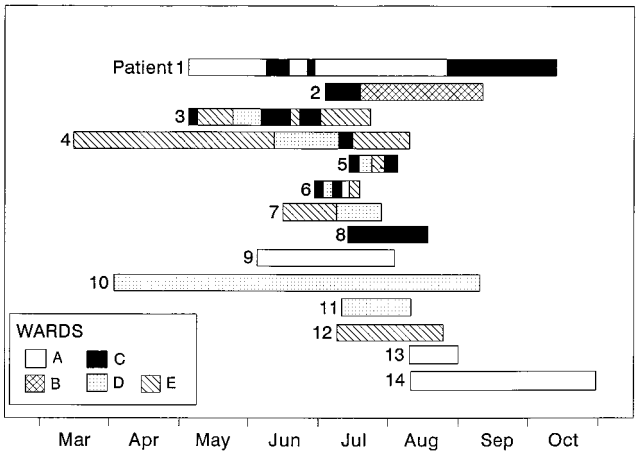

FIG. 3. Movements of patients between wards. The bars represent the hospital stay of each patient and show the wards to which each patient was admitted.

TABLE 2. β-Lactam MICs for low- and high-level SHV-5 producers[a]

Compound	MIC (μg/ml) for:	
	Low-level β-lactamase producers	High-level β-lactamase producers
Ampicillin	1,024	>8,192
Amoxicillin-clavulanic acid (2:1)	2	16
Piperacillin	256	>512
Piperacillin + tazobactam (4 μg/ml)	8	>64
Cefuroxime	4	64
Cefotaxime	2	32
Ceftazidime	32	256
Ceftazidime + sulbactam (4 μg/ml)	0.25	32
Ceftazidime + clavulanic acid (2 μg/ml)	0.5	2
Aztreonam	32	128
Cefoxitin	4	4
Imipenem	≤1	≤1

[a] The β-lactamase activities of the low- and high-level β-lactamase producers were 205 and 1,138 nmol of nitrocefin hydrolyzed per min per mg of protein, respectively.

DISCUSSION

Until recently, multiresistance in klebsiellas implied resistance to ampicillin-amoxicillin and gentamicin, together with various combinations of resistance to chloramphenicol, tetracycline, sulfonamide-trimethoprim combinations, and the narrow-spectrum cephalosporins. Hospital outbreaks with such strains used to be difficult to control because infected patients failed to respond to empirical treatment with gentamicin. Many isolates were susceptible to amikacin, especially in Great Britain, but some strains that were resistant to all available aminoglycosides were reported elsewhere (29, 42). Some outbreaks involved many patients in several different hospitals,

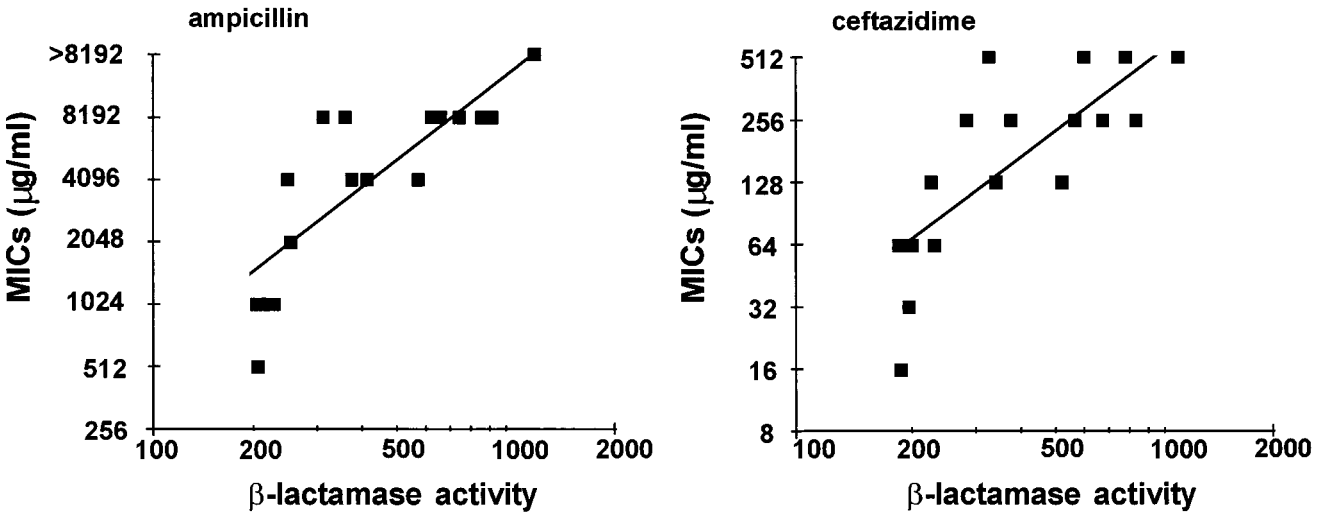

FIG. 4. Relationship between antibiotic MICs and β-lactamase activity (nanomoles of nitrocefin hydrolyzed per minute per milligram of protein) for *K. pneumoniae* K2 isolates from the outbreak.

and mortality rates could be high in compromised patients such as neonates (20). During these outbreaks, aminoglycoside resistance was often transferred to related enterobacteria such as *Enterobacter* and *Serratia* species (18, 27). However, the organisms remained susceptible to the extended- and broad-spectrum cephalosporins, and following the introduction of these agents, outbreaks with these multiresistant klebsiellas became uncommon.

Klebsiellas resistant to the extended- and broad-spectrum cephalosporins by the production of extended-spectrum β-lactamases have been isolated sporadically since the early 1980s (25, 39). They are usually also resistant to gentamicin and often are resistant to other aminoglycosides. They are sometimes resistant to the quinolones but are usually susceptible to the carbapenems. The genes responsible for cephalosporin and aminoglycoside resistances are usually encoded on transferable plasmids. Until recently, such strains of *K. pneumoniae* were regarded more as a nuisance than as a threat (38), first because they did not seem to have much capacity to cause clinical outbreaks or to disseminate their resistance genes and second because their extended-spectrum β-lactamases could usually be inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam (21). It is now obvious that these multiresistant klebsiellas can and do cause large hospital outbreaks, that their resistance traits readily pass to other bacterial species, and that β-lactam–β-lactamase inhibitor combinations may not be effective treatment.

In the last 5 years, these organisms have produced significant hospital outbreaks in France (2, 12, 13), Germany (4, 41), Great Britain (23), Australia (32), Greece (53), Tunisia (5), and the United States (31, 34). Although most outbreaks with single strains have been small, with the strain usually infecting or colonizing fewer than 20 patients, three have involved between 58 and 155 patients (2, 12, 13, 31). Two reports have indicated that the outbreak organisms appeared to have spread between different hospitals (13, 23). Several transferable extended-spectrum enzymes have been involved, including TEM-3 (1, 12, 13), TEM-26 (34, 52), SHV-2 (5), SHV-2a (41), SHV-3 (23), SHV-4 (CAZ-5) (2, 13), SHV-5 (4, 32, 53), and CAZ-6 (12). In both outbreaks and sporadic cases these enzymes have been transmitted in vivo to other bacteria, including *Klebsiella oxytoca* (5, 13), *E. coli* (5, 13, 34, 50, 53), and *Enterobacter* (13), *Serratia* (13), *Citrobacter* (13), and *Salmonella* (1, 5) species. In some outbreaks in France and in the United States, epidemic plasmids encoding extended-spectrum β-lactamases have spread among several different types of klebsiellas and several species of enterobacteria (6, 7, 31, 34, 51).

In the outbreak reported here, a multiresistant, SHV-5-producing strain of *K. pneumoniae* infected or colonized 14 pediatric patients and also colonized a nurse and the father of one of the children. The outbreak strain can, beyond reasonable doubt, be regarded as a single clone, since isolates were indistinguishable by three independent typing methods (capsular typing, bacteriophage typing, and ribotyping) and, furthermore, had the same plasmid profiles. Aminoglycoside and cephalosporin resistances transferred to *E. coli* in vitro on a 160-kb plasmid, and a similar resistant *E. coli* strain was isolated from the stools of one of the affected patients. Five wards were involved because of extensive patient transfers. The epidemic organism colonized the bowel and skin and was probably transmitted via staff hands. The outbreak was controlled by patient isolation and attention to handwashing. Several of the patients were neonates recovering from cardiac surgery for congenital defects and were highly compromised; four had septicemia, and three children died. One death was unrelated to *Klebsiella* infection, and in the other two cases the postoperative course was complicated by renal failure and terminal septicemia.

All of the isolates of the outbreak strain were identical by phage typing, ribotyping, plasmid profiling, and biochemical and serological testing, but they varied in their production of SHV-5. Some isolates produced normal amounts of SHV-5 and were susceptible to β-lactam–β-lactamase inhibitor combinations. Others were hyperproducers of SHV-5, producing up to five times as much enzyme as "normal" isolates. This hyperproduction resulted in increased MICs of several penicillins and cephalosporins and in vitro resistance or reduced susceptibility to amoxicillin-clavulanic acid, ampicillin-sulbactam, piperacillin-tazobactam, and ceftazidime-clavulanic acid. There are several mechanisms that could result in increased β-lactamase production. For example, there could be an increase in the number of copies of the β-lactamase gene, as a result of an increase either in the copy number of the plasmid or in the number of copies of the β-lactamase gene on the

plasmid. Since the plasmid encoding the SHV-5 β-lactamase was large (160 kb), a fivefold increase in its copy number seems unlikely, but duplication of the β-lactamase gene, as has been reported for TEM-1 in *E. coli* (47), is a likely explanation. Alternatively, an effect, such as mutation to a more efficient promoter, on the transcription or translation of the β-lactamase gene could result in increased β-lactamase activity.

Extended-spectrum β-lactamases of the TEM and SHV series are usually sensitive to β-lactamase inhibitors, and combinations of β-lactams with these inhibitors have been recommended for treatment (3). However, sulbactam is less effective than clavulanic acid for inhibition of the SHV enzymes (3, 22), a marked inoculum effect has been noted (8, 14), and these combinations have not always been successful in the treatment of experimental infections (8, 14, 26, 30, 44). The hyperproduction of SHV-5 by *K. pneumoniae* and *E. coli* seen in this outbreak at Guy's Hospital and some in vitro susceptibility results in the literature (3) suggest that β-lactam–β-lactamase inhibitor combinations may be unreliable for the treatment of these organisms. It is likely that hyperproduction of extended-spectrum β-lactamases will become more common in enterobacteria. These organisms may soon become resistant to all available agents and produce large hospital outbreaks of untreatable infection similar to the epidemics of gentamicin-resistant klebsiellas of the 1970s and 1980s.

ACKNOWLEDGMENTS

This study was supported in part by grants from the Special Trustees of Guy's Hospital and the Special Trustees of St. Thomas' Hospital.

We are grateful to T. L. Pitt, Central Public Health Laboratory, Colindale, London, United Kingdom, for typing the strains of *K. pneumoniae.*

REFERENCES

1. **Archambaud, M., G. Gerbaud, E. Labau, N. Marty, and P. Courvalin.** 1991. Possible in-vivo transfer of beta-lactamase TEM-3 from *Klebsiella pneumoniae* to *Salmonella kedougou*. J. Antimicrob. Chemother. **27:**427–436.
2. **Arlet, G., M. J. Sanson-le Pors, M. Rouveau, G. Fournier, O. Marie, B. Schlemmer, and A. Philippon.** 1990. Outbreak of nosocomial infections due to *Klebsiella pneumoniae* producing SHV-4 beta-lactamase. Eur. J. Clin. Microbiol. Infect. Dis. **9:**797–803.
3. **Bauernfeind, A.** 1990. Perspective of beta-lactamases inhibitors in therapy of infections caused by *Escherichia coli* or Klebsiella with plasmidic resistance to third generation cephalosporins. Infection **18:**48–52.
4. **Bauernfeind, A., E. Rosenthal, E. Eberlein, M. Holley, and S. Schweighart.** 1993. Spread of *Klebsiella pneumoniae* producing SHV-5 beta-lactamase among hospitalized patients. Infection **21:**18–22.
5. **Ben Hassen, A., G. Fournier, A. Kechrid, C. Fendri, S. Ben Redjeb, and A. Philippon.** 1990. Resistance enzymatique au cefotaxime chez cinquante-six souches de *Klebsiella* spp., *Escherichia coli* et *Salmonella* spp. dans un hopital Tunisien (1984–1988). Pathol. Biol. (Paris) **38:**464–469.
6. **Bingen, E., P. Desjardins, G. Arlet, F. Bourgeous, P. Mariani-Kurkdjian, N. Lambert-Zechovsky, E. Denamur, A. Phillipon, and J. Elion.** 1993. Molecular epidemiology of plasmid spread among extended broad-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates in a pediatric hospital. J. Clin. Microbiol. **31:**179–184.
7. **Brun-Buisson, C., P. Legrand, A. Philippon, F. Montravers, M. Ansquer, and J. Duval.** 1987. Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. Lancet **ii:**302–306.
8. **Caron, F., L. Gutmann, A. Buré, B. Pangon, J.-M. Vallois, A. Pechinot, and C. Carbon.** 1990. Ceftriaxone-sulbactam combination in rabbit endocarditis caused by a strain of *Klebsiella pneumoniae* producing extended-broad-spectrum TEM-3 β-lactamase. Antimicrob. Agents Chemother. **34:**2070–2074.
9. **Casewell, M. W., and I. Phillips.** 1981. Aspects of plasmid-mediated antibiotic resistance and epidemiology of *Klebsiella* species. Am. J. Med. **70:**459–462.
10. **Casewell, M. W., H. G. Talsania, and S. Knight.** 1981. Gentamicin-resistant *Klebsiella aerogenes* as a clinically significant source of transferable antibiotic resistance. J. Antimicrob. Chemother. **8:**153–160.
11. **Curie, K., D. C. E. Speller, R. A. Simpson, M. Stephens, and D. I. Cooke.** 1978. A hospital epidemic caused by a gentamicin-resistant *Klebsiella aerogenes*. J. Hyg. **80:**115–123.
12. **De Champs, C., D. Rouby, D. Guelon, J. Sirot, D. Sirot, D. Beytout, and J. M. Gourgand.** 1991. A case-control study of an outbreak of infections caused by *Klebsiella pneumoniae* strains producing CTX-1 (TEM-3) beta-lactamase. J. Hosp. Infect. **18:**5–13.
13. **De Champs, C., D. Sirot, C. Chanal, M. C. Poupart, M. P. Dumas, and J. Sirot.** 1991. Concomitant dissemination of three extended-spectrum beta-lactamases among different Enterobacteriaceae isolated in a French hospital. J. Antimicrob. Chemother. **27:**441–457.
14. **Fantin, B., B. Pangon, G. Potel, F. Caron, E. Valée, J.-M. Vallois, J. Mohler, A. Buré, A. Philippon, and C. Carbon.** 1990. Activity of sulbactam in combination with ceftriaxone in vitro and in experimental endocarditis caused by *Escherichia coli* producing SHV-2-like β-lactamase. Antimicrob. Agents Chemother. **34:**581–586.
15. **French, G., and T. Ling.** 1988. Amoxycillin/clavulanate resistant *Escherichia coli*. Lancet **i:**704.
16. **Garaizar, J., M. E. Kaufmann, and T. L. Pitt.** 1991. Comparison of ribotyping with conventional methods for the type identification of *Enterobacter cloacae*. J. Clin. Microbiol. **29:**1303–1307.
17. **Gaston, M. A., B. A. Ayling-Smith, and T. L. Pitt.** 1987. New bacteriophage typing scheme for subdivision of the frequent capsular serotypes of *Klebsiella* spp. J. Clin. Microbiol. **25:**1228–1232.
18. **Gerding, D. N., A. E. Buxton, R. A. Hughes, P. P. Cleary, J. Arbaczawaski, and W. E. Stamm.** 1979. Nosocomial multiply resistant *Klebsiella pneumoniae*: epidemiology of an outbreak of apparent index case origin. Antimicrob. Agents Chemother. **15:**608–615.
19. **Hansen, S. L., and P. K. Freedy.** 1983. Concurrent compatibility of automated systems and commercially prepared microdilution trays for susceptibility testing. J. Clin. Microbiol. **17:**878–886.
20. **Hart, C. A.** 1993. *Klebsiellae* and neonates. J. Hosp. Infect. **23:**83–96.
21. **Jacoby, G., and I. Carreras.** 1990. Activities of β-lactam antibiotics against *Escherichia coli* strains producing extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **34:**858–862.
22. **Jacoby, G. A., and A. A. Medeiros.** 1991. More extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **35:**1697–1704.
23. **Johnson, A. P., M. J. Weinbren, B. Ayling-Smith, S. K. Du Bois, S. G. B. Amyes, and R. C. George.** 1992. Outbreak of infection in two UK hospitals caused by a strain of *Klebsiella pneumoniae* resistant to cefotaxime and ceftazidime. J. Hosp. Infect. **20:**97–103.
24. **King, A., C. Warren, K. Shannon, and I. Phillips.** 1980. The *in vitro* antibacterial activity of cefotaxime compared with that of cefuroxime and cefoxitin. J. Antimicrob. Chemother. **6:**479–494.
25. **Knothe, H., P. Shah, V. Krcmery, M. Antal, and S. Mitsuhashi.** 1983. Transferrable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens*. Infection **11:**315–317.
26. **Leleu, G., M. D. Kitzis, J.-M. Vallois, L. Gutmann, and J. M. Decazes.** 1994. Different ratios of the piperacillin-tazobactam combination for treatment of experimental meningitis due to *Klebsiella pneumoniae* producing the TEM-3 extended-spectrum β-lactamase. Antimicrob. Agents Chemother. **38:**195–199.
27. **Markowitz, S. M., J. M. Veazly, F. L. Macrina, C. G. Mayhall, and V. A. Lamb.** 1980. Sequential outbreaks of infection due to *Klebsiella pneumoniae* in a neonatal intensive care unit: implication of a conjugative R plasmid. J. Infect. Dis. **142:**106–112.
28. **Martinez, J. L., E. Cercenado, M. Rodriguez-Creixems, M. F. Vincente-Perez, A. Delgado-Iribarren, and F. Baquero.** 1987. Resistance to β-lactam/clavulanate. Lancet **ii:**473.
29. **Mayer, K. H.** 1986. Review of epidemic aminoglycoside resistance worldwide. Am. J. Med. **80:**56–64.
30. **Mentec, H., J.-M. Vallois, A. Buré, A. Saleh-Mghir, F. Jehl, and C. Carbon.** 1992. Piperacillin, tazobactam, and gentamicin alone or combined in an endocarditis model of infection by a TEM-3-producing strain of *Klebsiella pneumoniae* or its susceptible variant. Antimicrob. Agents Chemother. **36:**1883–1889.
31. **Meyer, K. S., C. Urban, J. A. Eagan, B. J. Berger, and J. J. Rahal.** 1993. Nosocomial outbreak of *Klebsiella* infection resistant to late generation cephalosporins. Ann. Intern. Med. **119:**353–358.
32. **Mulgrave, L., and P. V. Attwood.** 1993. Characterization of an SHV-5 related extended broad-spectrum beta-lactamase in Enterobacteriaceae from Western Australia. Pathology **25:**71–75.
33. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
34. **Naumovski, L., J. P. Quinn, D. Miyashiro, M. Patel, K. Bush, S. B. Singer, D. Graves, T. Palzkill, and A. M. Arvin.** 1992. Outbreak of ceftazidime resistance due to a novel extended-spectrum β-lactamase in isolates from cancer patients. Antimicrob. Agents Chemother. **36:**1991–1996.
35. **Noreiga, E. R., R. E. Leibowitz, A. S. Richmond, E. Rubinstein, S. Schaefler, M. S. Simberkoff, and J. J. Rahal.** 1975. Nosocomial infection caused by gentamicin-resistant streptomycin-sensitive *Klebsiella*. J. Infect. Dis. **131**(Suppl.)**:**S45–S50.
36. **Nugent, M., and R. W. Hedges.** 1979. The nature of the genetic determinant

for the SHV-1 β-lactamase. Mol. Gen. Genet. **175:**239–243.
37. **Palyfreyman, J. M.** 1978. *Klebsiella* serotyping by countercurrent immunoelectrophoresis. J. Hyg. **18:**219–225.
38. **Payne, D. J., and S. G. B. Amyes.** 1991. Transferable resistance to extended-spectrum β-lactams: a major threat or a minor inconvenience? J. Antimicrob. Chemother. **27:**255–261.
39. **Philippon, A., R. Labia, and G. Jacoby.** 1989. Extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **33:**1131–1136.
40. **Phillips, I., S. Eykyn, A. King, W. R. Gransden, B. Rowe, J. A. Frost, and R. J. Gross.** 1988. Epidemic multiresistant *Escherichia coli* infection in West Lambeth Health District. Lancet **i:**1038–1041.
41. **Podbielski, A., J. Schonling, B. Melzer, K. Warnatz, and H. G. Leusch.** 1991. Molecular characterization of a new plasmid-encoded SHV-type beta-lactamase (SHV-2 variant) conferring high-level cefotaxime resistance upon *Klebsiella pneumoniae*. J. Gen. Microbiol. **137:**569–578.
42. **Price, K. E., P. A. Kresel, L. A. Farchione, S. B. Siskin, and S. A. Karpow.** 1981. Epidemiological studies of aminoglycoside resistance in the USA. J. Antimicrob. Chemother. **8**(Suppl. A)**:**89–105.
43. **Rennie, R. P., and I. B. R. Duncan.** 1977. Emergence of gentamicin-resistant *Klebsiella* in a general hospital. Antimicrob. Agents Chemother. **11:**179–184.
44. **Rice, L. B., J. D. C. Yao, K. Klimm, G. M. Eliopoulos, and R. C. Moellering.** 1991. Efficacy of different β-lactams against an extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strain in the rat intra-abdominal abscess model. Antimicrob. Agents Chemother. **35:**1243–1244.
45. **Shah, P., and W. Stille.** 1983. *E. coli* and *K. pneumoniae* strains more susceptible to cefoxitin than to third-generation cephalosporins. J. Antimicrob. Chemother. **11:**597–598.
46. **Shannon, K. P., A. King, and I. Phillips.** 1992. Prevalence of resistance to β-lactam antibiotics in *Escherichia coli* isolated from blood from 1969–1991. J. Antimicrob. Chemother. **30:**661–672.
47. **Shannon, K. P., H. Williams, A. King, and I. Phillips.** 1990. Hyperproduction of TEM-1 beta-lactamase in clinical isolates of *Escherichia coli* serotype O15. FEMS Microbiol. Lett. **67:**319–324.
48. **Simpson, I. N., P. B. Harper, and C. H. O'Callaghan.** 1980. Principal β-lactamases responsible for resistance to β-lactam antibiotics in urinary tract infections. Antimicrob. Agents Chemother. **17:**929–936.
49. **Sirot, D., C. De Champs, C. Chanal, R. Labia, A. Darfeuille-Michaud, R. Perroux, and J. Sirot.** 1991. Translocation of antibiotic resistance determinants including an extended-spectrum β-lactamase between conjugative plasmids of *Klebsiella pneumoniae* and *Escherichia coli*. Antimicrob. Agents Chemother. **35:**1576–1581.
50. **Sirot, D., J. Sirot, R. Labia, A. Morand, P. Courvalin, A. Darfeuille-Michaud, R. Perroux, and R. Cluzel.** 1987. Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel β-lactamase. J. Antimicrob. Chemother. **20:**323–334.
51. **Sirot, J., C. Chanal, A. Petit, D. Sirot, R. Labia, and G. Gerbaud.** 1988. *Klebsiella pneumoniae* and other Enterobacteriaceae producing novel plasmid-mediated beta-lactamases markedly active against third-generation cephalosporins: epidemiologic studies. Rev. Infect. Dis. **10:**850–859.
52. **Urban, C., K. S. Meyer, N. Mariano, J. J. Rahal, R. Flamm, B. A. Rasmussen, and K. Bush.** 1994. Identification of TEM-26 β-lactamase responsible for a major outbreak of ceftazidime-resistant *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **38:**392–395.
53. **Vatopoulos, A. C., A. Philippon, L. S. Tzouvelekis, Z. Komninou, and N. J. Legakis.** 1990. Prevalence of a transferable SHV-5 type beta-lactamase in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* in Greece. J. Antimicrob. Chemother. **26:**635–648.
54. **Weinstein, R. A., C. Nathan, R. Grunsfelder, and S. A. Kabins.** 1980. Endemic aminoglycoside resistance in gram-negative bacteria. J. Infect. Dis. **141:**338–345.
55. **Wilson, K.** 1990. Preparation of genomic DNA from bacteria, p. 2.4.1–2.4.5. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. Greene Publishing Associates, New York.
56. **Working Party on Antibiotic Sensitivity Testing of the British Society for Antimicrobial Chemotherapy.** 1991. A guide to sensitivity testing. J. Antimicrob. Chemother. **27**(Suppl. D)**:**1–48.