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Molecular Epidemiology of *Klebsiella pneumoniae* Producing SHV-5 β-Lactamase: Parallel Outbreaks Due to Multiple Plasmid Transfer

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Over a period of 22 months, 32 patients treated in three independent intensive care units of the Innsbruck University Hospital were infected with extended-spectrum β-lactamase-producing members of the family *Enterobacteriaceae* (30 *Klebsiella pneumoniae* isolates, 1 *Klebsiella oxytoca* isolate, and 1 *Escherichia coli* isolate). As confirmed by sequencing of a *bla* gene PCR fragment, all isolates expressed the SHV-5-type β-lactamase. Genomic fingerprinting of epidemic strains with *Xba*I and pulsed-field gel electrophoresis grouped 20 of 21 isolates from ward A into two consecutive clusters which included 1 of 3 ward B isolates. All six *K. pneumoniae* isolates from ward C formed a third cluster. Stool isolates of asymptomatic patients and environmental isolates belonged to these clusters as well. Additionally, 2,600 routine *K. pneumoniae* isolates from the surrounding provinces (population, 900,000) were screened for SHV-5 production. Only one of six nonepidemic isolates producing SHV-5 β-lactamase was matched with the outbreak strains by genomic fingerprinting. Plasmid fingerprinting, however, revealed the epidemic spread of a predominant R-plasmid, with a size of approximately 80 kb, associated with 29 of the 30 *K. pneumoniae* isolates. This plasmid was also present in the single *K. oxytoca* and *E. coli* isolates from ward C and in three nonepidemic isolates producing SHV-5. Our results underline that strain typing exclusively on the genomic level can be misleading in the epidemiological investigation of plasmid-encoded extended-spectrum β-lactamases. Our evidence for multiple events of R-plasmid transfer between species of the family *Enterobacteriaceae* in this nosocomial outbreak stresses the need for plasmid typing, especially because SHV-5 β-lactamase seems to be regionally spread predominantly via plasmid transfer.

Plasmid-encoded resistance to broad-spectrum cephalosporins and aztreonam is becoming a widespread phenomenon in clinical medicine. These antibiotics are inactivated by an array of different extended-spectrum β-lactamases (ESBLs) which have evolved by stepwise mutation of TEM- or SHV-type β-lactamases (for review, see references 10 and 13). Plasmids encoding these enzymes have been encountered in several members of the family *Enterobacteriaceae*, but are, for unknown reasons, most often harbored by *Klebsiella pneumoniae* (18).

Among the SHV-type β-lactamases, SHV-5 was responsible for outbreaks of nosocomial infections in several countries in the world (5, 16, 19, 23, 26, 29). As for all plasmid-mediated resistance factors, detailed molecular analysis of such outbreaks has to address a complex of transmission modes. Fingerprinting of genomic bacterial DNA with *Xba*I and pulsed-field gel electrophoresis (20), as well as with ribotyping (reviewed in reference 7), had been applied for other pathogens. Both methods were successfully adopted to analyze the clonal spread of epidemic *K. pneumoniae* strains (3, 8, 15). In contrast, plasmid transfer between strains as an alternative way of spreading genes encoding ESBLs was rarely found in small epidemic settings (8), although nationwide dissemination via a single plasmid occurred with other ESBLs (11, 28). The Innsbruck University Hospital, a 1,602-bed general hospital with five independent and spatially separated intensive care units (ICUs) is the central tertiary care facility in the western part of Austria (1995 population, 900,000). There, several periods of nosocomial spread of ESBL-producing *K. pneumoniae* were noticed from May 1993 until February 1995. The epidemic seemed to be restricted to two ICUs frequently exchanging patients (wards A and B) and a third, unrelated ICU (ward C). To support the epidemiological investigation, the outbreak isolates were analyzed by a combination of molecular typing methods. Additionally, because geriatric departments and nursing homes have been postulated to serve as a reservoir for ESBL-producing bacteria (24), we tried to assess the occurrence of outbreak strains in the general population. To this end, all *K. pneumoniae* isolates obtained in Western Austria over a 7-month period were screened for ESBL production.

MATERIALS AND METHODS

Patients and clinical data. Fifty-eight isolates of ESBL-producing *K. pneumoniae* and one isolate each of *Klebsiella oxytoca* and *Escherichia coli* were recovered from 32 patients treated at ICUs A, B, and C (neurology, neurosurgery, and neonatology, respectively). Only the first isolate of each patient was further analyzed, as detailed in Table 1. For wards A and B, urine and bronchial secretions were regularly cultured twice weekly. Thirty of the patient charts available were analyzed with respect to antibiotic treatment, clinical and laboratory parameters, infection due to other pathogens, and history of transfers to or from other wards.

Preventive measures and environmental investigations. Routine preventive measures in all three ICUs included the use of disposable gloves and disposable aprons during patient care. Wearing of masks was not implemented for nosocomial infections with ESBL-producing *K. pneumoniae*. The staff was informed about the epidemiologic background of the ongoing outbreak and advised to pay special attention to patients with diarrhea. Detailed environmental investigations

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TABLE 1. Characterization of SHV-5-producing epidemic and nonepidemic isolates

Isolate[a]	PFGE pattern	R-plasmid
Epidemic		
Ward A		
A1–A11[b]	a	pIBK1
A12	a.1[c]	pIBK1
A13–A16	a.2	pIBK1
A17	b	pIBK1
A18	c	pIBK1
A19–A21	b.1	pIBK1
Ward B		
B1[b]	d	pIBK2
Ba2[b]	a	pIBK1
Ba3	e	pIBK1
Ward C		
C1	*K. oxytoca*	pIBK1
C2	*E. coli*	pIBK1
C3–C8	f	pIBK1
Nonepidemic		
XF[b]	g	pIBK1
XN	h	No transconjugants obtained
XM	i	pIBK1
XH	a.2	pIBK1
XK	k	pIBK1
XC	l	pIBK3

[a] Isolates are numbered identically to the patients from which they were isolated (Fig. 1). All isolates except C1 and C2 are *K. pneumoniae.*
[b] Isolate(s) for which SHV-5 was confirmed by DNA sequencing.
[c] PFGE types showing close relatedness are indicated by extension numbers.

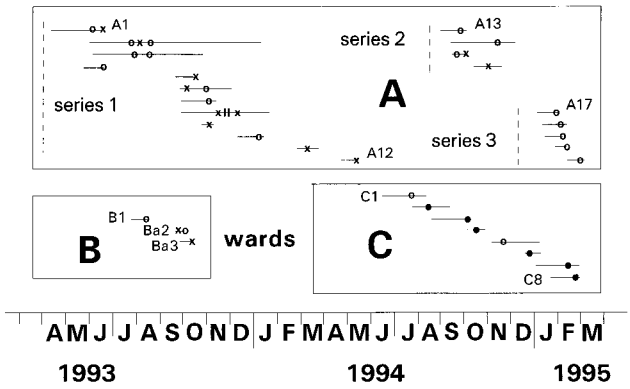

FIG. 1. Hospitalization periods of patients infected with SHV-5-producing *K. pneumoniae* in three different ICUs (wards A, B, and C). Sites of infections are marked with ○ (deep airways), × (urine), ● (blood culture), and ▮ (cerebrospinal fluid). Patients are numbered in chronological order (see Table 1). Letters at the bottom denote months.

were performed in wards A and C in October 1994 and January 1995 and included examination of beds, sinks, floors, respirators, patients' utensils, hands of the nursing and medical staff, and stool samples from patients and staff. Case control studies were not undertaken for ward A because cases of infection in series 2 and 3 (Fig. 1) had occurred only in one of the rooms and environmental investigations allowed conclusions about the mode of transmission to be drawn. In ward C, a retrospective analysis of patient charts permitted investigation of the impact of age or β-lactam pretreatment. The risk associated with exposure to nurses or neighbor patients, however, could not be reliably assessed from the charts because this was not recorded throughout the patients' stay.

Isolation of ESBL-producing members of the family *Enterobacteriaceae.* Biochemical identification of presumptive *K. pneumoniae* isolates from clinical specimens growing on MacConkey agar and exhibiting resistance against broad-spectrum cephalosporins by the disk diffusion method was done with API20E (BioMérieux, Marcy-l'Etoile, France). ESBL production was confirmed by the double-disk synergy test (9). Isolates were subcultured and frozen at −70°C until they were further analyzed. Testing of stool samples was done by addition of 1 g of stool to 5 ml of Columbia broth (Difco, Detroit, Mich.) without antibiotics. After overnight culture at 37°C, 10 μl was plated on MacConkey agar, and then *K. pneumoniae* colonies were tested for ESBL production. From October 1994 until April 1995, all regional *K. pneumoniae* routine diagnostic laboratory isolates (2,600 isolates) were screened for ESBL production by antibiotic susceptibility patterns and the double-disk diffusion test (9). ESBL producers were further analyzed by isoelectric focusing (IEF) and PFGE.

Antibiotic susceptibility testing. MICs were determined by E-test (AB Biodisk, Solna, Sweden) (4) according to the manufacturer's instructions for wild-type *K. pneumoniae* isolates and for the transconjugant *E. coli* C600. MICs were read after 16 h of incubation at 35°C. *E. coli* ATCC 25922 was used as a reference strain.

Transfer of oxyimino-β-lactam resistance. The wild-type strains and the recipient *E. coli* C600 strain (10^9 CFU/ml per strain) were suspended in Mueller-Hinton broth (Difco, Detroit, Mich.) and incubated for 18 h at 30°C. Transconjugants were selected on MacConkey agar (Oxoid, Basingstoke, United Kingdom) supplemented with nalidixic acid (64 mg/liter), rifampin (128 mg/liter), or sodium azide (100 mg/liter) to inhibit the growth of the donor strain and ceftazidime (1 mg/liter) to inhibit the growth of the recipient strain.

Phenotypic identification of β-lactamases. Supernatants (15 μl) of crude bacterial lysates obtained after sonication were subjected to IEF on 7.5% polyacrylamide gels (pH gradient 3.5 to 10) and separated with 1,500 V (50 W) for 1.5 h at 4°C with the Multiphor II system (Pharmacia, Uppsala, Sweden). β-Lactamases were visualized by the chromogenic cephalosporin nitrocefin as described previously (21).

Genotypic identification of the SHV-5 β-lactamase. Plasmid DNA of four transconjugant *E. coli* C600 isolates (Table 1) was prepared by alkaline lysis and purified by anion-exchange columns (QIAGEN midi-scale, Hilden, Germany). A 292-bp fragment of the SHV gene was amplified by PCR with the primers P1 (5′-ACT GAA TGA GGC GCT TCC-3′) and P2 (5′-TCC CGC AGA TAA ATC ACC-3′), corresponding to nucleotides 492 to 509 and 783 to 765, respectively, of the SHV-5 *bla* gene (6). The PCR products were sequenced by the Sanger dideoxy procedure with an automatic sequencer (Applied Biosystems, Foster City, Calif.).

Plasmid fingerprinting. R-plasmids encoding SHV-5 were prepared from transconjugant *E. coli* strains as described above. Purified plasmid DNA (1.5 μg) was either directly electrophoresed on 0.7% agarose gels or analyzed by electrophoresis on a 0.9% agarose gel after restriction digestion with *Eco*RI (Boehringer Mannheim, Mannheim, Germany). Bands were visualized with ethidium bromide. Southern transfer of *Eco*RI-digested plasmid DNA to nylon membrane (Hybond N^+; Amersham, Little Chalfont, United Kingdom) was done by capillary blotting by standard methods. The blot was hybridized with the 292-bp bla_{SHV}-specific PCR fragment generated as described above and labelled with peroxidase with the enhanced chemiluminescence direct nucleic acid labelling and detection system (Amersham). Hybridization (10 ng of labelled probe per ml), washing steps, and chemiluminescence detection were performed as suggested by the manufacturer.

Genome fingerprinting by PFGE. Agarose plugs containing chromosomal DNA were prepared by a method similar to that described by Maslow et al. (20). Bacteria grown overnight were resuspended in 1 M NaCl with 10 mM Tris (pH 7.6), mixed with an equal volume of 1.3% InCert agarose (FMC BioProducts, Rockland, Maine), dispensed at 100 μl into plug molds, and allowed to solidify on ice. The plugs were then incubated at 37°C with lysis buffer (1 M NaCl, 100 mM EDTA, 50 mM Tris [pH 7.5]) containing 1 mg of lysozyme (Sigma, St. Louis, Mo.) per ml. After 3 h, sodium dodecyl sulfate (1% final concentration) and proteinase K (Boehringer) at a final concentration of 500 μg/ml were added for a further 48 h at 55°C. After a washing with TE buffer (10 mM Tris [pH 8.0], 1 mM EDTA; second of three wash mixtures containing 1 mM phenylmethylsulfonyl fluoride), the chromosomal DNA was digested with *Xba*I (Promega, Madison, Wis.) according to the manufacturer's instructions. DNA fragments were separated by PFGE in a 1% agarose gel (SeaKem LE; FMC) in 0.5× TBE buffer (45 mM Tris borate, 1 mM EDTA [pH 8.0]) with the CHEF Mapper system (Bio-Rad, Richmond, Calif.). Electrophoresis conditions were 14°C at 6 V/cm for 21 h with pulse times ranging from 1 to 40 s. Bands were visualized after being stained with ethidium bromide.

RESULTS

Clinical cases. Of 30 ICU patients infected with ESBL-producing *K. pneumoniae*, 21 were found in ward A, 3 were found in ward B, and 6 were found in ward C. Single isolates of *K. oxytoca* and *E. coli* producing ESBL were obtained from ward C patients (Table 1 and Fig. 1).

Twenty-two ICU patients experienced overt infections, including pneumonia (11 patients), septicemia (9 patients), urinary tract infection (3 patients), meningitis (1 patient), and wound infection (1 patient). They responded well to treatment

TABLE 2. MIC for transconjugant *E. coli* C600 strains representative of plasmids pIBK1, pIBK2, and pIBK3 transferred from wild-type strains A1, Ba3, and XC, respectively

Antibiotic	MIC[a] (mg/liter) for:						
	Wild-type *K. pneumoniae*			Transconjugant *E. coli* C600 (plasmid harbored)			
	A1	Ba3	XC	R^-	R^+ [A1] (pIBK1)	R^+ [Ba3] (pIBK2)	R^+ [XC] (pIBK3)
Gentamicin	8	2	0.064	0.023	1	0.13	0.023
Tobramycin	16	2	0.064	0.064	4	1	0.064
Chloramphenicol	8	32	4	2	2	2	2
Tetracycline	>256	>256	0.75	0.38	4	0.38	0.38
Ciprofloxacin	0.38	0.25	0.023	0.008	0.012	0.008	0.008
Ceftazidime[b]	>256	>256	>256	0.19	32	32	32
Cefotaxime[b]	6	12	16	0.023	1.5	1.5	1.5
Aztreonam[b]	>256	>256	>256	0.094	64	32	32
Cefoxitin	8	32	2	0.25	0.25	0.25	0.25
Imipenem	0.25	0.25	0.19	0.125	0.125	0.125	0.125

[a] MICs were determined by E-test.
[b] Combination with clavulanic acid restored susceptibility.

with imipenem, ciprofloxacin, or, if appropriate, aminoglycosides. Eight patients were found to harbor *K. pneumoniae* in the urinary or upper respiratory tract without clinical or laboratory signs of pneumonia or urinary tract infection. Skin colonization was not observed.

Environmental and epidemiological investigations. A first investigation in ward A performed in October 1994 revealed that the dilution of the disinfectant used for room disinfection had been inappropriate for several months. After this practice was changed, the incidence of infections with the outbreak strain decreased. In a detailed environmental investigation after a flare-up in January 1995, the outbreak strain was recovered from inadequately disinfected respirator condensates and from a mortar used in one of the rooms to crush pills for administration through the gastric tube. All of the new cases of infection had appeared in this room. Other instruments, beds, and sinks or hand cultures from the personnel did not yield positive cultures. At the same time, ESBL-producing *K. pneumoniae* isolates were present in stool samples of three of five asymptomatic patients and two infected patients in ward A, but not in stool samples from the staff. Two of the asymptomatic colonized patients and both infected patients were fed by gastric tube. In ward C during repeated investigations, ESBL-producing pathogens could not be cultured from the ward environment or hand cultures (October 1994 and January 1995). With respect to therapy with β-lactam antibiotics, age, or prematurity, the infected infants did not differ from the uninfected ones. Patients C3 and C4 as well as C7 and C8 had been neighbors before klebsiellae were isolated, but for all cases of infection, overt infections were seen only for one child at a time.

Antibiotic susceptibility testing. As shown in Table 2, wild-type and transconjugant strains had a susceptibility pattern concordant with the SHV-5-type β-lactamase activity. Although most wild-type strains were also resistant to chloramphenicol, gentamicin, tetracycline, and cotrimoxazol, only resistance to tetracycline and cotrimoxazol was cotransferred to *E. coli* with the SHV-5 phenotype.

Characterization of ESBLs. IEF of crude bacterial homogenates revealed a major β-lactamase band at a pI of 8.2, suggesting the presence of SHV-5. This band at pI 8.2 was seen with all ESBL-producing isolates from wards A, B, and C. The SHV-5-specific mutations in the *bla* gene (arginine 205, serine 238, and lysine 240; numbering according to that of Ambler et al. [2]) were revealed by sequencing of a representative PCR fragment amplified from the R-plasmid of three selected ICU isolates and strain XF from a district hospital, thus confirming the presence of SHV-5-type β-lactamase. Among the 2,600 regional *K. pneumoniae* isolates screened, 9 were found to produce ESBL, originating from nonepidemic wards of the Innsbruck Hospital (7 isolates) or from district hospitals (2 isolates). Six of the nine nonepidemic regional isolates also expressed SHV-5, whereas, the remaining three produced β-lactamases with pIs of between 5.4 and 5.6.

Genomic typing of ESBL *K. pneumoniae* isolates. Genomic typing with *Xba*I and PFGE was performed to determine the extent of clonal spread of ESBL-producing *K. pneumoniae*. By PFGE patterns, 26 of 32 ICU isolates appeared to be epidemiologically linked (Fig. 1). Three clusters were observed. All *K. pneumoniae* isolates from ward C (C3 to C8) formed a unique cluster unrelated to ward A or ward B. The biggest cluster comprised 17 isolates from ward A (A1 to A16) and ward B (Ba2, a patient transferred from ward A to ward B). Twelve of them (A1 to A11 and Ba2) were identical, and a further 4 (A13 to A16) differed only by an additional band. Thus, the second series of infections in ward A (A13 to A16) was caused by a strain minimally different from the first series. In the third series occurring in the same ward only 2 months later, the emergence of a new cluster of strains (A17 and A19 to A21) unrelated to the former epidemic strain was evident.

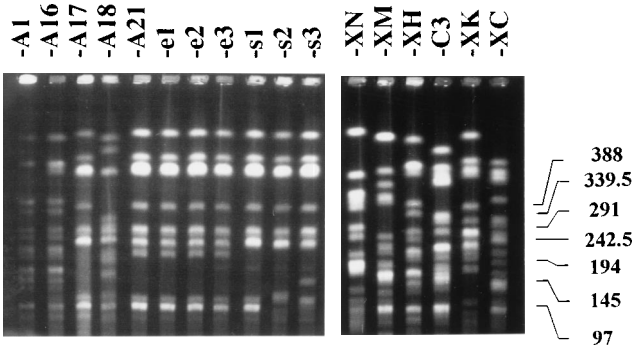

FIG. 2. Macrorestriction patterns with *Xba*I of epidemic patient isolates (lanes A1, A16, A17, A18, A21, and C3) compared with SHV-5-producing *K. pneumoniae* recovered from the ward A environment (lanes e1 to e3), from stools of asymptomatic patients in ward A (lanes s1 to s3), and from nonepidemic settings (XN to XC). Lambda concatemers served as a size marker (kilobases).

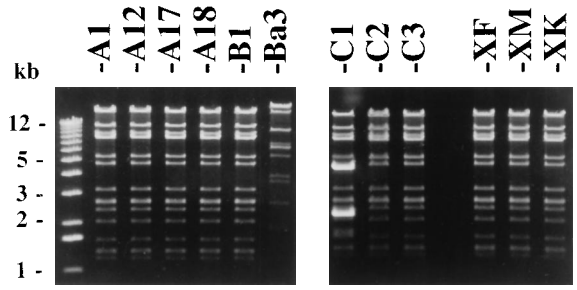

FIG. 3. *Eco*RI fingerprint of R⁻ plasmids isolated from transconjugants for epidemic and nonepidemic isolates which were different by PFGE. All isolates except Ba3 harbored plasmid pIBK1. The transconjugant of C1 possessed two additional small plasmids with sizes of 4.5 and 2 kb. Size markers (BRL ladder) are shown on the left.

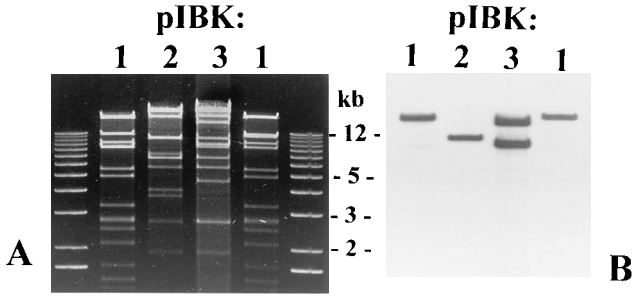

FIG. 4. Direct comparison of the bla_{SHV-5}-containing plasmids pIBK1, pIBK2, and pIBK3. (A) *Eco*RI digest of 1 μg of plasmid DNA. (B) Corresponding Southern blot hybridized with a bla_{SHV}-specific probe as described in Materials and Methods. The size marker (kilobases) applies to both A and B.

The environmental isolates obtained during this last outbreak period from ward A (e1 to e3 [Fig. 2]) were identical to this new epidemic strain. *K. pneumoniae* isolates producing ESBL and isolated from the stools of three asymptomatic patients (s1 to s3 [Fig. 2]) also belonged to this cluster, but showed slight individual differences of one to three bands.

In contrast, of six nonepidemic SHV-5-producing *K. pneumoniae* isolates, five had unique PFGE patterns (Fig. 2). Only one isolate (XH) had the PFGE pattern found with isolates A13 to A16. Interestingly, patient XH was treated in a district hospital for septicemia, but he had been admitted to a regular ward in the university hospital 3 weeks before. There he had stayed together with patient A14, who had recently been discharged from ward A. As shown on the occasion of a follow-up examination 4 months later, patient A14 still harbored *K. pneumoniae* with the outbreak PFGE type.

Plasmid typing. R-plasmids of epidemic and nonepidemic strains with different PFGE patterns were analyzed after transfer to *E. coli*. Restriction endonuclease analysis with *Eco*RI demonstrated one prevailing plasmid fingerprint (Fig. 3 and Table 1). The size of this epidemic plasmid (preliminarily termed pIBK1) was estimated to be approximately 80 kb, and it additionally harbored genes for resistance to tetracycline and trimethoprim (Table 2). The plasmid was present in all 21 ward A isolates, 2 of 3 ward B isolates, and all 8 ward C isolates, including the single *K. oxytoca* isolate and single *E. coli* isolate. Also, three of four nonepidemic isolates with unique PFGE patterns harbored this plasmid. Therefore, the presence of SHV-5 β-lactamase in the few *K. pneumoniae* outbreak strains and in *K. oxytoca* and *E. coli* reflected multiple transfer of pIBK1.

The two other bla_{SHV-5}-containing plasmids, pIBK2 and pIBK3, which were both observed only once, differed from pIBK1 in their *Eco*RI restriction pattern, as shown in Fig. 4A. pIBK2 and pIBK3, but not pIBK1, had several bands in common, indicating a potential genetic relationship. They also are responsible for very similar resistance patterns in the transconjugants (Table 2). However, they do not share the same location of the bla_{SHV-5} gene (Fig. 4B). pIBK3 harbors two copies of the bla_{SHV-5} gene, because the probed part of the gene normally does not contain an *Eco*RI restriction site, yet SHV-5 was the only β-lactamase observed in the transconjugant by IEF.

DISCUSSION

Nosocomial infections with *K. pneumoniae* expressing SHV-type or TEM-type ESBLs have become more and more frequent since the first SHV-2-related outbreak in 1984 (25).

However, the development of sensitive subtyping techniques has given microbiologists tools to detect origins and routes of hospital epidemics as a step toward control and elimination of infection.

In the study presented, the identification of SHV-5 as the ESBL produced by all epidemic isolates was the first step in analyzing this outbreak. However, as described previously (8), DNA fingerprinting methods analyzing both the bacterial genome and the R-plasmid are required for the complex epidemiology of ESBL. Genomic macrorestriction fragment analysis with *Xba*I and PFGE has been widely applied for subtyping of other pathogens (1, 17) and for small outbreaks, the clonal spread of an ESBL-producing *K. pneumoniae* strain could be proven by PFGE without the need for further typing methods (15). On a larger scale, PFGE revealed the dissemination of the SHV-4 *bla* gene in French hospitals via a single *K. pneumoniae* strain (3). Epidemiological connections between isolates differing in PFGE, however, are missed by this approach. The same is true for analysis of random amplified polymorphic DNA patterns (14).

Combining ribotyping and plasmid typing, Bingen et al. demonstrated the existence of many epidemic *K. pneumoniae* strains individually associated with differing R-plasmids in a large nosocomial outbreak (8). Apparently, also more than a single ESBL was involved in that epidemic. Identical plasmids in different strains were detected only in two isolates with similar ribotypes, suggesting that plasmid transfer was a relatively rare event in this single-hospital epidemic. Our findings apparently disclosed a different epidemiological situation, because several SHV-5-producing *K. pneumoniae* outbreak strains were linked by the same R-plasmid. Thus, although clonal spread of strains was seen in the individual wards and only a little dissemination was due to transfer of patients between the wards, propagation of the outbreak prominently included plasmid spread. Because the PFGE patterns of epidemic isolates were stable over several months but then changed suddenly (from A16 to A17 [Fig. 2]), this new strain is unlikely to have evolved by chromosomal mutation.

Transfer of this plasmid apparently also caused SHV-5 dissemination to regular wards of the Innsbruck Hospital and beyond to other regional hospitals. Although these cases remained sporadic and did not cause other epidemics, they illustrate that SHV-5 was by far the most frequently encountered ESBL in Western Austria.

Besides the epidemic plasmid, pIBK1, two further SHV-5-encoding plasmids were detected at the periphery of the outbreak. This fact and the presence of two bla_{SHV} copies on the pIBK3 gene may reflect the location of bla_{SHV-5} on a transposable element, as has been shown for other ESBLs in *K. pneumoniae* (27). This has to be addressed in further experiments.

In neighboring countries, outbreaks involving SHV-5 β-lactamase have been reported in Germany (5) and Northern Italy (19). Plasmid pMVP-5 from Southern Germany was clearly dissimilar to ours as shown by *Dra*I fingerprinting and an antibiogram. A comparison with the recent Italian isolates would be interesting, because many patients clinically evaluated in ward A have predominantly been transferred from Northern Italian ICUs.

Attempts to control ESBL-producing pathogens were partly successful. In ward A, the last outbreak episode was terminated by eliminating a potential source for infection. Although the spread of the *K. pneumoniae* outbreak strains ceased after series 2 and series 3, the outbreak plasmid pIBK1 reappeared within new strains—most recently in a singular *E. coli* isolate 5 months after the last isolation (not shown). Thus, although the number of cases of infection could be reduced, the plasmid did not disappear completely, which maintains the risk for further episodes. To us, the frequent intestinal colonization of ward A patients with klebsiellae harboring plasmid pIBK1 and transfer of these klebsiellae to others provide an explanation for the observed plasmid endemicity. The same may be true for the neonatal ward. ESBL-producing klebsiellae were found at up to 10^9 CFU/g of stool in other settings (8). It could be speculated that intestinal colonization in discharged ICU patients led to dissemination of pIBK1 from ICUs to the normal population. However, pIBK1-carrying *Klebsiella* strains may also have existed in the population prior to their introduction into the ICUs, where they subsequently expanded under antibiotic pressure. The endemicity of ESBL-producing bacteria in a hospital has also been observed by others (5, 24), although differentiation between bacterial strain or plasmid endemicity is rarely performed. Only a few authors have reported the termination of an ESBL outbreak (12). We conclude from this study that regular screening for intestinal colonization will be important to this end. Meyer et al. described a significant increase in overall mortality due to ESBL-producing *K. pneumoniae* infections (22). In our setting, these infections did not lead to higher mortality among the affected patients. In agreement with others (8), treatment was effective in most overt cases. This at least may lessen the threat immediately after the detection of ESBL in a clinical ward.

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