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Extended-Spectrum β-Lactamases in *Klebsiella pneumoniae* Bloodstream Isolates from Seven Countries: Dominance and Widespread Prevalence of SHV- and CTX-M-Type β-Lactamases

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A huge variety of extended-spectrum β-lactamases (ESBLs) have been detected during the last 20 years. The majority of these have been of the TEM or SHV lineage. We have assessed ESBLs occurring among a collection of 455 bloodstream isolates of *Klebsiella pneumoniae*, collected from 12 hospitals in seven countries. Multiple β-lactamases were produced by isolates with phenotypic evidence of ESBL production (mean of 2.7 β-lactamases per isolate; range, 1 to 5). SHV-type ESBLs were the most common ESBL, occurring in 67.1% (49 of 73) of isolates with phenotypic evidence of ESBL production. In contrast, TEM-type ESBLs (TEM-10 type, -12 type, -26 type, and -63 type) were found in just 16.4% (12 of 73) of isolates. The finding of TEM-10 type and TEM-12 type represents the first detection of a TEM-type ESBL in South America. PER (for *Pseudomonas* extended resistance)-type β-lactamases were detected in five of the nine isolates from Turkey and were found with SHV-2-type and SHV-5-type ESBLs in two of the isolates. CTX-M-type ESBLs ($bla_{\text{CTX-M-2}}$ type and $bla_{\text{CTX-M-3}}$ type) were found in 23.3% (17 of 73) of isolates and were found in all study countries except for the United States. We also detected CTX-M-type ESBLs in four countries where they have previously not been described—Australia, Belgium, Turkey, and South Africa. The widespread emergence and proliferation of CTX-M-type ESBLs is particularly noteworthy and may have important implications for clinical microbiology laboratories and for physicians treating patients with serious *K. pneumoniae* infections.

During the last 2 decades, extended-spectrum β-lactamases (ESBLs) found in gram-negative bacilli have emerged as a significant mechanism of resistance to oxyimino-cephalosporin antibiotics (8, 10, 21, 29, 37). The ESBLs mediate resistance to broad-spectrum cephalosporins (e.g., ceftazidime, ceftriaxone, and cefotaxime) and aztreonam. The genes encoding ESBLs are usually found on plasmids, along with genes encoding mechanisms of resistance to aminoglycosides and trimethoprim-sulfamethoxazole. Additionally, *Klebsiella pneumoniae* isolates harboring ESBLs are significantly more frequently found to be resistant to quinolones than non-ESBL-producing strains (22, 30). Finally, the combined effect of multiple β-lactamases and outer membrane protein (OMP) deficiencies may lead to resistance of ESBL-producing enteric bacteria to β-lactam–β-lactamase inhibitor combinations and, occasionally, even to cephamycins and carbapenems (24).

Most commonly, ESBLs derive from genes for the narrower-spectrum TEM-1, TEM-2 or SHV-1 β-lactamases by mutations that alter the amino acid configuration around the active site of these enzymes (27, 28). More than 100 genetically distinct TEM-type and SHV-type ESBLs have now been characterized (www.lahey.org/studies/webt.asp). Additionally, other types of ESBLs have been documented. These include the CTX-M-type ESBLs, β-lactamases that have less than 40% homology with TEM and SHV types, and PER (for *Pseudomonas* extended resistance)-type ESBLs (1, 2, 5, 33, 42). As a group, the CTX-M-type β-lactamases are closest in amino acid identity to the chromosomal cephalosporinases of *Kluyvera georgiana*, *Kluyvera cryorescens*, and *Kluyvera ascorbata* (14, 20, 34). PER-type β-lactamases represent a distinct class A cephalosporinase phenotype so far only restricted to South America and Europe (3, 42). Although possessing only 26% identity to the TEM-type ESBLs, PER β-lactamases also confer resistance to oxyimino-β-lactams, such as cefotaxime, ceftazidime, and aztreonam (6, 7, 40, 43, 44).

Curiously, ESBLs are most commonly detected in *K. pneumoniae*. This organism is a cause of significant community- and hospital-acquired infections. Bloodstream infections with *K. pneumoniae* may arise from the lungs (community- and ventilator-acquired pneumonia), the urinary tract, intra-abdominal pathologies, and central venous line-related infections. In this study, we have performed molecular characterization of ESBLs from bloodstream isolates of *K. pneumoniae* collected from 12 hospitals in seven countries. Our goal is to present a partial sequence analysis of the types of β-lactamases found in these isolates. The demographic features, clinical characteristics, and outcomes of the patients harboring these ESBLs have been reported (30, 31).

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MATERIALS AND METHODS

Study design. A prospective, observational study of consecutive, sequentially encountered patients with *K. pneumoniae* bacteremia was performed in 12 hospitals in the United States, Taiwan, Australia, South Africa, Turkey, Belgium, and Argentina (30). The study period was 1 January 1996 to 31 December 1997.

TABLE 1. Primer sets used in characterizing β-lactamases[a]

Gene sought	Primer sequence	Target amino acids
bla_{SHV}	5′-ATGCGTTATATTCGCCTGTG-3′ 5′-TGCTTTGTTATTCGGGCCAA-3′	8–249
bla_{TEM}	5′-AAACGCTGGTGAAAGTA-3′ 5′-AGCGATCTGTCTAT-3′	35–274
bla_{CTX-M}	5′-CGCTTTGCGATGTGCAG-3′ 5′-ACCGCGATATCGTTGGT-3′	Variable
$bla_{CTX-M-2}$	5′-ATGATGACTCAGAGCATTCGCCGCT-3′ 5′-TCAGAAACCGTGGGTTACGATTTTCG-3′	9–279
bla_{PER-1}	5′-ATGAATGTCATTATAAAAG-3′ 5′-TTGGGCTTAGGGCAG-3′	7–301
bla_{AmpC}	5′-ATCAAAACTGGCAGCCG-3′ 5′-GAGCCCGTTTTATGCACCCA-3′	141–311
bla_{TEM}	5′-CGCATACACTATTCTCAGAATG-3′ (TEM164 forward)[b] 5′-CTGAATGAAGCCATACCAAAC-3′ (TEM238 forward)[b] 5′-GTTAATAGTTTGCGCAACGTTG-3′ (TEM104 reverse)[b]	
bla_{SHV}	5′-GACGCCCGCGACACCACTACC-3′ (SHV238 forward)[b]	

[a] The nucleotide sequence of bla_{TEM} was determined by using primers based upon the published sequences (23, 38) AmpC primers were based upon conserved sequences of P99 (GenBank accession no. X0724), ACT-1 (GenBank accession no. U58495), and CMY-2 (GenBank accession no. X91840) β-lactamases. CTX-M primers were designed as described by Bonnet et al. (5) CTX-M-2 primers were based upon GenBank accession no. X92507. PER-1 primers were based upon GenBank accession no. Z21957.

[b] Cy5-labeled sequencing primer.

Patients older than 16 years of age with blood cultures positive for *K. pneumoniae* were enrolled, and a 188-item study form was completed. Patients were monitored for 1 month after the onset of bacteremia to assess clinical outcome, including mortality and infectious complications.

Microbiologic methods. The *K. pneumoniae* isolates potentially harboring ESBLs were those with a positive phenotypic confirmatory test for ESBLs according to current National Committee for Clinical Laboratory Standards (NCCLS) criteria (26). These isolates were initially screen positive in that the MICs of ceftazidime, cefotaxime, ceftriaxone, or aztreonam for these organisms were ≥1 μg/ml according to standard broth dilution techniques. A phenotypic confirmatory test was then performed by testing MICs for ceftazidime, ceftazidime-clavulanic acid, cefotaxime, and cefotaxime-clavulanic acid. A ≥threefold concentration decrease in a MIC of either ceftazidime or cefotaxime tested in combination with clavulanic acid versus its MIC when tested alone was indicative of phenotypic confirmation of ESBL production.

aIEF. We performed initial characterization of the β-lactamases in these clinical isolates by analytical isoelectric focusing (aIEF) as previously described (32). Ten microliters of the crude enzyme extract was loaded onto a precast gel (Ampholine PAGplate; Amersham Pharmacia Biotech, Piscataway, N.J.). We used gels with a pH range of 3.5 to 9.5 as part of the initial screen. Isolates with previously characterized β-lactamases were used as controls. These were obtained as a kind gift from P. Bradford (Wyeth Pharmaceuticals, Pearl River, N.Y.). In addition, RTEM-1 enzyme from *Escherichia coli* 205 (Sigma Chemical Co., St. Louis, Mo.) and SHV-1 from *K. pneumoniae* 15571 were loaded onto a gel as control β-lactamases (pI 5.4 and 7.6) (32).

PCR amplification and *bla* gene sequencing. A 10-μl aliquot of an overnight culture of the test isolate was diluted 1:10 with water and boiled for 10 min. PCR amplification was then performed with 10 μl of this dilution as the DNA template. The primer sets are described in Table 1. The PCR conditions used were 35 cycles of amplification at a denaturation temperature of 94°C for 30 s, an annealing temperature of 60°C for 1 min (70°C for the CTX-M-2 primers, 43°C for PER-1 primers, and 45°C for TEM primers), and an extension temperature of 72°C for 1 min. This step was followed by a final extension at 72°C for 10 min. PCR products were resolved on 1% agarose gels, stained with ethidium bromide, and photographed with UV illumination. ΦX174 replicative-form DNA *Hae*III fragments (GIBCO BRL Life Technologies, Rockville, Md.) were used to assess PCR product size.

Direct sequencing of amplified products was performed on an ALF Express automated DNA sequencer (Amersham Pharmacia Biotech, Piscataway, N.J.) by using the Thermo Sequenase fluorescent-labeled primer cycle sequencing kit (Amersham Pharmacia Biotech). Under some circumstances, amplicons were cloned into the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, Calif.) and sequenced with Cy5-labeled M13 reverse and M13 forward primers (17). We repeated amplification and sequencing twice for each isolate. Sequencing primers for bla_{SHV} were used as previously described (17).

ELISA. We examined *K. pneumoniae* isolates for the presence or absence of SHV- or CMY-2-type β-lactamases by using a sensitive and specific enzyme linked immunoabsorbent assay (ELISA) as a screening test (18). This assay detects these two β-lactamases with a sensitivity and specificity of greater than 94%.

RESULTS

Four hundred fifty-five episodes of *K. pneumoniae* bacteremia occurred in 440 patients during the study period; the isolates were from Argentina ($n = 41$), South Africa ($n = 116$), Europe ($n = 27$), the United States ($n = 58$), Australia ($n = 71$), and Taiwan ($n = 142$). Eighteen percent (85 of 455) of the isolates had phenotypic evidence of ESBL production. Of these, 73 isolates were available for aIEF and gene sequencing. These isolates came from Argentina ($n = 18$), South Africa ($n = 27$), Turkey ($n = 9$), the United States ($n = 11$), Australia ($n = 2$), Belgium ($n = 3$), and Taiwan ($n = 3$).

aIEF. All 73 isolates possessed at least one β-lactamase (mean, 2.7; range, 1 to 5) (Table 2). The numbers of β-lactamases produced by each isolate were one (1.4%; 1 isolate), two (49.3%; 36 isolates), three (34.2%; 25 isolates), four (12.5%; 9 isolates), and five (2.7%; 2 isolates). In certain isolates that possessed CTX-M-type β-lactamases, it was difficult to assess the precise number of β-lactamases by using aIEF. For certain isolates, it was not possible from aIEF to enumerate all of the β-lactamases detected.

TABLE 2. IEF data from 73 *K. pneumoniae* bloodstream isolates with phenotypic confirmation of ESBL production

Country (*n*)	No. of isolates with β-lactamases with pI in range:					
	5.1–5.6	5.7–6.0	6.1–7.0	7.1–8.2	8.3–8.8	>8.8
Argentina (18)	18	0	2	7	13	0
Australia (2)	2	0	0	1	1	1
Belgium (3)	3	0	2	3	2	1
Taiwan (3)	3	0	0	3	1	1
South Africa (27)	14	3	1	27	23	0
Turkey (9)	9	6	4	9	6	0
United States (11)	11	2	1	11	4	1

Analysis of *bla* gene sequencing results. We amplified and sequenced each bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M}$ at least twice. Among the isolates studied, 67 of 73 (91.8%) that had a positive phenotypic confirmatory test for ESBL production were found to produce a TEM-, SHV-, PER-, or CTX-M-type ESBL by sequencing (exceptions are isolates 456, 470, 438, 442, 104, and 140; see Table 4). The ceftazidime MICs for four of these isolates were ≥256 μg/ml. One of the 73 isolates was positive by ELISA for SHV β-lactamase, but we were not able to amplify bla_{SHV} by using specific primers. These isolates are undergoing further characterization of their plasmids, β-lactamases, and outer membrane protein profiles. From aIEF gels, it is clear that multiple TEM and SHV β-lactamases are present.

Although we did not sequence the entire bla_{TEM} and bla_{SHV} genes, we concentrated our analysis in the regions of these enzymes responsible for the ESBL phenotype (Table 1). In our analysis, 87.7% (64 of 73) isolates produced a TEM-type β-lactamase. These included the non-ESBL enzymes TEM-1A-type (8 isolates), TEM-1B-type (36 isolates), a novel TEM variant we designate TEM-1H-type (1 isolate), and TEM-2-type (7 isolates). The TEM-1H sequence differed from that of TEM-1B by a single nucleotide change at the codon encoding amino acid 171 (GAA to GAG). Only 16.4% (12 of 73) isolates produced a TEM-type ESBL (Table 4). Eight isolates possessed TEM-10-type (amino acid changes R164S and E240K; four isolates from the United States, two from South Africa, and two from Argentina), two isolates harbored TEM-12-type (amino acid change R164S; one isolate each from Argentina and South Africa), one isolate had TEM-26-type (amino acid changes E104K and R164S; from the United States), and one isolate contained TEM-63-type (amino acid changes E104K, R164S, and M182T; from South Africa). TEM-63 has been reported from Durban, South Africa (15).

Ninety percent (66 of 73) of the isolates produced an SHV-type β-lactamase. Notably, 49 isolates produced an SHV-type ESBL. We focused our attention upon the dominant amino acid substitutions that result in the ESBL phenotype for SHV-type β-lactamases and compared each sequence to the $bla_{SHV\text{-}1}$ sequence deposited in GenBank (accession no. AF124984). Fourteen isolates had an amino acid mutation at Ambler position 238 but not at site 240 (amino acid change G238S; therefore, ESBL types SHV-2, -2A, -3, -20, or -21 according to www.lahey/org/studies/webt.asp). These isolates were from Argentina ($n = 4$), South Africa ($n = 5$), Turkey ($n = 1$), the United States ($n = 2$), Australia ($n = 1$), and Taiwan ($n = 1$). We designated these ESBLs as SHV-2-type. Thirty-five isolates had mutations at amino acid positions 238 and 240 (amino acid changes G238S and E240K; therefore ESBL types SHV-4, -5, -7, -9, -10, -12, or -15; for simplicity we designated the isolates as SHV-5-type). These isolates were from Argentina ($n = 2$), South Africa ($n = 21$), Turkey ($n = 3$), United States ($n = 5$), Australia ($n = 1$), Belgium ($n = 2$), and Taiwan ($n = 1$) (Table 4). In our analysis, we observed that there were also bla_{SHV} genes with silent mutations. At amino acid position 240, Glu is normally encoded by GAG. We found GAA in SHV-2-type enzymes (designated SHV-2-type§ in Table 4). In SHV-5-type enzymes, we found both AAA (designated SHV-5-type ^) and AAG encoding Lys at 240. In sum, 35 isolates possessed SHV-5-type, and 14 isolates produced SHV-2-type enzymes.

The ESBL found in 23.3% (17 of 73) of our isolates was a CTX-M-type ESBL. CTX-M-type ESBL-producing *K. pneumoniae* isolates were found in all study countries, except the United States. The CTX-M β-lactamases identified were CTX-M-2 type (14 isolates; 11 from Argentina, 1 from South Africa, 1 from Turkey, and 1 from Belgium) and CTX-M-3 type (1 isolate each from Taiwan, Australia, and South Africa).

PER-1-type β-lactamases were detected in five of the nine isolates from Turkey and were found with both SHV and TEM β-lactamases. All five isolates possessed SHV and TEM β-lactamases.

More than one ESBL was found in 19.2% (14 of 73) of isolates (Table 4). Ten of the isolates had TEM- and SHV-type ESBLs; two had TEM-, SHV-, and CTX-M-type ESBLs; and two had SHV- and PER-type ESBLs.

DISCUSSION

To our knowledge, this study is among the first to give a snapshot of the SHV and TEM sequence variability of ESBLs found in bloodstream isolates of *K. pneumoniae* in different continents at a single point in time (1996 to 1997). This is a necessary first step in our quest to understand how the genotypes of these complex antibiotic resistance phenotypes emerge in the clinical setting. A major finding was that SHV-type ESBLs were by far the most dominant ESBL type. Although, we did not fully sequence all 49 of the ESBL bla_{SHV} genes, we focused our attention upon the amino acid sequence at the crucial 238 and 240 sites. This showed that 35 isolates with SHV-type ESBLs had mutations at both sites 238 and 240 and were therefore of the ESBL phenotype SHV-4, -5, -7, -9, -10, -12, or -15. Why this remains the preferred site of mutation to evolve an ESBL phenotype in SHV remains to be established. It has been demonstrated that the mutation G238S in the SHV β-lactamase (SHV-2) preserves efficient catalytic activity against both penicillins and cephalosporins (17). The mutation G238S coupled with the E240K mutation is also associated with increased steady-state β-lactamase expression relative to the G238S mutant β-lactamase. Other reports show that the molecular heterogeneity of bla_{SHV} associated with ESBLs centers about the mutations at amino acid positions 238 and 240 (4, 15, 29, 36). Data do not yet exist to support the notion that the E240K mutation functions in any way to stabilize the effect of other mutations in SHV, especially G238S. This mutation may simply be enhancing the affinity of broad-spectrum cephalosporins to the active site (19). We also found

TABLE 3. Organisms with multiple ESBL types

Source country	β-Lactamase-types[a]	MIC (μg/ml)			
		Cefotaxime	Ceftazidime	Cefepime	Piperacillin/tazobactam
Argentina	TEM-10 SHV-5	>256	128	128	>256
	TEM-10 SHV-2	16	64	4	>256
	TEM-12 SHV-5 CTX-M-2	>256	16	>256	>256
Australia	TEM-1B SHV-2 CTX-M-3	>256	32	4	64
South Africa	TEM-10 SHV-2	32	16	4	>256
	TEM-10 SHV-5	32	16	4	>256
	TEM-12 SHV-2 CTX-M-2	64	>256	4	>256
	TEM-63 SHV-5	128	>256	32	>256
Turkey	TEM-1B SHV-5 CTX-M-2	8	0.5	1	>256
	TEM-2 SHV-2 PER-1	32	>256	4	>256
	TEM-2 SHV-5 PER-1	32	>256	8	>256
United States	TEM-10 SHV-2	4	>256	8	16
	TEM-10 SHV-5	8	128	2	16
	TEM-26 SHV-5	16	>256	4	16

[a] Denotes gene not fully sequenced (see text).

that in 10 *K. pneumoniae* isolates, the amplified bla_{SHV} gene differed from wild type SHV-1 by one nucleotide that did not result in an amino acid change (Table 4). This was found in 3 SHV-2-type β-lactamase amplicons. Like the situation that exists in TEM (TEM-1A to -G), there is molecular heterogeneity of bla_{SHV} (23, 35).

It is perplexing to us that in seven of the isolates, our SHV PCR screening primers did not detect bla_{SHV} (isolates 8, 9, 10, 11, 465, 466, and 467 [see summary in Table 4]). bla_{SHV} is reported to be "universal" in *K. pneumoniae* (G. S. Babini and D. M. Livermore, Letter, Antimicrob. Agents Chemother. **44:** 2230, 2000). bla_{SHV} is mainly a chromosomally encoded species-specific enzyme (12). Isolates 8, 9, 10, 11, 465, 466, and 467 were positive with the PCR screen for CTX-M-2 by CTX-M-2-specific primers (hence explaining the phenotype) but did not amplify with bla_{AmpC} primers and did not demonstrate a signal that could be detected by the ELISA for AmpC or SHV β-lactamase. Since the aIEF was difficult to interpret due to the presence of a unique pattern of bands due to CTX-M enzymes (data not shown), we used PCR amplification and ELISA to detect these enzymes. We currently have no information that identifies a related β-lactamase in lieu of SHV, nor do we have data to propose that the presence of bla_{CTX-M} excludes bla_{SHV}. It has been previously observed that many isolates of *K. pneumoniae* possess LEN-1- or LEN-2-type β-lactamases migrating at pI 7.1 (16). We had a number of isolates with β-lactamases in this range (Table 2). In further studies, we plan to expand our analysis to seek LEN-1 and LEN-2.

Although more varieties of TEM-type ESBLs have been described than SHV-type ESBLs, TEM-type ESBLs were found less frequently in our study. TEM-10-type was the most frequently detected TEM-type ESBL and was found in disparate regions (the United States, South Africa, and Argentina). As far as we are aware, this finding of TEM-type β-lactamases in Argentina represents the first report of TEM-type ESBLs from South America. We also noted that TEM-1B-type was the most common TEM variant found, being sequenced in more than half of the isolates studied. TEM-1B-type occurred most often in association with SHV-2-type and SHV-5-type enzymes (23 of 73 isolates). A substantial proportion (19.2%) of organisms produced multiple ESBL types. Two isolates produced a TEM-type ESBL, an SHV-type ESBL, and a CTX-M-type ESBL.

Perhaps most significantly, this report extends the geographical spread of CTX-M-type ESBLs to Australia, Belgium, Turkey, and South Africa (9, 11, 13, 39, 41). There are no other published reports of the discovery of CTX-M-type β-lactamases in these nations. Additionally, in our study, CTX-M-type ESBLs were more numerous than TEM-type ESBLs. Since cefotaxime and ceftriaxone are used worldwide, it is not surprising that CTX-M-type ESBLs are now being found in multiple countries. Although no epidemiologic studies have yet been performed that have linked cefepime use with infection with a CTX-M-type ESBL, it is noteworthy that elevated cefepime MICs are frequent for *K. pneumoniae* isolates producing CTX-M-type ESBLs. Of increasing importance is the potential effect of the presence of a CTX-M-type ESBL on detection of ESBLs by the clinical microbiology laboratory. Those laboratories, which rely on resistance to ceftazidime as a surrogate marker for ESBL production, will likely not be aware of organisms producing CTX-M-type ESBLs.

As noted above, PER-type enzymes were detected in more than half of the *K. pneumoniae* isolates from Turkey. PER-type β-lactamases have recently been recovered from *E. coli*, *Proteus mirabilis*, *Salmonella enterica* serovar Typhimurium, *K. pneumoniae*, *Acinetobacter baumannii*, and *Alcaligenes faecalis* (33, 42–44). PER-2 shares 86% homology with PER-1 and has been found predominately in South America (3). The PER-type ESBLs are among the most efficient β-lactamases, able to hydrolyze broad-spectrum cephalosporins (11-fold dilution increase when transformed into *E. coli* C600) (43). The residues responsible for the ESBL phenotype in PER-1 are distinct from SHV and TEM, and the binding cavity of this β-lacta-

TABLE 4. Summary of *bla* sequencing results of each isolate grouped by country[a]

Isolate no.	TEM[b]	SHV[b]	CTX-M[b]	PER
South Africa				
16	1B	5		
26		5		
28		5		
33		5		
51	1B	2		
62		5		
71	10	2		
93	1A	5		
186		2		
190	1B	2		
195	1B	5		
198	1B	5		
202		5		
223	1B	5		
231	1B	5		
236	10	5		
237	1B	5		
238	12	2	2	
243	63	5		
262	1A/1C	5		
264	1B	5		
266		5		
271	1B	5		
273	1B	1*	3	
276		5		
278	1B	5		
281		5		
Argentina				
8	1B		2	
9	1B		2	
10	1B		2	
11	1B		2	
181	10	5		
182	10	2		
183	1B	2§		
253	12	5	2	
255	1A	2		
257	2	1	2	
261	1B	1*	2	
456	1A	1*		
458	1A	2		
465	1B		2	
466	1B		2	
467	1B		2	
468	1H	1*	2	
470	1A	1*		
Australia				
158	1B	5		
160	1B	2	3	
Belgium				
163	1A/1B	5		
165	1B	5		
172	1B	1*	2	
Taiwan				
312	1B	2		
335	1B	5		
427	1B	1	3	
Turkey				
59	1B	5	2	
60	2	2		+
438	2	1		
440	1A	5		
441	2	1		+
442	1B	1*		
444	2	5		+
447	2	1		+
449	2	1		+
United States				
104	1B	1		
111	10	1*		
126	10	1*		
131	10	2§		
140	1B	1*		
146	10	5		
157	26	5		
8642	1B	2§		
10045	1B	5		
10627	1B	5		
14733	1B	5^		

[a] For the bla_{TEM} genes, 10 = TEM-10-type (amino acid changes R164S and E240K), 12 = TEM-12-type (amino acid change R164S), 26 = TEM-26-type (amino acid changes E104K and R164S), and 63 = TEM-63-type (amino acid changes E104K, R164S, and M182T). For the bla_{SHV} genes, 2 = SHV-2-type (amino acid change G238S), and 5 = SHV-5 (amino acid changes G238S and E240K). *, bla_{SHV-1} with silent nucleotide change from reported sequence at amino acid 240 (Glu240, GAG to GAA); §, SHV-2-type with a silent mutation at 240 (Glu240, GAG to GAA); 5^, SHV-5-type with nucleotide change from reported sequence at amino acid 240 (Glu240Lys, AAG to AAA); +, presence of PER-1 β-lactamase. For the bla_{CTX-M} genes, 2 = CTX-M-2-type and 3 = CTX-M-3-type.

[b] Denotes genes not fully sequenced (see the text).

mase is quite different (6, 40). Our finding of this ESBL in *K. pneumoniae* in Turkey raises significant clinical concern. These PER β-lactamases were detected in *K. pneumoniae* isolates possessing TEM-2 and either SHV-2 or SHV-5 type (in isolates with three β-lactamases).

In summary, we have documented the dominance of *K. pneumoniae* SHV ESBL types worldwide and highlighted the emergence of CTX-M-type ESBLs in numerous countries. It is important to consider that the number of reports of novel ESBLs of SHV- and TEM-type have diminished in recent years. The growing number of ESBLs of different varieties challenges us to ponder if *K. pneumoniae* is one of the main pathogens in which ESBLs evolve. A limitation to our study is that only a select number of hospitals in each country were assessed. There may be peculiarities in prescribing antibiotics or other forces that may have biased the ESBL types seen in the study hospitals. It has not escaped our attention that an isolate may have more than one bla_{TEM} or bla_{SHV} gene present, and amplification and sequencing efforts only detected a single genotype. If multiple bla_{TEM} or bla_{SHV} genes are present, the predominant one will preferentially amplify and produce sequence. Nevertheless, we have been able to gain a unique assessment of ESBL-types occurring in consecutive patients with *K. pneumoniae* bacteremia at the same point in time. As antibiotic usage changes over time, we speculate that types of β-lactamase produced by *K. pneumoniae* may progress as well. In turn, the antibiotic susceptibilities of this organism will evolve, and it behooves us to constantly reevaluate both laboratory detection of ESBLs and potential treatment options for organisms producing ESBLs (45). This study complements

investigations directed at increasing the awareness of β-lactamases in *K. pneumoniae* in the United States and other hospitals worldwide (25). Studies are planned to examine the number and type of plasmids present in these isolates.

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