

# Molecular Correlation for the Treatment Outcomes in Bloodstream Infections Caused by *Escherichia coli* and *Klebsiella pneumoniae* with Reduced Susceptibility to Ceftazidime

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**Data are limited on outcomes of treatment with extended-spectrum cephalosporins (ESCs) for infections caused by Enterobacteriaceae that produce extended-spectrum  $\beta$ -lactamases (ESBLs). This study describes the largest treatment experience of a nonoutbreak series of bloodstream infections caused by strains of *Escherichia coli* (23 episodes) and *Klebsiella pneumoniae* (13 episodes) with a ceftazidime minimal inhibitory concentration of  $\geq 2$   $\mu\text{g}/\text{mL}$ . *E. coli* isolates produced a greater variety of  $\beta$ -lactamase types than did *K. pneumoniae* isolates, among which ESBL production was predominant. Five ESBL types were identified: TEM-12, TEM-71, TEM-6, SHV-12, and SHV-5. Most patients were treated empirically with an ESC-based regimen. A favorable response to treatment with a nonceftazidime ESC was observed when the causative pathogen produced either TEM-6 or TEM-12; ceftazidime treatment was associated with failure of therapy in all patients. Despite the limited clinical success, ESCs are currently not recommended for the treatment of serious infections caused by ESBL-producing strains.**

During the past 2 decades, antibiotic-resistant mutant strains that produce extended-spectrum  $\beta$ -lactamases (ESBLs) have emerged among the Enterobacteriaceae, predominantly *Escherichia coli* and *Klebsiella pneumoniae*. The emergence of such strains has important clinical and therapeutic implications. First, ESBLs are often

derived from TEM and SHV enzymes, which are present in 75% of Enterobacteriaceae [1]. The resistance determinants for ESBLs are often found on transmissible plasmids, which facilitate the spread of the determinants to other organisms [1]. Second, because of the lack of an obvious marker to indicate the presence of such enzymes, routine susceptibility testing may not detect the presence of ESBLs. To improve laboratory detection, the National Committee on Clinical Laboratory Standards (NCCLS) currently recommends that laboratories perform special screening to identify possible ESBL producers, using a break point MIC of  $\geq 2$   $\mu\text{g}/\text{mL}$  for  $\geq 1$  of the following antimicrobial agents: cefpodoxime, cefotaxime, ceftriaxone, ceftazidime, or aztreonam. This testing should be followed by a confirmatory test that depends on the observation of increased susceptibility to cefotaxime or ceftazidime in the presence of clavulanic acid [2]. However, according

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acetate (pH 5.5); extracts were subjected to 4 freeze-thaw cycles, then centrifuged. We determined isoelectric points for  $\beta$ -lactamases in the supernatants by means of isoelectric focusing, using Multiphor apparatus and Ampholine PAGplates (Amersham-Pharmacia) with a pH of 3.5–9.5. As standards, we used TEM-1, TEM-26, SHV-1, and P99  $\beta$ -lactamases.  $\beta$ -Lactamase activity was detected by use of a nitrocefin overlay. AmpC-type cephalosporinases were identified by observing  $\beta$ -lactamase inhibition when an overlay of aztreonam (concentration, 1  $\mu$ g/mL) was applied 10 min before the application of nitrocefin.

**OMP characterization and transfer of resistance.** Selected *E. coli* isolates underwent characterization of OMP and transfer of resistance. Bacterial cell membranes were isolated from strains grown overnight in trypticase soy broth by means of sonication (3 pulses of 30 s alternated with 15 s of cooling on ice). The suspension was then centrifuged at 100,000 g for 40 min at 4°C. OMPs were separated from total membrane preparations by solubilizing the inner membrane with 20% Sarkosyl, followed by centrifugation at 40,000 g for 30 min at 4°C. These pellets were then resuspended in 1% Sarkosyl and centrifuged a second time. The inner membrane was dissolved facilitating the extraction of the insoluble outer membrane. OMP was analyzed by use of electrophoresis on a 10% sodium dodecyl sulfate–polyacrylamide gel and visualized by staining with 0.125% Coomassie blue stain.

Mating experiments were performed with *E. coli* C<sub>600</sub>Rif<sup>R</sup>/NA<sup>R</sup> as the recipient. Ceftazidime (concentration, 10  $\mu$ g/mL), nalidixic acid (concentration, 50  $\mu$ g/mL), and rifampin (concentration, 60  $\mu$ g/mL) were used as selecting agents.

**PCR amplification and sequencing of PCR products for bla<sub>TEM</sub> and bla<sub>SHV</sub>.** Genomic DNA from KP3160 (isolated from case 15) and EC2859 (isolated from case 12) was digested with *Hind*III and ligated into *Hind*III digested pACYC184 (New England Biolabs) and transformed into *E. coli* DH5 $\alpha$  and selected on trypticase soy agar plus 50  $\mu$ g/mL of ampicillin. Clones were tested for the presence of the desired SHV enzyme by means of isoelectric focusing. The full-length SHV PCR product was generated with use of *Pfu* polymerase (Stratagene) and the primers SHV162F (5'-GCC TTT ATC GGC CCT CAC TCA-3') and SHV1135B (5'-ATG CCG CCG CCA GTC ATA TC-3'). The purified PCR products were sequenced (2 PCR reactions per sample) with the primers SHVF, SHVB, SHV671F (5'-GCT GGT TTA TCG CCG ATA AG-3'), and SHV671B (5'-TCT TAT CCG CGA TAA ACC AGC-3'). Isolates EC1924 (from case 10), EC3022 (from case 15), and KP2679 (from case 17) had their TEM-containing plasmid isolated and transformed into *E. coli* DH5 $\alpha$  or HB101. E-test ESBL strips were used to confirm that the resulting clones were ESBL producers. The TEM gene was amplified by PCR with use of *Pfu* polymerase and the primers BLAOT3 (5'-ATG AGT ATT CAA CAT TTC CG-3') and BLAOT4 (5'-CCA ATG CTT AAT CAG TGA GG-

3') [7]; it was then placed into PCR blunt vector (Invitrogen). Plasmid DNA (2 clones for each enzyme) was sequenced with the primers BLAOT3, BLAOT4, BLA189F (5'-ACG TTT TCC AAT GAT GAG CAC T-3'), and BLA608B (5'-CGG GAA GCT AGA GTA AGT AGT-3'). All sequencing was performed by ACTG, Inc. (Northbrook, IL), and analyses were performed in-house by use of Vector NTI 5.2 (InforMax)

**Assessment of outcomes.** An episode of bloodstream infection was defined by the isolation of *E. coli* and *K. pneumoniae* strains that had a ceftazidime MIC of  $\geq 2$   $\mu$ g/mL from at least 1 blood culture and the presence of at least 1 of the following clinical signs or symptoms of infection: temperature of  $>38^\circ\text{C}$ , chills, or hypotension (for patients aged  $\leq 12$  months: temperature of  $>38^\circ\text{C}$ , hypothermia [temperature  $<37^\circ\text{C}$ ], apnea, or bradycardia). Bloodstream infection was classified as primary or secondary, and acquisition was classified as nosocomial or community by use of a standard surveillance definition [8].

Clinical outcomes were classified as one of the following: complete response, partial response, failure, relapse, or unassessable. Complete response was defined as resolution of fever, leukocytosis, and local signs of infection. Partial response was defined as improvement of fever, leukocytosis, and local signs of infection without complete resolution. Treatment failure was defined as absence of resolution or worsening of signs and symptoms of infection. Relapse was defined as recurrence of infection with same organism at any body site in  $\leq 1$  month after discontinuation of therapy. An outcome was defined as unassessable if medical records were incomplete.

Clinical outcome was assessed for all assessable episodes of bloodstream infection according to strain type, initial antimicrobial regimen, and type of treatment. A patient who had complete or partial response to treatment was considered a responder, whereas those who experienced a relapse or those whose infections failed to respond to treatment were considered nonresponders.

**Statistical analysis.** Treatment outcomes were analyzed according to strain type (*E. coli* or *K. pneumoniae*), antimicrobial regimen (including cephalosporin or not including cephalosporin) initially and at the end of treatment, and type of treatment (monotherapy or combination therapy). To compare treatment outcomes for each of the subgroups,  $\chi^2$  or Fisher's exact test was used, as appropriate.  $P < .05$  indicated statistical significance. All analyses were performed with GraphPad Prism software, version 3.00 for Windows (GraphPad Software).

## RESULTS

**Patients and bacterial strains.** A total of 44 episodes of bacteremia for which tests were positive for *E. coli* or *K. pneumoniae* with ceftazidime MICs of  $\geq 2$   $\mu$ g/mL were identified from the computerized microbiology database at UCLA Med-

ical Center for the years 1986–1997. Of those 44 episodes, 36 (in 34 patients) were treated with  $\geq 3$  days of antimicrobial therapy and were included in the analysis of treatment outcome. Two patients each had 2 separate episodes of bloodstream infections due to different organisms. *E. coli* was more frequently isolated from study patients than was *K. pneumoniae* (23 vs. 13 patients, respectively). At least 1 blood isolate was saved for 21 episodes of bloodstream infection; 14 *E. coli* isolates and 7 *K. pneumoniae* isolates were available for phenotypic and molecular analysis.

Patient characteristics are shown in table 1. Bacteremia was primary in 14 (39%) of 36 episodes and secondary in 22 episodes (61%). For patients with secondary bacteremia, urine was the most frequent source of primary infection. Half of the episodes occurred in solid-organ transplant recipients. End-stage hepatic or renal disease and malignancy were the next most common underlying conditions. Most episodes (24 [67%] of 36) were classified as nosocomial. Approximately one-third of the patients had a recent history of surgery or ceftazidime exposure ( $\leq 30$  days before the first positive blood culture result). At the time that the positive blood culture result was obtained, most patients with episodes were hospitalized (in 24 [67%] of 36 episodes) in an acute-care unit. The crude mortality rate was 22% (8 of 36 episodes), and attributable mortality due to sepsis was 8% (3 of 36 episodes).

**Phenotypic characterization: antimicrobial susceptibility testing.** Table 2 summarizes the results of in vitro antimicrobial susceptibility tests of selected antimicrobial agents. None of the 21 saved isolates were fully susceptible to ticarcillin and piperacillin, except 1 strain that had a piperacillin MIC of 8  $\mu\text{g}/\text{mL}$ ; this strain produced an AmpC enzyme at a low level. Combination with tazobactam restored the activity of piperacillin (MIC,  $\leq 8 \mu\text{g}/\text{mL}$ ) against 11 (79%) of 14 *E. coli* strains, regardless of whether the strains produced ESBL, but restored its activity against only 2 (29%) of 7 *K. pneumoniae* strains, each of which produced 2  $\beta$ -lactamases (TEM-6 and SHV-1; table 3). On the other hand, combination with clavulanic acid restored the activity of ticarcillin against only 2 *E. coli* strains, neither of which produced ESBL. All isolates were susceptible to imipenem and meropenem at an MIC of  $\leq 0.5 \mu\text{g}/\text{mL}$ . Most isolates were susceptible to gentamicin (14 of 21 isolates) and to ciprofloxacin (17 of 21 isolates). All strains but 2 were resistant to trimethoprim-sulfamethoxazole (data not shown).

**Phenotypic characterization: ESBL screening and confirmatory tests.** Among the antimicrobial agents recommended by NCCLS for identifying strains that produce ESBL, ceftazidime, aztreonam, and cefpodoxime at an MIC of  $\geq 2 \mu\text{g}/\text{mL}$  had sensitivities of 100%, 90%, and 70% and positive predictive value of 52%, 55%, and 55%, respectively, for identifying ESBL-producing strains. Strains that have either TEM-12 or TEM-6 are less efficient in hydrolyzing ceftriaxone and cefotaxime [8];

**Table 1. Demographic characteristics of 34 patients with bacteremia due to ceftazidime-resistant *Escherichia coli* (EC) and *Klebsiella pneumoniae*.**

Characteristic	Value
Assessable episodes, no.	36 <sup>a</sup>
Sex, M/F	12/22
Age, mean years $\pm$ SD	53.5 $\pm$ 24
Underlying condition(s), n/N (%)	
Malignancy	4/36 (11)
End-stage kidney or liver disease	4/36 (11)
Organ transplantation	18/36 (50)
Neutropenia	3/36 (8)
Other	6/36 (17)
None	1/36 (3)
Days in hospital, mean $\pm$ SD (range)	36.7 $\pm$ 24 (4–139)
Overall mortality, n/N (%)	8/36 (22)
Attributable mortality, n/N (%)	3/36 (8)

<sup>a</sup> In 34 patients

for such strains, both agents have MICs in the range of 0.5–2  $\mu\text{g}/\text{mL}$ . Cefoxitin MICs were generally higher for strains that produced AmpC enzyme (MIC<sub>50</sub>, 64  $\mu\text{g}/\text{mL}$ ) than they were for strains that produced ESBLs (MIC<sub>50</sub>, 16  $\mu\text{g}/\text{mL}$ ; tables 3 and 4). It is of note that cefoxitin susceptibility (MIC,  $\leq 8 \mu\text{g}/\text{mL}$ ) was observed in only 5 (45%) of 11 ESBL-producing strains.

Using either the original ESBL E-test strip or broth microdilution tests, we performed an ESBL confirmatory test of ceftazidime alone and in combination with clavulanic acid and found that it discriminated between ESBL-producing and non-ESBL-producing strains (sensitivity, 100%; positive predictive value, 78%; specificity, 70%; negative predictive value, 100%; tables 3 and 4). Broth microdilution tests with cefotaxime alone or in combination with clavulanic acid were less sensitive but more specific in confirming ESBL production (sensitivity, 91%; positive predictive value, 83%; specificity, 80%; negative predictive value, 89%). Two *E. coli* strains had a positive ESBL confirmatory test result for all 3 of these tests but did not produce an ESBL; both strains produced an atypical cephalosporinase that was significantly inhibited by clavulanic acid. For 1 *K. pneumoniae* strain that produced SHV-1, there were false positive ESBL confirmatory test results for the ESBL E-test and broth microdilution test of clavulanic acid alone and in combination with ceftazidime, but not clavulanic acid in combination with cefotaxime. On the other hand, for 1 strain of *E. coli* that produced TEM-12 (isolated from patient 10), there was a false negative results for the ESBL confirmatory broth microdilution test of cefotaxime alone and in combination with clavulanic acid.

The clinical relevance of performance of ESBL confirmatory test for isolates with elevated MICs for the ESCs was demon-

**Table 2. In vitro susceptibilities of *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) blood isolates from episodes (cases) of bacteremia.**

Case no.	Isolate		MIC, $\mu\text{g}/\text{mL}$											
	Organism	Type <sup>a</sup>	Caz	Atm	Cpd	Ctx	Ctrx	Tic	Tic + CA	Pip	Pip + Taz	Gm	Amk	Cip
1	EC	A	>32	16	>16	8	8	64	64	64	$\leq 8$	4	16	0.12
2	EC	A	>32	32	>16	32	>32	256	256	128	$\leq 8$	10	1	0.25
3	EC	A	2	4	>16	2	<b>0.5</b>	> 512	32	8	$\leq 8$	10	2	2
4	EC	A	32	16	>16	4	4	>512	128	64	16	0.5	2	0.25
5	EC	B	16	>32	>16	2	2	>512	$\leq 8$	128	$\leq 8$	4	8	0.25
6	EC	B	16	32	>16	2	8	>512	$\leq 8$	128	$\leq 8$	0.5	4	0.25
7	EC	C	>32	16	>16	32	2	128	256	256	$\leq 8$	6	2	2
8	EC	D	8	16	>16	4	<b>1</b>	>512	128	64	$\leq 8$	10	2	0.25
9	EC	D	>32	16	>16	16	32	>512	256	>512	32	1	2	0.25
10	EC	E	32	2	>16	<b>0.5</b>	<b>0.5</b>	>512	16	512	8	1	4	0.12
11	EC	F	32	16	>16	8	8	>512	16	512	$\leq 8$	0.5	2	0.25
12	EC	F	16	32	>16	4	2	>512	256	>512	$\leq 8$	10	1	0.25
13	EC	F	16	32	>16	2	4	>512	256	>512	32	0.5	1	0.25
14	KP	F	>32	>32	>16	>32	>32	>512	512	>512	>512	10	8	2
15	EC	G	>32	>32	>16	32	32	>512	64	256	$\leq 8$	0.5	2	2
16	KP	H	2	$\leq 0.5$	<b>0.5</b>	$\leq 0.12$	<b>0.5</b>	>512	512	>512	>512	0.5	1	0.12
17	KP	I	>32	>32	>16	<b>1</b>	2	>512	128	512	$\leq 8$	6	4	0.25
18	KP	I	>32	>32	>16	<b>1</b>	2	>512	128	512	$\leq 8$	0.5	2	1
19	KP	J	>32	>32	>16	16	32	>512	512	>512	>512	0.5		0.25
20	KP	K	>32	>32	>16	>32	>32	>512	512	>512	>512	0.5	1	0.25
21	KP	K	>32	>32	>16	>32	>32	>512	512	>512	>512	1	1	0.25

**NOTE.** Values bold indicate that the MIC would not have characterized the strain as a possible extended-spectrum  $\beta$ -lactamase producer on the basis of the National Committee on Clinical Laboratory Standards screening break points [2]. All isolates except those from cases 13 and 16 were resistant to trimethoprim-sulfamethoxazole (MIC  $>4 \mu\text{g}/\text{mL}$  and  $>76 \mu\text{g}/\text{mL}$ , respectively). Resistance to aminoglycosides and quinolones varied among the isolates, with no apparent differences between the AmpC versus extended-spectrum  $\beta$ -lactamase type strains. Amk, amikacin; Atm, aztreonam; CA, clavulanic acid; Caz, ceftazidime; Cip, ciprofloxacin; Cpd, cefpodoxime; Ctrx, ceftriaxone; Ctx, cefotaxime; Gm, gentamicin; Pip, piperacillin; Taz, tazobactam; Tic, ticarcillin.

<sup>a</sup> Isolate types are based on the isoelectric points of the  $\beta$ -lactamases detected, as shown in tables 3 and 4.

strated by cases 8 and 12 (tables 2 and 5). These 2 cases of bloodstream infection were caused by an *E. coli* isolate that had cefotaxime and ceftriaxone MICs of 1–4  $\mu\text{g}/\text{mL}$  and ceftazidime MICs of 8 and 16  $\mu\text{g}/\text{mL}$ . Both patients were treated with ceftizoxime; the patient with case 8 had a complete response (ceftizoxime MIC, 4  $\mu\text{g}/\text{mL}$ ), but the patient with case 12 died as a result of urosepsis after 6 days of treatment (ceftizoxime MIC, 2  $\mu\text{g}/\text{mL}$ ). The first case was associated with a non-ESBL-producing strain that produced both a TEM-1 and AmpC  $\beta$ -lactamase, which indicates that a combination of non-ESBL enzymes can result in an ESBL screening phenotype that has negative ESBL confirmatory test results. The second case was caused by a strain that produced the enzymes SHV-12 and TEM-1; the ESBL phenotype was confirmed by both the ESBL E-test and the broth microdilution test of ceftazidime alone and in combination with clavulanic acid. In this case, the failure to detect ESBL-mediated ESC resistance and modify therapy from ceftizoxime likely contributed to the fatal outcome. The difference in responses to treatment with an ESC in these 2 cases may be attributable to ESBL production in one strain but

not in the other. Undoubtedly, if there had been a positive ESBL confirmatory test result at the time the bacterial strain was isolated and its identification and antibiotic susceptibility profile were reported to the clinician, as well as a lack of clinical response, these findings would have guided the clinician to promptly change therapy and, possibly, would have prevented the patient's death from sepsis.

**Molecular analysis.** Tables 3 and 4 show the isoelectric points for each isolate and the  $\beta$ -lactamases identified for selected isolates. The isolates were grouped into 11 types (designated A–K) on the basis of the isoelectric points of the  $\beta$ -lactamases detected. The isolates were further grouped into AmpC-producing strains ( $n = 9$ ) and putative ESBL-producing strains ( $n = 12$ ) on the basis of the results of  $\beta$ -lactamase extraction and isoelectric focusing studies. PCR amplification and sequencing of the PCR products for *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> were performed for each of the non-AmpC enzymes that had a unique isoelectric point.

A majority (9 [64%] of 14) of the *E. coli* strains (types A, B, C, and D) produced an AmpC enzyme, compared with 0 of the 7 *K. pneumoniae* strains. Among the AmpC-producing

**Table 3. Profiles of putative extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) isolates from episodes (cases) of bacteremia.**

Case no.	Organism isolated	Isolate pl profile										E-test ESBL result	Putative $\beta$ -lactamase(s) identified		
		Type	Approximate pls					MIC, $\mu$ g/mL							
			5.2	5.4	6.0	7.5	8.2	Cfox	Caz	Caz + CA	Ctx			Ctx + CA	
10	EC	E	S						8	32	0.5	0.5	0.12	P	TEM-12
11	EC	F		X			X	>16	32	<0.12	8	0.12	P	TEM-1 + SHV-12	
12	EC	F		X			S	4	16	0.25	4	0.12	P	TEM-1 + SHV-12	
13	EC	F		X			X	16	16	0.5	16	0.12	P	TEM-1 + SHV-12	
14	KP	F		X			X	8	> 32	2	>16	0.12	P	TEM-1 + SHV-12	
15	EC	G			S			32	> 32	2	>16	0.25	P	TEM-71	
16	KP	H				S		2	2	<0.12	0.12	0.12	P	SHV-1	
17	KP	I			S	S		8	> 32	2	8	0.12	P	TEM-6 + SHV-1	
18	KP	I			X	X		4	> 32	1	4	0.12	P	TEM-6+ SHV-1	
19	KP	J				S	X	4	> 32	0.5	>16	0.12	P	SHV-1 + SHV-5	
20	KP	K				X	S	16	> 32	0.5	>16	0.25	P	SHV-1 + SHV-5	
21	KP	K				X	X	16	> 32	0.5	>16	0.12	P	SHV-1 + SHV-5	

**NOTE.** CA, clavulanic acid; Caz, ceftazidime; Cfox, ceftioxitin; Ctx, cefotaxime; ESBL, extended-spectrum  $\beta$ -lactamase; N, negative; P, positive; pl, isoelectric point; S, the enzyme has been sequenced.

*E. coli* strains, type B strains were unique because the  $\beta$ -lactamase had a high isoelectric point (9.0), a finding consistent with the presence of AmpC, but the enzyme was inhibited by clavulanic acid and tazobactam, whereas type D strains produced concomitant TEM-1 enzyme. In addition, OMP characterization was performed for 3 *E. coli* isolates. The ceftioxitin-susceptible isolate from patient 5 showed 3 proteins in the 31,000–42,700-kDa range. The ceftioxitin-resistant isolates from cases 2 and 8 exhibited only 2 OMP bands and showed the loss of a 38-kDa protein. In addition, ceftioxitin resistance could not be transferred to the *E. coli* C<sub>600</sub>Rif<sup>R</sup>/NA<sup>R</sup> recipient by conjugation with either of the 2 strains examined. These data suggest that decreased permeability contributed to ceftioxitin resistance in these strains.

On the basis of PCR amplification and sequencing of the PCR products for *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>, 5 of 14 *E. coli* strains and 6 of 7 *K. pneumoniae* strains produced ESBLs, as follows: TEM-12 (*n* = 1), SHV-12 (*n* = 4), TEM-71 (*n* = 1), TEM-6 (*n* = 2), and SHV-5 (*n* = 3). Nine of 11 strains produced 2  $\beta$ -lactamases. No strain produced multiple ESBLs. For 1 *K. pneumoniae* strain (isolated from patient 16), the ceftazidime MIC was elevated (2  $\mu$ g/mL), the ESBL E-test result was positive, and the strain produced SHV-1 enzyme. One *K. pneumoniae* strain (isolated from patient 14) had an isoelectric point profile identical to that of *E. coli* type F isolates, which suggests that the gene encoding for this ESBL (SHV-12) may have been transferred between *E. coli* and *K. pneumoniae* strains.

**Treatment.** Initial treatment regimens for the 36 clinically assessable episodes of bloodstream infection included administration of an ESC agent (22 episodes [61%]), most commonly

ceftazidime (7 episodes [31%]) or ceftioxitin (7 episodes [19%]). Therapy with an agent other than a cephalosporin was primarily with ampicillin-sulbactam (1 episode [3%]), ciprofloxacin (5 episodes [14%]), and imipenem (4 episodes [11%]) alone or in combination with an aminoglycoside. Initial treatment regimens included a single agent or combination of agents (18 episodes each). Most patients received multiple courses of antimicrobial therapy for the episode of bloodstream infection, for a total of 49 courses of treatment (range, 1–4 regimens per episode). Table 5 summarizes the treatment regimens, duration, and associated outcomes for 21 episodes from which an isolate was available for phenotypic and molecular analysis.

**Outcomes.** Overall, patients responded to treatment in 20 (56%) of 36 assessable episodes. Patients with bloodstream infections caused by *E. coli* strains with elevated ceftazidime MICs were almost twice as likely to respond to treatment as patients with infections caused by *K. pneumoniae*, although the difference was not statistically significant (15 [65%] of 23 vs. 5 [38%] of 13 episodes; *P* = .12). A trend toward better treatment response was observed for noncephalosporin regimens compared with regimens containing an ESC, both for *E. coli* infections (6 [86%] of 7 vs. 9 [56%] of 16 episodes; *P* = .34) and *K. pneumoniae* infections (3 [50%] of 6 vs. 2 [28%] of 7 episodes; *P* = .59; figure 1). When treatment response was analyzed according to regimens that contained at least 1 agent to which the strain was susceptible in vitro, monotherapy and combination therapy were equally effective for the treatment of *E. coli* bacteremia (4 [44%] of 9 vs. 4 [57%] of 7 episodes; *P* = 1.0). In contrast, none of the episodes of *K. pneumoniae* bacteremia responded to monotherapy, whereas 4 (57%) of 7 ep-

**Table 4. Profiles of AmpC-producing strains of *Escherichia coli* (EC) isolated from episodes (cases) of bacteremia.**

Case no.	Organism isolated	Isolate pI profile			MIC, µg/mL					E-test ESBL result	β-Lactamase type	38-kDa OMP result
		Type	Approximate pls		Cfox	Caz	Caz + CA	Ctx	Ctx + CA			
1	EC	A		X	64	>32	>16	4	4	N	AmpC (derepressed)	NT
2	EC	A		X	64	>32	>16	>16	>16	N	AmpC (derepressed)	N
3	EC	A		X	64	2	1	2	0.5	N	AmpC (low level)	NT
4	EC	A		X	>64	32	8	4	2	N	AmpC	NT
5	EC	B		X	2	16	<0.12	8	0.12	P	Atypical AmpC?; not SHV or TEM	P
6	EC	B		X	2	16	<0.12	16	0.12	P	Atypical AmpC?; not SHV or TEM	NT
7	EC	C		X	>64	>32	>16	>16	>16	N	AmpC type	NT
8	EC	D	S	X	>64	8	2	4	1	N	TEM-1 + AmpC (low level)	N
9	EC	D	X	X	64	>32	>16	16	16	N	TEM-1 + AmpC	NT

**NOTE.** CA, clavulanic acid; Caz, ceftazidime; Cfox, cefoxitin; Ctx, cefotaxime; ESBL, extended-spectrum β-lactamase; N, negative; NT, not tested; OMP, outer membrane protein; P, positive; pI, isoelectric point; S, enzyme has been sequenced.

isodes responded to combination therapy. However, the small number of patients in each subgroup precluded meaningful statistical comparison.

**Clinical and microbiologic correlation.** The relationship between β-lactamase type and treatment outcomes, particularly with the ESCs, was examined in detail for the 21 episodes of bloodstream infection for which isolates were saved (table 5). Overall, 35 courses of antimicrobial therapy were prescribed for these 21 episodes of bloodstream infection; 19 courses for 11 ESBL-associated episodes and 16 courses for non-ESBL-associated episodes. The clinical response to the initial treatment regimen was significantly lower for episodes of bloodstream infection caused by ESBL-producing strains than for episodes of infection with non-ESBL-producing strains (2 [18%] of 11 vs. 8 [80%] of 10 episodes;  $P = .009$ ). However, when clinical response was determined at the end of treatment, a similar result was observed (ESBL vs. non-ESBL-associated infection: 7 [64%] of 11 vs. 9 [90%] of 10 episodes;  $P = .31$ ). Among the 11 episodes associated with ESBL-producing strains, the initial and end-of-treatment response rates did not differ according to whether the strains had TEM or SHV-type enzymes (data not shown).

When clinical response was assessed by treatment type, we observed that the response to empiric therapy was similar for ESC-containing courses of antimicrobials and for non-ESC-based courses (5 [38%] of 13 vs. 5 [63%] of 8 episodes;  $P = .39$ ; figure 2); a similar response was noted at the end of treatment (7 [70%] of 10 vs. 9 [82%] of 11 episodes;  $P = .64$ ). Among the 16 ESC-based courses, the response rate for non-ceftazidime ESCs was more than double that for ceftazidime (7 [70%] of 10 vs. 2 [33%] of 6 episodes;  $P = .3$ ), although this difference did not reach statistical significance. Partial or

complete response to nonceftazidime ESCs was observed for 2 patients infected with strains that produced TEM-6 or TEM-12 (1 of each). Two patients died of sepsis as a result of failure to respond to treatment with ceftazidime therapy alone or in combination with ciprofloxacin. Both of these patients had bloodstream infections with *K. pneumoniae* strains that harbored either a TEM-6 or SHV-5 ESBL. In addition, imipenem was prescribed for 4 courses of therapy, 2 of which resulted in relapse. One case of relapse was caused by infection with a strain of *E. coli* that produced the TEM-71 enzyme; *K. pneumoniae* that produced both SHV-1 and SHV-5 enzymes was associated with the other case of relapse. When treatment response was analyzed according to regimens that contain at least 1 agent to which the strain is susceptible in vitro, response rates for monotherapy and combination empiric therapy were similar (3 [38%] of 8 episodes, vs. 2 [40%] of 5 episodes;  $P = 1.0$ ). Response rates noted at the end of treatment for the same comparison (10 [83%] of 12 vs. 4 [57%] of 7 episodes;  $P = .3$ ) favor monotherapy, but the difference in rates did not reach statistical significance (figure 2). Of the 5 non-ESC-containing treatment courses that resulted in a positive response, 4 (80%) were combination therapy.

## DISCUSSION

We identified 36 episodes of bloodstream infection that occurred during a 12-year period among patients at a large tertiary-referral medical center and were associated with strains of *E. coli* and *K. pneumoniae* that had reduced susceptibility to ceftazidime. Infections occurred sporadically throughout the study period and included both nosocomial and community-acquired cases of bacteremia. Blood isolates from 21 of the 36

**Table 5. Antimicrobial regimens and outcomes for patients with episodes (cases) of bacteremia due to ceftazidime-resistant strains of *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP).**

Isolate type, case no.	Patient's underlying condition(s)	Organism isolated	Enzyme(s) identified	Antimicrobial therapy <sup>a</sup>		Therapy outcome (patient outcome)	Comment
				Agent (duration, days)	MIC, $\mu\text{g/mL}$		
ESBL nonproducer							
1	Recurrent cholangitis	EC	AmpC (Derep/OMP-negative?)	Caz (3) Amk (13) Cper (5)	Caz, 32 Amk, 16 Cper, 8.0	Failure Failure CR (alive)	
2	OLT	EC	AmpC (Derep/OMP-negative)	Caz (3) Cip (9)	Caz, 32 Cip, 0.25	PR CR (alive)	Discharged home receiving oral Cip therapy
3	ESRD, DM	EC	AmpC (low level)	Cip (3) + Gm (5) Amk (5)	Cip, 2.0; Gm, 10.0 Amk, 2.0	PR CR (alive)	Discharged home receiving Amk therapy
5 <sup>b</sup>	ESRD, ESLD	EC	Atypical AmpC	Czox (10)	Czox, 1	CR (alive)	
16	OLT	KP	SHV-1	Cper (3) + Pip (3) Ctx (18)	Cper, NT; Pip, 512 Ctx <0.5	Failure CR (alive)	
8	Kidney-pancreas TR	EC	TEM-1 + AmpC (low level), OMP-negative	Czox (9)	Czox, 4	CR (alive)	
4	OLT	EC	AmpC	Cip (14) + Gm (22)	Cip, 0.25; Gm, 0.5	PR (died)	Died of polymicrobial sepsis
6	Malignancy	EC	Atypical AmpC	Clfx (8)	Clfx <0.008	PR (alive)	Defervescence occurred after neutropenia resolved
9	OLT	EC	TEM-1 + AmpC	Caz (3) + Amp-Sulb (3)	Caz, 32; Amp-Sulb, 32; Cip, 0.25	PR (alive)	Discharged home receiving oral Cip therapy
7	Malignancy	EC	AmpC	Cip (3) + Gm (3) Imi (11) + Amk (11)	Cip, 2.0; Gm, 6.0 Imi, 0.25; Amk, 2.0	PR Relapse (alive)	Readmitted to the hospital for urosepsis due to ESBL-EC

## ESBL producer

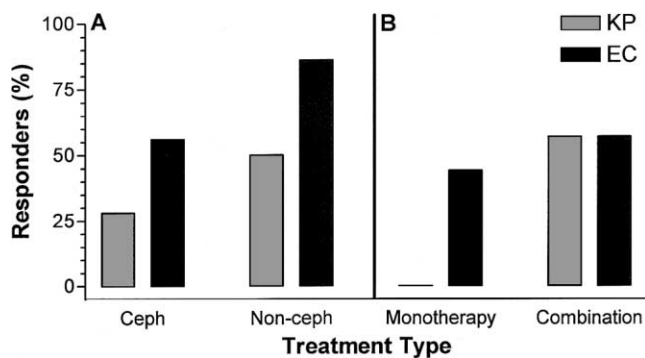
10	OLT	EC	TEM-12	Cper (3) Czox (9)	Cper, NT Czox, 0.5	PR CR (alive)	
11	OLT	EC	TEM-1 + SHV-12	Czox (17) Cip (7) + Amp-Sulb (7)	Czox, 4 Cip, 0.25; Amp-Sulb, 32	Relapse CR (alive)	
14	Biliary atresia	KP	TEM-1 + SHV-12	Imi (10) + Amk (10)	Imi, 0.25; Amk, 8.0	CR (alive)	
17	OLT	KP	TEM-6 + SHV-1	Caz (3) + Amp-Sulb (3) Czox (5)	Caz, 32; Amp-Sulb, 8.0 Czox, 0.5	Failure PR (alive)	
19	OLT	KP	SHV-1 + SHV-5	Cip (8) Imi (45) + Gm (5)	Cip, 0.25 Imi, 0.25; Gm, 0.5	Relapse PR (alive)	
20 <sup>c</sup>	OLT	KP	SHV-1 + SHV-5	Imi (17) Cip (34)	Imi, 0.25 Cip, 0.25	Relapse PR (alive)	Discharged home receiving oral Cip therapy
15	BPH	EC	TEM-71	Czox (8) + Gm (5) Imi (4) + Gm (5) Czox (5)	Czox, 8; Gm, 0.5 Imi, 0.25; Gm, 0.5	Relapse Relapse PR	Died 1 month after treatment was completed
13 <sup>c</sup>	OLT	EC	TEM-1 + SHV-12	Pip-Taz (8) + Gm (8) Cip (17)	Pip-Taz, 8/4; Gm, 0.5 Cip, 0.25	PR (died) Relapse (alive)	Irreparable bile leak, persistently positive blood culture results
12	Recurrent UTI	EC	TEM-1 + SHV-12	Czox (6)	Czox, 2	Failure (died)	Died of urosepsis
18	OLT	KP	TEM-6 + SHV-1	Caz (4)	Caz, 32	Failure (died)	Died of sepsis
21 <sup>b</sup>	ESRD, ESLD	KP	SHV-1 + SHV-5	Caz (3) + Cip (3)	Caz, 32; Cip, 0.25	Failure (died)	Died of sepsis

**NOTE.** BPH, benign prostatic hypertrophy; CR, complete response; DM, diabetes mellitus; EC, *E. coli*; ESBL, extended-spectrum  $\beta$ -lactamase; ESLD, end-stage liver disease; ESRD, end-stage renal disease; KP, *K. pneumoniae*; NT, not tested; OLT, orthotopic liver transplantation; PR, partial response; TR, transplantation; UTI, urinary tract infection.

<sup>a</sup> Amp-Sulb, ampicillin-sulbactam; Amk, amikacin; Caz, ceftazidime; Cip, ciprofloxacin; Clfx, clinafloxacin; Cper, cefoperazone; Czox, ceftizoxime; Gm, gentamicin; Imi, imipenem; Pip, piperacillin; Taz, tazobactam.

<sup>b</sup> Cases 5 and 21 occurred in the same patient. The patient experienced separate episodes of bacteremia due to *Escherichia coli* and *Klebsiella pneumoniae*, 21 days apart. The patient had a complete response to treatment with Czox alone for *E. coli* bacteremia; however, the patient died as a result of overwhelming sepsis due to *K. pneumoniae* after 3 days of therapy with ceftazidime and ciprofloxacin.

<sup>c</sup> Cases 13 and 20 occurred in the same patient. The patient experienced separate episodes of bacteremia due to *E. coli* and *K. pneumoniae*, 1.5 months apart; the patient responded to prolonged therapy with ciprofloxacin for *K. pneumoniae* bacteremia but experienced persistent positive blood cultures of *E. coli* after therapy with ciprofloxacin.



**Figure 1.** Response to the initial empirical regimen for patients with bacteremia due to ceftazidime-resistant *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP) by strain type. A, Regimens that included an extended-spectrum cephalosporin (ESC) compared with other regimens ( $n = 36$  patients). B, Monotherapy compared with combination therapy (all regimens contained  $\geq 1$  agent to which the strain was susceptible;  $n = 25$  patients). Ceph, ESC-containing regimen; non-ceph, non-ESC-containing regimen; responders, patients who responded to treatment.

episodes were available for phenotypic and molecular analysis, which facilitated the correlation of phenotypic and genotypic characteristics of the strains with treatment outcome for most of the episodes. Previous studies have focused on the epidemiology, laboratory detection methods, and molecular characterization of ESBL-producing organisms. Data on treatment outcomes are limited to case reports or descriptions of nosocomial outbreaks [6, 9–25]. Thus, sufficient clinical data to establish the relationship between the type of ESBLs produced by the infecting strains and treatment outcomes is lacking. To our knowledge, this is the largest reported series (not associated with an outbreak investigation) to examine treatment of bloodstream infections caused by strains of *E. coli* and *K. pneumoniae* with reduced susceptibility to ceftazidime.

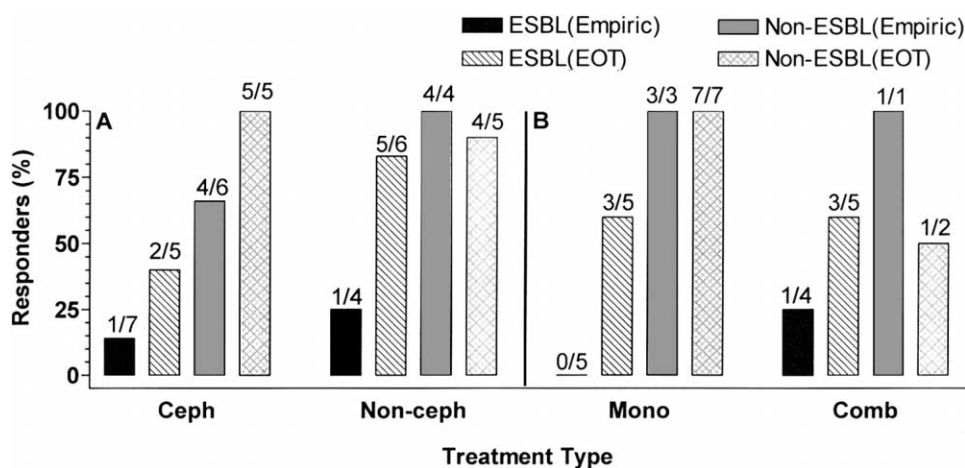
On the basis of in vitro susceptibility results and limited reports of clinical experience, imipenem has emerged as the agent of choice for the treatment of serious infections associated with ceftazidime-resistant strains of *E. coli* and *K. pneumoniae*. This has occurred despite infrequent reports of the failure of ESCs treatment of infections caused by strains that appear to be susceptible in vitro [6]. Lacking sufficient outcome data on other agents, many investigators recommend the use of imipenem as the treatment of choice for serious infections due to ESBL-producing isolates [12, 23]. Several investigators have concluded that initial treatment of bloodstream infections caused by ESBL-producing strains with noncarbapenem agents may be associated with higher mortality than is treatment with a carbapenem agent; however, details on treatment information for most patients were not available in those studies [26, 27]. Currently, the NCCLS recommends that ESBL-producing strains should be reported by microbiology laboratories as resistant to all penicillins, cephalosporins, and aztreonam [2].

These criteria and other expert recommendations may promote the exclusive use of imipenem for the treatment of serious infections caused by ceftazidime-resistant *E. coli* and *K. pneumoniae* strains, limiting clinical experience with the use of other agents, particularly the ESCs. Increased use of imipenem also may promote antimicrobial resistance. Rahal and associates [28] found that reduction in the use of ESC resulted in a dramatic decrease in the incidence of ESBL-producing strains, but reduction in use was also associated with a 140% increase in the use of imipenem and a 70% increase in the incidence of infection with imipenem-resistant strains of *Pseudomonas aeruginosa*. Therefore, therapeutic options other than carbapenems would be an attractive alternative for ceftazidime-resistant ESBL-producing organisms.

In this study, we retrospectively assessed treatment outcomes and examined clinical response after both initial empirical therapy and at end of treatment. Of 36 episodes, more than one-half were treated with an ESC, and only 4 courses of imipenem were administered. In addition, although other studies have reported treatment experience with outbreak strains of *K. pneumoniae* that produced only 1 or 2 different types of ESBLs, we evaluated treatment outcomes for bloodstream infections associated with 5 different types of ESBL-producing *E. coli* and *K. pneumoniae* strains in a situation in which these strains were endemic.

Our study confirms that the current NCCLS-recommended ESBL screening and confirmatory tests (which use ceftazidime or cefotaxime, each alone and in combination with clavulanic acid) for *E. coli* and *K. pneumoniae* are highly sensitive in detecting a variety of ESBL phenotypes. Although strains producing atypical AmpC or SHV-1, or a combination of a TEM-1 with an AmpC enzyme, yielded false positive screening results for ESBL production, the ESBL confirmatory tests were associated with high positive predictive value (78%–83%). It is of note that, because cefotaxime is less susceptible to hydrolysis by most North American ESBLs than is ceftazidime, the cefotaxime-based ESBL confirmatory test is likely to be less sensitive than are ceftazidime-based tests. Promptly informing clinicians of an *E. coli* or *K. pneumoniae* strain that is suspected to produce ESBL may assist in directing or altering antimicrobial therapy.

We found that the overall treatment response for bloodstream infections due to ceftazidime-resistant *E. coli* strains was higher than for *K. pneumoniae* strains. The difference in treatment response by strain could be explained largely by the variation in the mechanisms of resistance observed among study organisms.  $\beta$ -Lactamase extraction and OMP characterization of available isolates revealed that the underlying mechanism of ceftazidime resistance in the majority of *E. coli* isolates was the activity of an AmpC enzyme with or without a concomitant TEM-1 enzyme and loss of OMPs. On the other hand, 86% of



**Figure 2.** Response to the initial empiric regimen (empiric) versus response at the end of treatment (EOT), according to the isolates' extended-spectrum  $\beta$ -lactamase (ESBL) production status, for patients with bacteremia due to ceftazidime-resistant *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KP). A, Regimens with ESC compared with other regimens ( $n = 21$  cases). B, Monotherapy versus combination therapy (all regimens contained  $\geq 1$  agent to which the strain was susceptible;  $n = 13$  cases with initial response to empirical therapy,  $n = 19$  cases with response at EOT). Ceph, extended-spectrum cephalosporin (ESC)-containing regimen; comb, combination therapy; mono, monotherapy; non-ceph, non-ESC-containing regimen; responders, patients who responded to treatment.

the *K. pneumoniae* strains were found to produce single or multiple TEM- or SHV-type ESBLs. The difference in responses may be attributable in part to the enhanced ability of TEM and SHV ESBLs to hydrolyze ESCs, especially ceftazidime, compared with the ability of AmpC enzymes [29]. Cases 5, 8, and 16 were due to AmpC-producing organisms, which had ceftazidime MICs of 16, 8, and 2  $\mu\text{g}/\text{mL}$ , respectively, and all had complete response to nonceftazidime ESC monotherapy. In contrast, cases 12, 18, and 21 were due to organisms that had 1 of the following combinations of enzymes: SHV-12 and TEM-1, TEM-6 and SHV-1, or SHV-5 and SHV-1. All 3 patients died as a result of sepsis after treatment with an ESC-based regimen. Of note, we did not study porin changes for the *K. pneumoniae* isolates. Thus it is possible that a concomitant loss of OMPs may have contributed to the resistance profile as well as the lower response to treatment.

When outcomes of episodes of bacteremia caused by ESBL-producing strains were analyzed with respect to treatment regimen, no significant difference was found between regimens containing an ESC and other regimens. It is noteworthy that positive response to treatment with an ESC-containing regimen was observed for bacteremia caused by pathogens that produced either TEM-6 or TEM-12. Response to treatment with a nonceftazidime-ESC may be explained by the relatively less efficient ceftazidime-hydrolyzing capability of TEM-6 and TEM-12 ESBLs compared with TEM-71. Ceftazidime treatment was associated with treatment failure in all patients, as expected, because the isolates involved were all resistant to this agent (MIC  $\geq 32 \mu\text{g}/\text{mL}$ ). In contrast to other reports, only a few of our

patients received imipenem therapy; treatment success was not observed in all patients despite an MIC of  $\leq 0.25 \mu\text{g}/\text{mL}$ .

We recognize that we could not evaluate the impact of host and other nondrug factors on the overall clinical response because of the retrospective design of our study. Nonetheless, several important insights can be gained. First, an NCCLS confirmatory test or equivalent should be performed for all *E. coli* and *K. pneumoniae* isolates that test positive for an ESBL phenotype, and the results of these confirmatory tests should be used to guide decisions about therapy in the context of clinical response. Second, we found that ceftazidime-resistant *E. coli* strains produced a greater variety of  $\beta$ -lactamase types than did *K. pneumoniae* strains. Finally, we observed treatment success with the nonceftazidime ESCs for bloodstream infections caused by pathogens that produce ESBLs, which are known to be relatively weak hydrolyzers of the ESCs. Other investigators have also shown that serious infections, including meningitis, caused by ESBL-producing isolates can be successfully treated with an ESC [9, 10].

On the basis of the results of our study, we strongly recommend that the use of ceftazidime for empirical therapy should be avoided whenever possible, both because previous drug exposure may promote acquisition of ESBL-producing organisms and because ceftazidime is more susceptible to hydrolysis by most North American ESBLs than are ceftriaxone or cefotaxime. Our findings support the current NCCLS recommendation that ESBL-producing strains should be reported by microbiology laboratories as resistant to all penicillins, cephalosporins, and aztreonam. [2]. Despite the fact that a minority

of strains that produced an atypical cephalosporinase were falsely confirmed to produce ESBL and that limited cases of clinical success were observed for the treatment of bloodstream infections with an ESC in the patients we reviewed, we currently recommend that the use of ESCs be avoided for the treatment of serious infections caused by a strain with a confirmed ESBL phenotype.

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