JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1997, p. 2061–2067 Vol. 35, No. 8
0095-1137/97/$04.00+0

Detection and Clinical Significance of Extended-Spectrum β-Lactamases in a Tertiary-Care Medical Center

CHRISTOPHER L. EMERY AND LISA A. WEYMOUTH*

Department of Pathology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298

Received 2 January 1997/Returned for modification 4 March 1997/Accepted 1 May 1997

The prevalence of extended-spectrum β-lactamase (ESBL)-mediated resistance remains unknown for most hospitals, and national guidelines for testing and reporting ESBL-mediated resistance have not yet been developed. We undertook a study to determine the prevalence of ESBLs and the clinical need for testing in our tertiary-care medical center. Members of the family *Enterobacteriaceae* isolated over a 6-month period for which ceftazidime or ceftriaxone MICs were greater than 1 μg/ml were tested for production of ESBLs by the double-disk synergy method. Approximately 1.5% of isolates of the family *Enterobacteriaceae* (50 of 3,273), which were isolated from 1.2% of patients (23 of 1,844), were found to express ESBLs. ESBL-producing strains included eight different species and were isolated from patients located throughout the hospital, including outpatient clinics. By using the interpretive guidelines of the National Committee for Clinical Laboratory Standards, 26 to 39% of the isolates would have been reported to be susceptible to ceftazidime, depending upon the routine susceptibility method used. However, tests with cefpodoxime found all of the ESBL-producing strains to be resistant or intermediate. Nine patients infected with ESBL-producing isolates were treated with therapy which included an expanded-spectrum cephalosporin. Seven were cured. The deaths of the other two patients were not attributed to bacterial resistance missed by routine susceptibility testing. These observations suggest that in our tertiary-care medical center, it may not be clinically necessary or cost-effective at this time to institute additional testing on a routine basis to detect ESBL production in all clinical isolates of the family *Enterobacteriaceae*.

Members of the family *Enterobacteriaceae* commonly express plasmid-encoded β-lactamases (e.g., TEM-1, TEM-2, and SHV-1) which confer resistance to penicillins but not to expanded-spectrum cephalosporins (4, 21, 25, 31). In the mid-1980s a new group of enzymes, the extended-spectrum β-lactamases (ESBLs), was detected. ESBLs confer resistance to expanded-spectrum cephalosporins (e.g. ceftriaxone, cefotaxime, and ceftazidime), aztreonam, and related oxyimino-β-lactams (4, 11, 12, 20, 31). These enzymes are usually encoded by mutated TEM-1 and SHV-1 genes on plasmids and are easily transmissible from one organism to another. Nosocomial outbreaks of infection caused by ESBL-producing bacteria have been reported (2, 6, 15, 19, 20, 24). However, the prevalence of ESBL-producing bacteria at most hospitals remains unknown.

ESBL-mediated resistance poses problems for in vitro susceptibility testing and reporting. The optimal substrate profile varies from one ESBL to another (1, 8, 11, 13, 19, 21, 22, 27). As a result, a susceptibility panel with only one expanded-spectrum cephalosporin cannot predict resistance to other expanded-spectrum cephalosporins (8, 19, 23, 30). Some ESBL-producing isolates test susceptible to certain expanded-spectrum cephalosporins when the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) are used (7, 10, 14, 30) but test resistant when a much higher inoculum of 10^7 CFU/ml is used (5, 6, 9, 11, 23, 28). ESBL activity can be detected directly by adding a second test, i.e., the double-disk synergy (DDS) test, the three-dimensional test, or the E test (7, 13, 30). However, these tests increase cost and have variable sensitivities. Finally, the clinical significance of ESBL-mediated resistance remains unclear. Data from animal models and anecdotal cases of ESBL-mediated resistance in humans suggest caution (3, 6, 9, 23), but they do not yet provide clear guidelines for recommendations for therapy. Current NCCLS guidelines state that until more data are available, laboratories may choose to report confirmed ESBL-producing strains as resistant to all cephalosporins and aztreonam (18).

National consensus guidelines have not yet been developed for laboratory testing, i.e., when laboratories should test for ESBLs, what method should be used, and how the results should be reported. Lacking these guidelines, we undertook a 6-month retrospective study to assess whether our hospital microbiology laboratory should routinely perform screening tests for ESBL-producing strains. We attempted to determine (i) the prevalence of patients with infections caused by ESBL-producing members of the family *Enterobacteriaceae*, (ii) the percentage of ESBL-producing organisms that would be reported as resistant or intermediate to expanded-spectrum cephalosporins by routine antimicrobial susceptibility testing methods, and (iii) the outcomes for patients infected with ESBL-producing bacteria.

MATERIALS AND METHODS

Experimental design. The Medical College of Virginia Hospital is a 750-bed tertiary-care center with approximately 31,000 admissions and 300,000 ambulatory visits to the emergency room and outpatient clinics per year. Over a 6-month period, the antibiograms of all clinical isolates of the family *Enterobacteriaceae* routinely tested for susceptibility were reviewed. Isolates for which the ceftazidime and/or ceftriaxone MICs were greater than 1 μg/ml were tested for ESBL production by the DDS test of Jarlier et al. (13). The first ESBL-producing isolate from each patient was then tested by different routine susceptibility methods to determine what percentage would be detected as resistant or intermediate (i.e., not susceptible) to extended spectrum β-lactam agents. Finally, medical records were reviewed for each patient with an ESBL-producing isolate to obtain clinical information and patient outcome.

* Corresponding author. Mailing address: Department of Pathology, Box 980210, Medical College of Virginia, 403 N. 13th St., Richmond, VA 23298-0210. Phone: (804) 828-3494. Fax: (804) 828-6142.

TABLE 1. Prevalence of ESBLs in members of the family *Enterobacteriaceae*

Test group	Routine susceptibility test		DDS test for ESBL		Minimum prevalence (%)[c]
	No. tested	No. (%) for which MICs were elevated[a]	No. tested[b]	No. (%) with ESBLs	
Isolates	3,273	478 (14.6)	400	50 (12.5)	1.5
Patients[d]	1,844	246 (13.3)	197	23 (11.7)	1.2

[a] Elevated MIC is an MIC of ceftazidime and/or ceftriaxone of greater than 1 μg/ml by the breakpoint agar dilution method.
[b] Subset of isolates available for DDS testing for which MICs were elevated.
[c] Calculated as number with ESBL divided by number tested by routine susceptibility testing. Because 78 isolates from 49 patients were not available for testing by the DDS test, this represents a minimum prevalence.
[d] Values are numbers of individual patients with isolates in each category listed.

Susceptibility tests. Routine susceptibility testing in the clinical laboratory was performed by the agar dilution method using breakpoint concentrations and NCCLS guidelines (17, 18). The concentrations of ceftazidime and ceftriaxone tested were 1, 8, and 16 μg/ml. After detection of ESBL production by the DDS test (see below), the first ESBL-producing isolate from each patient was tested in triplicate by the agar dilution, broth microdilution, and disk diffusion methods and once by the E test. Agar dilutions included an additional plate with ceftriaxone at 32 μg/ml. Broth microdilution was performed with custom-made Microscan panels (Dade, West Sacramento, Calif.) according to the manufacturer's directions, and the results were read visually. Panels contained serial twofold dilutions of ceftazidime (1 to 32 μg/ml) and ceftriaxone (1 to 64 μg/ml). The final result was the mode or the median MIC or MIC breakpoint (i.e., the median MIC within a fourfold dilution range) from triplicate determinations. The disk diffusion method and the E test (AB BIODISK, Piscataway, N.J.) were performed according to NCCLS guidelines (16, 18). Disk diffusion zone diameters were averaged to obtain the final zone diameter (in millimeters). All susceptibility results were interpreted according to NCCLS guidelines (18). The only susceptibility results available to physicians were those obtained by the agar dilution breakpoint method. Therapy decisions were sometimes made before an organism had been isolated and tested for susceptibility by the laboratory.

DDS test. After inoculating a Mueller-Hinton plate as described above for the routine disk diffusion method, 30-μg disks of aztreonam, ceftazidime, and ceftriaxone and a 10-μg disk of cefpodoxime were placed 25 mm (center to center) from an amoxicillin-clavulanate (20 and 10 μg, respectively) disk (13, 30). After overnight incubation at 35°C, enhancement of the zone of inhibition between the clavulanate disk and any one of the extended-spectrum β-lactam disks indicated the presence of an ESBL (13).

Isolate identification and storage. Each ESBL-producing isolate was initially identified by using the BBL Crystal Enteric/Non-Fermenter system (Becton Dickinson, Cockeysville, Md.), and the identity was then confirmed by using the API 20E system (bioMérieux, Hazelwood, Mo.). Isolates were stored at −70°C in brain heart infusion broth with 20% glycerol.

Patient information. Medical records were reviewed for each patient from whom an ESBL-producing organism was being isolated. Patient outcome was categorized as (i) cure of the infection caused by the ESBL-producing isolate, (ii) death with ESBL-producing isolate present, or (iii) death due to other causes with prior cure of the infection caused by ESBL-producing bacteria. Cure was defined as resolution of the patient's clinical symptoms with negative cultures (if cultures were performed). Patient death was death occurring during the patient's current hospital admission. Urinary tract infection (UTI) was defined as quantitative urine cultures yielding at least 50,000 CFU of bacteria per ml and one clinical finding of infection (e.g., fever or dysuria) and/or one laboratory finding of infection (e.g., urinalysis with nitrites or with pyuria or leukocytosis). Pneumonia was defined as lower respiratory tract symptoms, with an elevated leukocyte count or fever and sputum yielding moderate to heavy growth of the ESBL-producing organism.

Statistics. Fisher's exact test (two-tail) was used to evaluate differences in mortality rates between patient groups. A P value of ≤ 0.05 was considered statistically significant.

RESULTS

During a 6-month period (1 August 1995 to 31 January 1996) clinical isolates of the family *Enterobacteriaceae* for which the MICs of ceftazidime or ceftriaxone were greater than 1 μg/ml were tested for the presence of an ESBL by the DDS test (Table 1). ESBLs were found in 50 isolates from 23 individual patients. The prevalence of ESBL resistance was estimated to be approximately 1.2% for all patients from whom an organism from the family *Enterobacteriaceae* was isolated.

TABLE 2. Species of the family *Enterobacteriaceae* with ESBLs

Organism	No. of patients whose isolate was tested for susceptibility[a]	No. of patients with ESBL-producing isolate	Minimum prevalence of ESBLs within species (%)
Escherichia coli	1,143	2[b]	0.2
Klebsiella pneumoniae	341	12	3.5
Klebsiella oxytoca	45	2	4.4
Enterobacter cloacae	156	1[b]	0.6
Enterobacter aerogenes	87	1	1.1
Enterobacter sakazakii	6	1	17
Citrobacter freundii	68	3	4.4
Serratia marcescens	40	2	5.0

[a] Number of individual patients from whom the specific organism was isolated and tested in a routine susceptibility assay. Patients from whom more than one species was isolated are counted once for each species.
[b] *E. coli* and *E. cloacae* were both isolated from one patient (patient 22).

Table 2 demonstrates which members of the family *Enterobacteriaceae* were found to have ESBLs by the DDS test. The data are expressed by patient (i.e., one species of an isolate per patient). The most common ESBL-producing isolate was *Klebsiella pneumoniae*, detected in 52% (12 of 23) of the patients. No other organism predominated, with eight different species isolated from the other 23 patients. However, when expressed as the prevalence of ESBL production within each species, several members of the family *Enterobacteriaceae* appeared to harbor ESBLs with a frequency similar to or greater than that for *K. pneumoniae*, i.e., *Klebsiella oxytoca*, *Enterobacter sakazakii*, *Citrobacter freundii*, and *Serratia marcescens.*

The first ESBL-producing organism isolated from each patient was tested for its susceptibility to ceftazidime, ceftriaxone, and aztreonam by several routine methods (Table 3). These methods detected resistance in only some of the isolates (30 to 87%). Combining the results for the three drugs increased the percentage of isolates found to be resistant or intermediate, but it did not reach 100%. Two patients (patients 5 and 16) were infected with isolates which were sensitive to ceftazidime, ceftriaxone, and aztreonam by all test methods. Therefore, a routine susceptibility panel containing these extended-spectrum β-lactams would not have detected resistance in all

TABLE 3. Numbers of patients with ESBL-producing isolates found to be resistant or intermediate by routine susceptibility testing

Antibiotic	No. (%) of patients whose isolates tested resistant or intermediate by the following usceptibility test method[a]:			
	Agar dilution	Broth microdilution	Disk diffusion	E test
Ceftazidime	17 (74)	16 (70)	14 (61)	ND
Ceftriaxone	7 (30)	19 (83)	14 (61)	ND
Aztreonam	ND	19 (83)	20 (87)	18 (78)
Ceftazidime plus ceftriaxone	19 (83)	21 (91)	17 (74)	ND
Ceftazidime, ceftriaxone, and aztreonam	ND	21 (91)	20 (87)	ND
Cefpodoxime	ND	ND	23 (100)	23 (100)

[a] The total number of patients was 23. ND, test not done.

TABLE 4. Patient and culture information

Patient no.	Organism with ESBL[a]	Culture source	Patient location[b]	Age (yr)	Sex[c]	Medical history[d]	Medical condition[e]	Outcome[f]
1	KP	Urine, catheter tip	Rehabilitation-1	54	M	Quadraplegia	Recurrent UTI	Cure
2	KP	Urine	Rehabilitation-2	41	M	Quadraplegia	Recurrent UTI	Cure
3	KP	Urine	Prison ward	30	M	Gun shot	Abdominal wound, recurrent UTI	Cure
4	ES	Blood, urine	Medicine ward-1	68	M	CLL, ethanol abuse	Sepsis, brain atrophy	Died (+)
5	KP	Peritoneal fluid	Medicine ward-2	56	M	Pancreatitis, prior pancreatectomy	Bowel necrosis after ileostomy closure	Died (+)
6	KP	Blood, urine	Rehabilitation-1	73	M	Hemiplegia	Recurrent UTI, bacteremia	Cure
7	EA	Catheter tip	Medical ER	34	M	Chronic cellulitis	Catheter infection	Cure
8	KO	Sputum	ICU-1	78	F	Liver cancer	MOSF	Died (+)
9	KP	Abscess	Medicine ward-2	66	M	Chronic pancreatitis	Abdominal abscess	Died (0)
10	KO	Urine	Medicine ward-2	54	M	Cervical fracture	UTI	Cure
11	EC	Blood	Pediatric ER	1	M	ALL, prior marrow transplant	Sepsis	Cure
12	KP	Sputum	Oncology	66	M	AML	Nosocomial pneumonia	Cure
13	KP	Urine	Medicine ward-1	43	F	Paraplegia	UTI	Cure
14	KP	Urine, catheter tip	Rehabilitation-2	47	M	Quadraplegia	UTI	Cure
15	CF	Abscess	Outpatient clinic	39	M	HIV (CDC class B3)	Buttock abscess	Cure
16	CF	Urine	Medical ER	62	M	Paraplegia	Recurrent UTI	Cure
17	SM	Blood, urine, sputum, catheter tip	ICU-2	37	M	Prior kidney/pancreas transplant	*S. aureus* endocarditis, mitral valve replacement, gangrenous gallbladder	Died (0)
18	SM	Sputum	ICU-3	78	M	COPD	Pneumonia	Cure
19	KP	Urine	Rehabilitation-1	23	M	Paraplegia	UTI	Cure
20	KP	Urine	Outpatient clinic	43	M	Paraplegia	UTI	Cure
21	CF	Urine	Medicine ward-3	71	F	COPD	UTI	Cure
22	EC, ECL	Sputum, catheter tip	ICU-1	37	F	Chronic pancreatitis	Splenectomy, ARDS, pneumonia	Died (+)
23	KP	Urine	Rehabilitation-1	31	M	Quadraplegia, HIV	Recurrent UTI	Cure

[a] CF, *Citrobacter freundii*; EC, *Escherichia coli*; ECL, *Enterobacter cloacae*; ES, *Enterobacter sakazakii*; KO, *Klebsiella oxytoca*; KP, *Klebsiella pneumoniae*; SM, *Serratia marcescens.*

[b] ER, emergency room; ICU, intensive care unit; 1, 2, and 3 indicate different wards.

[c] M, male; F, female.

[d] CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; HIV, human immunodeficiency virus infection; COPD, chronic obstructive pulmonary disease.

[e] UTI, urinary tract infection; MOSF, multiorgan system failure; ARDS, adult respiratory distress syndrome.

[f] Died (+), died with ESBL-producing isolate present; Died (0), died with prior cure of infection caused by ESBL-producing bacterium; Cure, cleared infection caused by ESBL-producing organism.

ESBL-producing isolates, no matter which method had been used. However, testing with cefpodoxime determined that 100% of these ESBL-producing isolates were resistant or intermediate by either the disk diffusion method or the E test.

Clinical outcome. Clinical information about the 23 patients infected or colonized with ESBL-producing isolates is provided in Table 4. Patient outcomes are summarized in Table 5. Twenty-two of the 23 patients had infections caused by an ESBL-producing isolate, and 1 patient was colonized.

ESBL-producing bacteria were isolated from cultures of blood from four patients. Two patients were treated with expanded-spectrum cephalosporins, and one patient (patient 11) was cured. Patient 4, who had leukemia, died of urosepsis caused by ESBL-producing *E. sakazakii*. Ceftriaxone and gentamicin therapy was instituted 2 days before the patient's death, but the organism was resistant to these drugs in vitro. Patient 11, whose blood cultures grew both a *Pseudomonas aeruginosa* isolate and an ESBL-producing *Escherichia coli* isolate, responded to therapy after ceftazidime plus gentamicin was switched to ceftazidime plus amikacin, a response consistent with in vitro susceptibility testing results. Of five patients from whom ESBL-producing bacteria were isolated from catheter tips, only one patient (patient 7) had a catheter-tip-related infection. He was not treated with an expanded-spectrum cephalosporin (Table 5).

ESBL-producing organisms were isolated from abscesses in two patients, one of whom received expanded-spectrum cephalosporin therapy. Patient 9 had an abdominal abscess which was drained and treated with ceftriaxone plus trimethoprim-sulfamethoxazole. Although the ESBL-producing *K. pneumoniae* isolate was resistant in vitro to ceftriaxone, treatment was successful, with no new abscess formation. The patient died weeks later from other causes (Table 6). One patient with massive bowel necrosis (patient 5) was infected with an ESBL-producing *K. pneumoniae* strain; in addition, two other organisms were isolated from the peritoneal fluid of patient 5. Ceftazidime and metronidazole therapy was begun, but the patient died after receiving only one dose of antibiotics.

For five patients, ESBL-producing members of the family *Enterobacteriaceae* were isolated from sputum specimens, but only three patients (patients 12, 18, and 22) had pneumonia. One of these patients (patient 18) was treated with expanded-spectrum cephalosporins plus an aminoglycoside. He improved. Eleven patients had UTIs only. Four of them were treated with expanded-spectrum cephalosporins. The isolate from one patient (patient 16) tested susceptible in vitro; isolates from the other three patients tested resistant or intermediate. All four patients were cured of their UTIs. One patient (patient 8) with advanced liver cancer was considered colonized only. A few colonies of ESBL-producing *K. pneumoniae* were isolated from two sputum specimens from patient 8. Therapy was begun with ceftriaxone and clindamycin, but pa-

TABLE 5. Outcomes of infections with ESBL-producing members of the family *Enterobacteriaceae*

Infection	Source of isolate	No. of patients	Treatment included expanded-spectrum cephalosporin		Treatment excluded expanded-spectrum cephalosporin	
			No. of patients	No. cured[d]	No. of patients	No. cured[d]
Sepsis	Blood (patients 4, 6, 11, and 17)	4	2	1[e]	2[f]	2[g]
Catheter tip infection	Catheter tip (patient 7)[a]	1	0		1[h]	1
Abscess	Abdominal (patient 9) Buttock (patient 15)	2	1[i]	1	1[i,j]	1
Bowel necrosis	Peritoneal fluid (patient 5)	1	1	0[n]	0	
Pneumonia	Sputum (patients 12, 18, and 22)[b]	3	1	1	2[k]	1[l]
Urinary tract infection	Urine (patients 1, 2, 3, 10, 13, 14, 16, 19, 20, 21, and 23)[c]	11	4	4	7[m]	7
None (probable colonization)	Sputum (patient 8)	1	1	0	0	
Total		23	10	7	13	12

[a] For four other patients catheter tip isolates probably represented colonization (patients 1 and 14 with UTIs, patient 22 with pneumonia, and patient 17 with sepsis).
[b] Two other patients did not have pneumonia but their sputa contained isolates which probably represented colonization (patient 17 with sepsis and patient 8 with no verified infection).
[c] Three other patients with septicemia also had isolates in their urine (patients 4, 6, and 17).
[d] Cured of infection caused by ESBL-producing bacteria.
[e] Patient 4 received antibiotics to which the isolates were resistant in vitro and was not cured.
[f] Therapy, ticarcillin-clavulanate plus ciprofoxacin (patient 6) and amikacin and imipenem (patient 17).
[g] Patient 17 was cleared of infection but later died from an unrelated cerebral infarct.
[h] Therapy, trimethoprim-sulfamethoxazole plus catheter removal.
[i] Abscess drained in addition to antimicrobial therapy.
[j] Therapy, cephradine plus trimethoprim-sulfamethoxazole.
[k] Therapy, ciprofloxacin (patient 12) and trimethoprim-sulfamethoxazole (patient 22).
[l] Patient 22 had severe underlying disease with adult respiratory distress syndrome and died.
[m] Therapy, ciprofloxacin (patients 1, 19, and 23), gentamicin (patients 13 and 14), cephradine (patient 2), and nitrofurantoin (patient 20).
[n] Patient died 1 after one dose of antibiotics.

tient 8 died 1 day later from multiorgan failure and liver cancer. All other cultures had been negative within 1 week of death, and the patient had no signs of pneumonia.

Table 6 summarizes the nine cases of infection and one case of colonization with ESBL-producing members of the family *Enterobacteriaceae* in which antibiotic therapy included an expanded-spectrum cephalosporin. Organisms from only 2 of the 10 patients tested susceptible to the expanded-spectrum cephalosporin used. These organisms were from patient 16, who had a UTI and who was cured, and patient 5, who died of massive bowel necrosis after receiving only one dose of antibiotic therapy. The ESBL-producing isolates from the other eight patients tested resistant or intermediate to the cephalosporin used. Patient 3 had a UTI which was cleared by ceftazidime monotherapy. For the other patients whose infections were cured, antibiotic therapy included a second drug to which the organism was susceptible or to which its susceptibility was not tested. In addition to antimicrobial therapy, abscesses were drained.

Isolates of the family *Enterobacteriaceae* from 197 patients were tested for ESBL production. Of 33 patients who died, an ESBL-producing organism was isolated from 6 (18%) of them. Of 164 patients who lived, an ESBL-producing organism was isolated from 17 (10%) of them. There was no significant difference in crude mortality rates between patients infected or colonized with isolates producing ESBLs (6 of 23; 26%) and patients not infected or colonized with ESBL-producing isolates (27 of 174; 16%) ($P = 0.14$).

DISCUSSION

We do not yet have clear guidelines on how clinical laboratories should approach the problem of ESBL-mediated resistance. Because these enzymes display substrate specificity (1, 8, 11, 13, 21, 22, 26), it has been recognized that clinical laboratories can no longer rely upon the class concept of in vitro susceptibility testing for expanded-spectrum cephalosporins and should instead test the specific expanded-spectrum cephalosporins which will be used therapeutically (8, 19, 22).

However, there is additional concern, because it is feared that in vitro susceptibility results performed by routine methods may not detect clinically relevant resistance to some extended-spectrum β-lactams. This concern originates from the fact that for ESBL-producing bacteria there is a dramatic rise in the MICs of expanded-spectrum cephalosporins as the inoculum is increased beyond that used in routine susceptibility tests. Some isolates test susceptible at the standard inoculum of 10^5 CFU/ml but resistant at an inoculum of 10^7 CFU/ml (5, 6, 9, 11, 23, 28). Rabbit endocarditis (5, 9) and rat intraperitoneal abscess (23) animal models have demonstrated an in vivo inoculum effect with ESBL-producing members of the family *Enterobacteriaceae*, in which high numbers of bacteria were more effectively sterilized after the addition of the inhibitor sulbactam (5, 9, 23). The animal model results suggest that routine susceptibility tests may not detect ESBL resistance expressed in vivo by high numbers of organisms sequestered in endocarditis vegetations or in abscesses. However, many bac-

TABLE 6. Patients who received expanded-spectrum cephalosporin therapy

Patient no.	Source	Antibiotics (no. of doses)	MIC (μg/ml), interpretation[a]	Isolate(s)[b]	Quantity	Comment	Outcome[d]
4	Blood	Ceftriaxone (5) Gentamicin (3)	64, R 16, R	***E. sakazakii***	NA[c] (>10^6 CFU/ml in urine)	Antibiotics started 2 days before death, urosepsis	Died (+)
11	Blood	Ceftazidime (41) Gentamicin (10) Amikacin (26)	≥64, R ≥32, R ≤4, S	***E. coli*** *P. aeruginosa*	NA	Patient improved after change to amikacin which was active against *E. coli*	Cured
9	Abdominal abscess	Ceftriaxone (16) Trimethoprim-sulfamethoxazole (17)	32, I ND	***K. pneumoniae*** *C. diversus*	Many Many only	After drainage, abscess resolved without relapse; patient died weeks later of *S. aureus* and *P. aeruginosa* sepsis and necrotizing pancreatitis	Died (0)
5	Peritoneal fluid	Ceftazidime (1) Metronidazole (1)	≤1, S ND	***K. pneumoniae*** *E. coli* *Enterococcus* spp.	Moderate Moderate Rare	Antibiotics started 1 day before death due to massive bowel necrosis	Died (+)
18	Sputum	Ceftriaxone (4) Ceftazidime (18) Gentamicin (5)	32, I 32, R ≤1, S	***S. marcescens*** *P. aeruginosa*	Moderate Moderate	Ceftriaxone given first followed by ceftazidime and gentamicin	Cured
3	Urine	Ceftazidime (23)	≥64, R	***K. pneumoniae***	5×10^4 CFU/ml	Patient febrile, no urinalysis	Cured
10	Urine	Ceftriaxone (17) Clindamycin (24)	16, I ND	***K. oxytoca*** *E. cloacae* *E. coli*	>10^6 CFU/ml (combined)		Cured
16	Urine	Ceftriaxone (1) Ceftazidime (5)	2, S ≤1, S	***C. freundii*** *K. pneumoniae* *M. morganii*	5×10^5 CFU/ml (combined) See above See above	Ceftriaxone given first followed by ceftazidime; discharged on ciprofloxacin	Cured
21	Urine	Ceftriaxone (2) Gentamicin (3)	≥128, R ≤1, S	***C. freundii***	>10^6 CFU/ml	Ceftriaxone and gentamicin followed by trimethoprim-sulfamethoxazole	Cured
8	Sputum (colonized)	Ceftriaxone (2) Clindamycin (3)	64, R ND	***K. oxytoca***	Few	Antibiotics started 1 day before death due to Multiorgan system failure and liver cancer	Died (+)

[a] MIC by broth microdilution method for first ESBL-producing organism isolated from each patient. ND, not determined.
[b] ESBL-producing isolates are in boldface.
[c] NA, not applicable.
[d] Died (+), died with ESBL-producing isolate present; Died (0), died but with prior cure of infection caused by ESBL-producing organism; Cured, cleared of infection caused by ESBL-producing organism.

teria which do not produce ESBLs also test resistant in vitro when an inoculum 2 orders of magnitude higher than the standard inoculum is used. In addition, it is recognized that clinical antibiotic failure may occur when high numbers of non-ESBL-producing bacteria cause infection in sequestered sites. Therefore, treatment failure in these models does not provide conclusive evidence that in vitro susceptibility results for ESBL-producing bacteria are uniquely misleading.

The literature on human infections caused by ESBL-producing members of the family *Enterobacteriaceae* also remains inconclusive about whether an organism which tests susceptible to a particular cephalosporin in vitro may in fact be clinically resistant. This question is difficult to answer because for all organisms, in vitro resistance is expected to predict clinical failure, but often does not, while in vitro susceptibility never ensures clinical success. Other factors such as the actual antibiotic concentration achieved at the infection site, the host's immune competence, and noninfectious contributors to morbidity all affect the ultimate outcome for the patient.

Several published studies include outcomes for patients infected with ESBL-producing organisms and treated with expanded-spectrum cephalosporins. In some reports (15, 19, 28), the isolates tested resistant in vitro to the antibiotics used for treatment, so failure of therapy in these patients is not surprising.

Two studies have suggested that ESBL-producing bacteria may appear falsely susceptible when tested by routine in vitro susceptibility methods. Brun-Buisson et al. (3) described an outbreak of ESBL-producing *K. pneumoniae* in a French hospital. The isolates tested susceptible to expanded-spectrum cephalosporins by disk diffusion. The modal MICs for 30 selected isolates were 2 μg/ml for cefotaxime and ceftriaxone and 4 μg/ml for ceftazidime. Three patients (two with mediastinitis and one with empyema), two of whom had bacteremia, were treated with an expanded-spectrum cephalosporin–aminoglycoside combination, without success (two failed therapy and one relapsed). Casellas and Goldberg (6) identified 46 ESBL-producing strains (44 *K. pneumoniae* and 2 *E. coli* strains) in Argentinean hospitals. The MICs by agar dilution ranged from 1 to 256 μg/ml for cefotaxime and ceftriaxone and from 0.5 to 256 μg/ml for ceftazidime (i.e., some strains tested susceptible and others tested resistant). Among 16 patients who failed

therapy with expanded-spectrum cephalosporins, a therapy switch was successful for 13 patients (to imipenem in 10 of 12 patients and to ciprofloxacin in 3 of 4 patients). However, neither report provides salient details such as the in vitro susceptibility of the organism to the specific antimicrobial agents used or the duration of therapy, so the reader cannot determine whether in vitro susceptibility results were in fact misleading with regard to the clinical outcome.

In contrast, other studies suggest that in vitro susceptibility results which reflect the drug specificities of ESBLs are in fact predictive of clinical outcome. Quinn et al. (22) described two patients infected with a *K. pneumoniae* strain resistant in vitro to ceftazidime (MIC, 64 μg/ml) and aztreonam (MIC, 32 μg/ml) but susceptible to ceftriaxone (MIC, 1 μg/ml) and cefotaxime (MIC, 0.5 μg/ml). One patient with positive blood cultures had been treated empirically with antibiotics to which the isolate was resistant, and the patient died. The other patient with positive cerebrospinal fluid cultures was successfully treated with ceftriaxone, to which the isolate was susceptible in vitro, and the patient fully recovered. Smith et al. (27) reported the case of a multiple-trauma patient who developed bacteremia and meningitis with an ESBL-producing *K. pneumoniae* strain. The isolate was resistant to ceftazidime but susceptible to cefotaxime and amikacin. The patient failed therapy with ceftazidime plus amikacin, but improved and recovered after the antibiotics were switched to cefotaxime plus amikacin. Rice and colleagues (24) described the dissemination throughout a chronic-care hospital of members of the family *Enterobacteriaceae* resistant to ceftazidime. Resistance was caused by two different ESBLs, one leading to an MIC of 32 μg/ml and the other leading to an MIC of 256 μg/ml for ceftazidime. Four infected patients, one of whom had septicemia, were treated with cefotaxime, to which the isolates were susceptible in vitro, and all had favorable clinical responses. These three studies suggest that the in vitro susceptibility results for ESBL-producing organisms, in which the organism appears resistant to one expanded-spectrum cephalosporin but susceptible to another, may in fact provide accurate results predictive of clinical outcome.

Because the clinical significance of ESBL-mediated resistance has not yet been resolved and national consensus for testing and reporting ESBL-mediated resistance does not yet exist, we undertook a 6-month retrospective study to determine whether supplemental testing for ESBL production is clinically necessary at our hospital at the present time.

Our first question concerned the prevalence of infections caused by ESBL-producing members of the family *Enterobacteriaceae* at our tertiary-care medical center. By the DDS test, ESBL production was detected in only 1.5% (50 of 3,273) of isolates of the family *Enterobacteriaceae* and in only 1.2% (23 of 1,844) of patients tested. A similarly low prevalence of 1.5% (139 of 9,382) among isolates of the family *Enterobacteriaceae* was reported by Sirot et al. (26) in a survey of French hospitals performed in 1990. Our estimate is a minimum since all ESBL producers may not have been detected. Seventy-eight isolates from 49 patients were not available for DDS testing. DDS testing is not standardized. The choice of drugs tested and the distance between disks has varied from study to study (7, 13, 30). We used 25 mm between the amoxicillin-clavulanate and the other disks, whereas Jarlier et al. (13) used 30 mm and Thomson and Saunders (30) used 30 mm, with a repeat test at 20 mm if the result with a distance of 30 mm was negative. Most important, the DDS test does not detect all ESBL-producing isolates (1, 7, 10, 30). Its sensitivity has been reported to be 79% compared to the results obtained by the three-dimensional test (30) and as 87% compared to the results obtained by the E test ESBL screen (7). For these reasons, the number of ESBL-producing organisms detected in our study must be regarded as a minimum estimate. Nevertheless, the prevalence of ESBL-producing *Enterobacteriaceae* in our medical center appears to be quite low.

Our second question concerned what percentage of isolates with ESBLs from patient specimens would test resistant or intermediate to expanded-spectrum cephalosporins by routine susceptibility methods, thereby alerting the physicians to resistance without the need for an ESBL screening test. We found that some of our ESBL-producing isolates appeared susceptible in vitro to expanded-spectrum cephalosporins (Table 3). Our data are similar to those of other studies which have reported that some ESBL-producing clinical isolates test susceptible in vitro by using NCCLS guidelines (6, 7, 10, 14, 20, 30). For example, Thomson and Saunders (30) reported that 50% of ESBL-producing isolates tested susceptible to ceftazidime and that 36% tested susceptible to ceftriaxone by disk diffusion. Philippon et al. (20) reported that 43% tested susceptible to ceftazidime by disk diffusion. Casellas and Goldberg (6) reported that for 50% of 46 ESBL-producing clinical isolates, the ceftriaxone MIC was equal to or less than 8 μg/ml by agar dilution, which would be interpreted as susceptible by NCCLS guidelines (18). In our study, cefpodoxime, tested by disk diffusion or the E test, detected 100% of the ESBL-producing isolates as either resistant or intermediate (Table 3), consistent with a report by Thomson (29). Further studies are needed to determine whether cefpodoxime may provide a screening test for ESBL production of sufficient sensitivity and specificity for use in the clinical laboratory.

Our results suggested that there were no criteria which our laboratory could effectively use to select a subset of isolates for limited supplemental ESBL testing. Only 12.5% of isolates with a suspicious antibiogram (i.e., MIC of ceftriaxone or ceftazidime, >1 μg/ml) actually had an ESBL by the DDS test (Table 1). Although the literature has emphasized *K. pneumoniae* and *E. coli* as the organisms most likely to harbor ESBLs (2, 3, 10, 13), our ESBL-producing isolates included eight different species (Table 2). This observation is consistent with those in other reports that several species of the family *Enterobacteriaceae* produce ESBLs (11, 20, 26). Patients with ESBL-producing isolates in our hospital were not limited to intensive care units, but were from many different locations, including the emergency room and outpatient clinics (Table 4).

Our study did not explore the number or type of ESBL enzymes expressed in these isolates. Isoelectric focusing of ESBLs found in our institution in 1993 and 1994 has suggested the presence of three or more different enzymes (14a). ESBLs are so numerous and diverse that observations made in any one institution with isolates harboring only a few ESBL types may not be representative of the situation at other institutions, because each institution may have its own set of indigenous ESBL enzymes.

Our third question concerned the outcomes for patients infected with ESBL-producing members of the family *Enterobacteriaceae*. In our study, 22 of 23 patients with ESBL-producing *Enterobacteriaceae* had infections, as confirmed by clinical and/or laboratory findings. Only nine patients infected with ESBL-producing strains received expanded-spectrum cephalosporin therapy. This number of patients is too low to provide conclusions about the relationship between clinical outcome and in vitro susceptibility results. However, seven of the nine patients were cured. Two patients died, but one patient received only one dose of antibiotics before death, and the other was treated with antibiotics to which his isolate tested resistant in vitro. Consequently, their deaths cannot be attributed to

antibiotic failure due to ESBL-mediated resistance not detected in routine susceptibility tests. Our results, although limited to 6 months of data, provide no evidence that additional screening for ESBLs would have favorably affected patient outcome.

Our study has demonstrated that, in our medical center, the prevalence of patients with ESBL-producing bacteria was low. These patients responded favorably to antibiotic therapy, based upon routine susceptibility testing which did not include supplemental testing for ESBL production. No clinical antibiotic failure could be attributed to bacterial resistance missed during routine testing. These observations suggest that in our tertiary-care center, at this time, it may not be clinically necessary or cost-effective to institute additional testing to detect ESBL production on a routine basis.

ACKNOWLEDGMENTS

We thank all members of the clinical microbiology laboratory, Charles Moncure, Michael Edmond, Sara Cottrell, Kathleen Kiley, Betty Herndon, Mauritta Baker-Hill, Ellen Basinger, Barbara Attema, Karen Schnell, Donna Mowan, and foremost, Lin Kerrick. Without the assistance of these kind individuals, this study would not have been possible.

REFERENCES

1. **Bauernfeind, A., Y. Chong, and S. Schweighart.** 1989. Extended broad spectrum β-lactamase in *Klebsiella pneumoniae* including resistance to cephamycins. Infection **17:**316–321.
2. **Bingen, E. H., P. Desjardins, G. Arlet, F. Bourgeois, P. Mariani-Kurkdjian, N. Y. Lambert-Zechovsky, E. Denamur, A. Philippon, and J. Elion.** 1993. Molecular epidemiology of plasmid spread among extended broad-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates in a pediatric hospital. J. Clin. Microbiol. **31:**179–184.
3. **Brun-Buisson, C., P. Legrand, A. Philippon, F. Montravers, M. Ansquer, and J. Duval.** 1987. Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistant *Klebsiella pneumoniae*. Lancet **ii:**302–306.
4. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
5. **Caron, F., L. Gutmann, A. Bure, B. Pangon, J.-M. Vallois, A. Pechinot, and C. Carbon.** 1990. Ceftriaxone-sulbactam combination in rabbit endocarditis caused by a strain of *Klebsiella pneumoniae* producing extended broad-spectrum TEM-3 β-lactamase. Antimicrob. Agents Chemother. **34:**2070–2074.
6. **Casellas, J. M., and M. Goldberg.** 1989. Incidence of strains producing extended spectrum β-lactamases in Argentina. Infection **17:**434–436.
7. **Cormican, M. G., S. A. Marshall, and R. N. Jones.** 1996. Detection of extended-spectrum β-lactamase (ESBL)-producing strains by the E test ESBL screen. J. Clin. Microbiol. **34:**1880–1884.
8. **Dudley, M.** 1995. Bacterial resistance mechanisms to β-lactam antibiotics: assessment of management strategies. Pharmacotherapy **15**(1 Pt. 2)**:**9S–14S.
9. **Fantin, B., B. Pangon, G. Potel, F. Caron, E. Vallee, J.-M. Vallois, J. Mohler, A. Bure, A. Philippon, and C. Carbon.** 1990. Activity of sulbactam in combination with ceftriaxone in vitro and in experimental endocarditis caused by *Escherichia coli* producing SHV-2-like β-lactamase. Antimicrob. Agents Chemother. **34:**581–586.
10. **Jacoby, G. A., and P. Han.** 1996. Detection of extended-spectrum β-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. J. Clin. Microbiol. **34:**908–911.
11. **Jacoby, G. A., and A. A. Medeiros.** 1991. More extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **35:**1697–1704.
12. **Jacoby, G. A., and G. L. Archer.** 1991. New mechanisms of bacterial resistance to antimicrobial agents. N. Engl. J. Med. **324:**601–612.
13. **Jarlier, V., M.-H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
14. **Katsanis, G. P., J. Spargo, M. J. Ferraro, L. Sutton, and G. A. Jacoby.** 1994. Detection of *Klebsiella pneumoniae* and *Escherichia coli* strains producing extended-spectrum β-lactamases. J. Clin. Microbiol. **32:**691–696.

14a. **Lofland, D., and L. A. Weymouth.** Unpublished data.

15. **Meyer, K. S., C. Urban, J. A. Eagan, B. J. Berger, and J. J. Rahal.** 1993. Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. Ann. Intern. Med. **119:**353–358.
16. **National Committee for Clinical Laboratory Standards.** 1993. Performance standards for antimicrobial disk susceptibility tests, 5th ed. Approved standard M2-A5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
17. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd ed. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
18. **National Committee for Clinical Laboratory Standards.** 1995. Performance standards for antimicrobial susceptibility testing, 6th informational supplement. M100-S5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
19. **Naumovski, L., J. P. Quinn, D. Miyashiro, M. Patel, K. Bush, S. B. Singer, D. Graves, T. Palzkill, and A. M. Arvin.** 1992. Outbreak of ceftazidime resistance due to a novel extended-spectrum β-lactamase in isolates from cancer patients. Antimicrob. Agents Chemother. **36:**1991–1996.
20. **Philippon, A., S. Ben Redjeb, G. Fournier, and A. Ben Hassen.** 1989. Epidemiology of extended-spectrum β-lactamases. Infection **17:**347–354.
21. **Philippon, A., R. Labia, and G. Jacoby.** 1989. Extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **33:**1131–1136.
22. **Quinn, J. P., D. Miyashiro, D. Sahm, R. Flamm, and K. Bush.** 1989. Novel plasmid-mediated β-lactamase (TEM-10) conferring selective resistance to ceftazidime and aztreonam in clinical isolates of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **33:**1451–1456.
23. **Rice, L. B., J. D. C. Yao, K. Klimm, G. M. Eliopoulos, and R. C. Moellering, Jr.** 1991. Efficacy of different β-lactams against an extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* strain in the rat intra-abdominal abscess model. Antimicrob. Agents Chemother. **35:**1243–1244.
24. **Rice, L. B., S. H. Willey, G. A. Papanicolaou, A. A. Medeiros, G. M. Eliopoulos, R. C. Moellering, Jr., and G. A. Jacoby.** 1990. Outbreak of ceftazidime resistance caused by extended-spectrum β-lactamases at a Massachusetts chronic-care facility. Antimicrob. Agents Chemother. **34:**2193–2199.
25. **Sanders, C. C., and W. E. Sanders, Jr.** 1992. β-Lactam resistance in gram-negative bacteria: global trends and clinical impact. Clin. Infect. Dis. **15:**824–839.
26. **Sirot, D. L., F. W. Goldstein, C. J. Soussy, A. L. Courtieu, M. O. Husson, J. Lemozy, M. Meyran, C. Morel, R. Perez, C. Quentin-Noury, M. E. Reverdy, J. M. Scheftel, M. Rosembaum, and Y. Rezvani.** 1992. Resistance to cefotaxime and seven other β-lactams in members of the family *Enterobacteriaceae*: a 3-year study in France. Antimicrob. Agents Chemother. **36:**1677–1681.
27. **Smith, C. E., B. S. Tillman, A. W. Howell, R. N. Longfield, and J. H. Jorgensen.** 1990. Failure of ceftazidime-amikacin therapy for bacteremia and meningitis due to *Klebsiella pneumoniae* producing an extended-spectrum β-lactamase. Antimicrob. Agents Chemother. **34:**1290–1293.
28. **Spencer, R. C., P. F. Wheat, T. G. Winstanley, D. M. Cox, and S. J. Plested.** 1987. Novel β-lactamase in a clinical isolate of *Klebsiella pneumoniae* conferring unusual resistance to β-lactam antibiotics. J. Antimicrob. Chemother. **20:**919–927.
29. **Thomson, K. S.** 1995. Improved detection of extended-spectrum β-lactamases (ESBLs) from disk diffusion susceptibility tests, abstr. C-87, p. 16. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
30. **Thomson, K. S., and C. C. Sanders.** 1992. Detection of extended-spectrum β-lactamases in members of the family *Enterobacteriaceae*: comparison of the double-disk and three-dimensional tests. Antimicrob. Agents Chemother. **36:**1877–1882.
31. **Thornsberry, C.** 1995. Trends in antimicrobial resistance among today's bacterial pathogens. Pharmacotherapy **15**(1 Pt. 2)**:**3S–8S.