SUPPLEMENT ARTICLE

Variations in the Prevalence of Strains Expressing an Extended-Spectrum β-Lactamase Phenotype and Characterization of Isolates from Europe, the Americas, and the Western Pacific Region

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To evaluate the prevalence of extended-spectrum β-lactamase (ESBL)–producing strains among species of Enterobacteriaceae, a microdilution susceptibility test was performed with strains of *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis,* and *Salmonella* species that were isolated as part of the SENTRY project. The highest percentage of ESBL phenotype (defined as a minimum inhibitory concentration [MIC] ⩾2 μg/mL for ceftazidime, ceftriaxone, or aztreonam) was detected among *K. pneumoniae* strains from Latin America (45%), followed by those from the Western Pacific region (25%), Europe (23%), the United States (8%), and Canada (5%). *P. mirabilis* and *E. coli* strains for which MICs of extended-spectrum cephalosporins or monobactams were elevated also were more prominent in Latin America. Testing with ceftazidime revealed more isolates with elevated MICs than did testing with ceftriaxone or aztreonam. ESBL strains showed high levels of co-resistance to aminoglycosides, tetracycline, trimethoprim-sulfamethoxazole, and ciprofloxacin. Imipenem remains highly effective against ESBL strains. Organisms expressing an ESBL are widely distributed worldwide, although prevalence rates are significantly higher in certain geographic regions.

Extended-spectrum β-lactamases (ESBLs) were first identified in the early 1980s. Since this time, ESBLs have been identified worldwide and have been found in a number of different organisms, including *Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis,* and *Salmonella* species [1–3]. ESBLs hydrolyze the oxyimino cephalosporins and monobactams and are commonly inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam [1–5]. Many of these enzymes have evolved from the TEM-1, TEM-2, and SHV-1 β-lactamases that are widely distributed among the Enterobacteriaceae [1–5]. The TEM- and SHV-derived ESBL genes typically encode point mutations that result in 1–4 amino acid substitutions. These substitutions induce changes in the active site of the enzyme that reduce the activity of the extended-spectrum cephalosporins and monobactams. More recently, a number of ESBL enzymes that are unrelated to the TEM and SHV enzymes have been described [6].

Current guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) recommend screening all *K. pneumoniae, Klebsiella oxytoca,* and *E. coli* isolates for which MICs of cefpodoxime, ceftazidime, aztreonam, cefotaxime, or ceftriaxone are ⩾2 μg/mL for evidence of an ESBL [7]. Several tests have been developed to confirm the presence of ESBLs, including the double-disk diffusion assay, the ESBL Etest strip (AB Biodisk), and several automated susceptibility systems [3, 8]. In each system, enhanced oxyimino cephalosporin or monobactam inhibition by clavulanic acid suggests the presence of an ESBL [8–12]. ESBL detec-

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tion can be difficult, however, and each system has failed to identify certain ESBLs [12–15].

ESBLs are typically encoded on large, 80- to 300-kb plasmids that can be exchanged between bacterial species [16]. In many cases, these plasmids also encode other antimicrobial resistance genes. Therefore, it is common for organisms expressing an ESBL to express co-resistances to aminoglycosides, trimethoprim-sulfamethoxazole, and tetracyclines [16].

The purpose of this report is to discuss trends in resistance to cephalosporins over time in species known to carry ESBLs, with an emphasis on occurrence rates by species, site of infection, and resistance patterns in various geographic locations, in addition to co-resistances common to different geographic locations and the molecular epidemiology and diagnostic techniques in particular areas in which ESBLs are highly prevalent.

MATERIALS AND METHODS

Study design. The SENTRY Antimicrobial Surveillance Program was established to monitor the prominent pathogens and antimicrobial resistance patterns of nosocomial and community-acquired infections via a broad network of sentinel hospitals distributed by geographic location and size. The monitored infections include bacteremia and fungemia (objective A), outpatient respiratory infections (objective B), pneumonia in hospitalized patients (objective C), wound infections (objective D), and urinary tract infections in hospitalized patients (objective E). Participating institutions in 1997 included 30 medical centers in the United States, 8 in Canada, 10 in Latin America, and 24 in Europe. In 1998, 16 medical centers in the Western Pacific region and Asia were added.

Each participating center contributed results (organism identification, date of isolation, and antimicrobial susceptibility profile) for the first 20 consecutive episodes of bacteremia per month, 100 consecutive episodes of pneumonia per year, 50 consecutive isolates from wound infections per year, and 50 consecutive isolates from urinary tract infections per year. All isolates were saved on agar slants and sent to the University of Iowa College of Medicine (Iowa City, IA) for storage and further characterization by reference identification and susceptibility testing methods. All isolates recovered in 1997 and 1998 were analyzed for this study, as were a subset of isolates recovered in 1999.

Identification of organisms. All isolates were identified at the participating institution by the routine methodology in use at each laboratory. On receipt at the University of Iowa, isolates were subcultured on blood agar to ensure viability and purity. Species identifications were confirmed with use of the Vitek system (bioMérieux Vitek) or API (bioMérieux Vitek) products and standard reference methods [17]. Isolates were frozen at −70°C until they were processed.

Susceptibility testing. Antimicrobial susceptibility testing of isolates was performed by reference broth microdilution methods as described by the NCCLS [7]. Microdilution trays were purchased from MicroScan, TREK, and PML Microbiologicals. Antimicrobial agents were obtained from their respective manufacturers. Quality control was performed by testing *E. coli* American Type Culture Collection (ATCC) 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus pneumoniae* ATCC 49619, and *Enterococcus faecalis* ATCC 29212. Interpretive criteria for each antimicrobial tested were those published by the NCCLS or cited in text and tables.

K. pneumoniae, E. coli, P. mirabilis, and *Salmonella* species isolates expressing an ESBL phenotype, as defined by a ceftazidime, ceftriaxone, or aztreonam MIC ≥ 2 μg/mL, were characterized with ESBL Etest strips containing antimicrobial gradients ranging from 0.016 to 256 μg/mL, paired with full strips containing the same cephalosporin gradient in the presence of 2 μg/mL of clavulanic acid (generously provided by AB Biodisk) or with the commercial ESBL Etest strips that contain a stable gradient of ceftazidime (1–32 μg/mL) on one half and ceftazidime plus clavulanic acid (2 μg/mL) on the other half. The Etest inoculum was adjusted to the turbidity of a 0.5 MacFarland standard from a 24-h subculture and swabbed onto the surface of a 150 mm^2 plate of Mueller-Hinton agar. An 8-fold or greater reduction in MIC with clavulanic acid in comparison with the MIC with the substrate oxyimino cephalosporin alone was considered evidence of a positive ESBL test [8].

Pulsed-field gel electrophoresis. Genomic DNA was isolated and digested with Spe1 (New England Biolabs) as described elsewhere [18]. Pulsed-field gel electrophoresis (PFGE) was performed on the CHEF-DRII (Bio-Rad Laboratories under the following conditions: 0.5× Tris-Borate-EDTA buffer, 1% agarose, at 13°C and 200 V for 23 h, with the switch interval ramped for *K. pneumoniae.* Gels were stained with ethidium bromide and photographed with a Bio-Rad Gel Doc 1000 system (Bio-Rad Laboratories). Strains that contained restriction-fragment patterns that differed by more than 3 bands were considered unique.

Ribotyping. Automated ribotyping was performed on the RiboPrinter Microbial Characterization System (Qualicon) according to the manufacturer's protocol and as described by Pfaller et al. [19]. In brief, colonies were resuspended in lysis buffer and placed into the RiboPrinter system. Bacterial DNA was digested with Eco RI, and the restriction fragments were separated by electrophoresis and then transferred to a nylon membrane. Ribosomal DNA was hybridized to a chemiluminescent-labeled *E. coli* rRNA operon (rrnB) probe. The chemiluminescent patterns were electronically imaged and analyzed with the RiboPrinter Microbial Characterization System computer. Assignment to a particular ribogroup is based on dif-

Table 1. Frequency of occurrence of selected enteric bacillus organisms in the SENTRY Antimicrobial Surveillance Program, 1997–1999.

Country or region, organism	Isolates of selected bacterial species, by SENTRY objective category,[a] %				
	A	C	D	E	Total
Canada	(*n* = 3840)	(*n* = 1659)	(*n* = 635)	(*n* = 651)	(*n* = 6785)
Klebsiella pneumoniae	5.5	4.7	2.2	8.9	5.3
Escherichia coli	18.4	6.3	8.3	45.9	17.1
Proteus mirabilis	1.2	0.8	2.5	2.8	1.4
Salmonella species	0.3	0.0	0.0	0.2	0.2
Europe	(*n* = 10,815)	(*n* = 2572)	(*n* = 2369)	(*n* = 2289)	(*n* = 18,045)
K. pneumoniae	5.3	4.9	2.9	7.2	5.1
E. coli	20.9	6.9	9.1	49.1	20.9
P. mirabilis	1.8	1.7	3.3	5.4	2.4
Salmonella species	1.5	0.0	<0.1	0.0	0.9
Latin America	(*n* = 5295)	(*n* = 1914)	(*n* = 1353)	(*n* = 1430)	(*n* = 9992)
K. pneumoniae	8.9	9.4	5.8	10.3	8.8
E. coli	17.7	4.4	3.4	56.0	20.1
P. mirabilis	1.1	1.1	3.0	5.1	1.9
Salmonella species	2.0	0.1	0.3	0.3	1.2
United States	(*n* = 17,399)	(*n* = 6711)	(*n* = 2328)	(*n* = 2996)	(*n* = 29,434)
K. pneumoniae	6.7	8.3	2.7	9.8	6.6
E. coli	17.6	4.0	6.8	44.6	16.4
P. mirabilis	1.5	1.3	3.1	4.9	1.9
Salmonella species	0.4	0.0	0.1	<0.1	0.3
Western Pacific	(*n* = 3162)	(*n* = 1704)	(*n* = 791)	(*n* = 959)	(*n* = 6616)
K. pneumoniae	7.4	11.0	4.7	10.2	8.5
E. coli	20.0	4.2	4.8	37.9	16.7
P. mirabilis	1.5	0.3	2.5	4.0	1.7
Salmonella species	2.7	0.0	0.3	0.0	1.3

[a] Objective A, bloodstream infections; C, suspected pneumonia in hospitalized patients; D, skin and soft-tissue infections; E, urinary tract infections.

ferences in both band position and the signal intensity of each band. Isolates were considered to be highly related if the coefficient of similarity was >0.93.

Isoelectric focus analysis of β-lactamases. Crude β-lactamase extracts were prepared by freeze-thaw lysis of bacterial cultures grown exponentially in tryptic soy broth, as described elsewhere [20]. Analytical isoelectric focusing was performed with a Multiphore II electrophoresis system (Amersham Pharmacia Biotech) and prepared ampholine-polyacrylamide plates (isoelectric point [pI], 3.5–9.5 and 5.5–8.5; Amersham Pharmacia Biotech) with limits of 1500 V, 30 mA, and 30 W for 1.5 h and 1600 V, 50 mA, and 25 W for 2.5 h, respectively. β-Lactamase activity was detected with 500 mg/mL nitrocephin (Becton Dickinson Microbiology Systems). Gels were photographed with a Bio-Rad Gel Doc 1000 system fitted with a Tiffen 58 dark green filter. TEM-1, TEM-4, SHV-1, SHV-3, and SHV-5 β-lactamases expressed in *E. coli* C600 control strains (generously provided by G. Jacoby) were used as pI standards [21]. Known pI values of each standard were plotted against the distance from the cathode, and a regression analysis was performed (Microsoft Excel 98; Microsoft).

RESULTS

During the study period (1997–1999), >70,000 bacterial isolates were tested, as shown in table 1. Of these, the bacteria selected for this analysis represent 27.4% of the total collected (12,876 *E. coli,* 4668 *K. pneumoniae,* 1406 *P. mirabilis,* and 455 *Salmonella* species isolates). In general, the relative distribution of the isolates was similar in all areas, although some trends were observed; *K. pneumoniae* strains were more prevalent in Latin America and the Western Pacific region, *E. coli* in Europe and Latin America, and *Salmonella* species in Latin America and the Western Pacific region. *Proteus* isolates were fairly evenly distributed.

Table 2 shows the percentages of bacterial isolates with an

Table 2. Percentage of organisms expressing an extended-spectrum β-lactamase (ESBL) phenotype in the SENTRY Antimicrobial Surveillance Program, 1997–1999.

Organism, country or region (no. of isolates tested)	No. (%) of isolates with ESBL phenotype			
	All substrates[a]	Ceftazidime[b]	Ceftriaxone[b]	Aztreonam[b]
Klebsiella pneumoniae				
Canada (n = 368)	18 (4.9)	15 (83.3)	9 (50.0)	14 (77.8)
Europe (n = 946)	214 (22.6)	204 (95.3)	192 (89.7)	199 (93.0)
Latin America (n = 897)	407 (45.4)	397 (97.5)	385 (94.6)	371 (91.2)
United States (n = 2017)	153 (7.6)	133 (86.9)	111 (72.5)	113 (73.9)
Western Pacific (n = 560)	138 (24.6)	134 (97.1)	128 (92.8)	129 (93.5)
Escherichia coli				
Canada (n = 1203)	51 (4.2)	40 (78.4)	20 (39.2)	33 (64.7)
Europe (n = 3822)	202 (5.3)	160 (79.2)	125 (61.9)	154 (76.2)
Latin America (n = 2026)	173 (8.5)	164 (94.8)	146 (84.4)	157 (90.8)
United States (n = 4966)	166 (3.3)	117 (70.5)	83 (50.0)	128 (77.1)
Western Pacific (n = 1104)	87 (7.9)	79 (90.8)	62 (71.3)	80 (92.0)
Proteus mirabilis				
Canada (n = 97)	3 (3.1)	2 (66.7)	2 (66.7)	1 (33.3)
Europe (n = 442)	49 (11.1)	42 (85.7)	34 (69.4)	23 (46.9)
Latin America (n = 196)	44 (22.4)	26 (59.1)	39 (88.6)	28 (63.6)
United States (n = 589)	29 (4.9)	21 (72.4)	12 (41.4)	16 (55.2)
Western Pacific (n = 111)	2 (1.8)	1 (50.0)	1 (50.0)	1 (50.0)
Salmonella species				
Canada (n = 11)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Europe (n = 128)	1 (0.8)	1 (100.0)	0 (0.0)	0 (0.0)
Latin America (n = 125)	3 (2.4)	3 (100.0)	2 (66.7)	2 (66.7)
United States (n = 79)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Western Pacific (n = 88)	3 (3.4)	3 (100.0)	3 (100.0)	3 (100.0)

[a] MIC of ceftazidime, ceftriaxone, or aztreonam, ⩾2 μg/mL.
[b] MIC, ⩾2 μg/mL. Percentages were calculated using the total no. of isolates with ESBL phenotype detected by any of the three substrates.

ESBL phenotype (i.e., ceftazidime, ceftriaxone, and/or aztreonam MIC, ⩾2 μg/mL). As shown, *K. pneumoniae* isolates with an ESBL phenotype were more prevalent in Latin America (45.4%), followed by the Western Pacific region (24.6%), Europe (22.6%), the United States (7.6%), and Canada (4.9%). The rank order for *E. coli* isolates with an ESBL phenotype was similar, although the MIC was ⩾2 μg/mL for many fewer isolates. *Salmonella* isolates expressing an ESBL phenotype were much less common (Western Pacific region, 3.4%; Latin America, 2.4%; and Europe, 0.8%). Regional differences could also be seen within a particular country. In the United States, an elevated ceftriaxone or ceftazidime MIC was more common in the northeast and south-central United States than in the western states (table 3).

Different ESBLs hydrolyze each oxyimino cephalosporin to varying degrees. Data were analyzed for ceftazidime, ceftriaxone, and aztreonam to determine whether there were regional or organism-specific differences in which substrate better identified an ESBL phenotype. In all countries, ceftazidime detected the greatest number of *K. pneumoniae* isolates with an ESBL phenotype. For *E. coli* isolates, the ESBL phenotype was slightly more likely to be detected with aztreonam in the Western Pacific region and the United States. *Proteus* isolates were better detected with ceftriaxone in Latin America, whereas ceftazidime was a better substrate in the United States and Europe. The differences in ESBL detection in *P. mirabilis* from Latin America likely reflect the fact that a specific epidemic strain of *P. mirabilis* in which ESBL was more easily detected with ceftriaxone was recovered in a single Brazilian hospital [22].

A subset of isolates expressing an ESBL phenotype were evaluated for evidence of an ESBL with use of ESBL Etest technology (table 4). As shown, 42.9% (North America), 83.8% (Latin America), and 82.4% (Western Pacific region) of the tested isolates expressed an ESBL, as evidenced by an 8-fold decrease in the ceftazidime MIC in the presence of a fixed concentration of clavulanic acid (2 μg/mL). *E. coli* strains were also evaluated: 27.8% of North American isolates, 61.9% of Latin American isolates, and 75% of Western Pacific isolates for which the MIC

Table 3. Geographic distribution of *Klebsiella pneumoniae* strains isolated in North American hospitals included in the SENTRY Antimicrobial Surveillance Program, 1997–1998.

Country, region (no. of strains)	Cephalosporin tested	Susceptible[a] isolates, %	No. (%) of isolates with ESBL phenotype[b]
Canada (n = 268)	Ceftriaxone	98.5	7 (2.6)
	Ceftazidime	98.5	12 (4.5)
United States			
West (n = 296)	Ceftriaxone	100.0	5 (1.7)
	Ceftazidime	99.3	9 (3.0)
North-central (n = 404)	Ceftriaxone	96.8	22 (5.4)
	Ceftazidime	95.0	26 (6.4)
South-central (n = 248)	Ceftriaxone	96.0	16 (6.5)
	Ceftazidime	94.8	22 (8.9)
Northeast (n = 270)	Ceftriaxone	93.7	26 (9.6)
	Ceftazidime	91.5	29 (10.7)
Southeast (n = 202)	Ceftriaxone	98.5	9 (4.5)
	Ceftazidime	98.5	13 (6.4)

NOTE. ESBL, extended-spectrum β-lactamase.

[a] According to the National Committee for Clinical Laboratory Standards interpretative criteria [7].

[b] MIC, $\geq$2 μg/mL.

was $\geq$2 μg/mL showed an 8-fold decrease in ceftazidime MIC with clavulanic acid. These results suggest that the predictive value of an ESBL phenotype is highest in a high-prevalence region. In addition, there was no correlation between isolates with a higher MIC of the oxyimino cephalosporins or monobactams. Elevated cefoxitin MICs ($\geq$32 μg/mL) were noted in 37%–54% of *E. coli* strains with elevated cephalosporin or monobactam MICs, a finding suggesting that at least some of the isolates for which the ESBL screening test was negative could express an ampC-like β-lactamase (data not shown).

Table 5 shows the results of screening for multiresistance in isolates from 1998 that expressed an ESBL phenotype. Co-resistances to tobramycin, gentamicin, tetracyclines, and trimethoprim-sulfamethoxazole were common throughout each geographic region. Nearly 85% of *K. pneumoniae* strains from Latin America and 81% of strains from Europe showed resistance to tobramycin. In general, rates of resistance to amikacin were lower than those to gentamicin and tobramycin, particularly in the United States and Canada. However, amikacin resistances were still quite prevalent in the other geographic regions. Rates of co-resistance to ciprofloxacin ranged from 14% to 80%, with particularly high levels of quinolone resistance noted among the *P. mirabilis* isolates.

Molecular epidemiological analyses and β-lactamase characterizations were focused on regions in which the prevalence of ESBLs was high. Table 6 shows molecular epidemiological data from a representative study concerning *K. pneumoniae* and *E. coli* bloodstream isolates from Latin America. Evidence of intrahospital spread of organisms with identical ribotype and PFGE patterns was found in many circumstances. However, clonal spread did not explain the overall number of isolates expressing an ESBL in many hospitals, and there was little evidence of clonal spread from hospital to hospital or country to country. Isoelectric focus analysis of β-lactamases did suggest some commonality among β-lactamases found in isolates from different hospitals in the same geographic region. For example, a 6.8-pI β-lactamase appeared more common in isolates from Latin American hospitals than in those from US hospitals. These findings could be explained by the spread of a common

Table 4. Results of testing *Klebsiella pneumoniae* with extended-spectrum β-lactamase phenotypes for inhibition using a marketed β-lactamase inhibitor (clavulanic acid).

Region (no. of phenotypes)[a]	No. tested[b]	No. (%) positive
North America (n = 63)	28	12 (42.9)
Latin America (n = 134)	80	67 (83.8)
Western Pacific (n = 85)	85	70 (82.4)

[a] Phenotypes selected from isolates with reproducible ceftazidime, ceftriaxone, or aztreonam MICs $\geq$2 μg/mL [7].

[b] Test performed with the Etest using up to 5 different β-lactam substrates (cefotaxime, ceftriaxone, ceftazidime, cefuroxime, aztreonam), with and without clavulanic acid (2 μg/mL) [8].

Table 5. Co-resistances identified in 1998 isolates that expressed an extended-spectrum β-lactamase (ESBL) clinical phenotype.

Organism, country or region (no. of strains with ESBL phenotype)	Resistant isolates, %					
	Tobramycin	Gentamicin	Amikacin	Tetracycline	TMP-SMZ[a]	Ciprofloxacin
Klebsiella pneumoniae						
Canada (n = 18)	16.7	16.7	5.6	61.1	5.6	22.2
Europe (n = 214)	80.8	65.0	54.2	49.5	6.4	24.3
Latin America (n = 407)	83.5	66.3	66.1	52.0	12.1	23.1
United States (n = 153)	54.2	49.0	11.1	44.4	17.0	34.6
Western Pacific (n = 138)	72.5	58.7	37.7	55.1	25.4	44.2
Escherichia coli						
Canada (n = 51)	13.7	15.7	5.9	45.1	5.9	13.7
Europe (n = 202)	32.2	25.7	10.9	61.4	8.9	34.2
Latin America (n = 173)	68.8	57.8	48.0	70.5	19.7	52.6
United States (n = 166)	21.7	21.1	7.8	48.8	6.0	19.3
Western Pacific (n = 87)	69.0	75.9	11.5	77.0	26.4	65.5
Proteus mirabilis						
Canada (n = 3)	33.3	77.7	33.3	100.0	0.0	77.7
Europe (n = 49)	59.2	61.2	24.5	93.9	8.2	61.2
Latin America (n = 44)	84.1	90.9	47.7	97.7	18.2	79.5
United States (n = 29)	24.1	34.5	3.4	100.0	6.9	34.5
Western Pacific (n = 2)	50.0	100.0	0.0	100.0	0.0	50.0
Salmonella species						
Canada (n = 0)	—	—	—	—	—	—
Europe (n = 1)	0.0	0.0	0.0	0.0	0.0	0.0
Latin America (n = 3)	66.7	66.7	33.3	33.3	66.7	0.0
United States (n = 0)	—	—	—	—	—	—
Western Pacific (n = 3)	33.3	33.3	0.0	100.0	66.7	66.7

NOTE. TMP-SMZ, trimethoprim-sulfamethoxazole.

[a] Ratio, 1:19.

plasmid from organism to organism rather than clonal spread of a particular strain.

Imipenem remains almost uniformly active against *K. pneumoniae, E. coli, P. mirabilis,* and *Salmonella* isolates that express an ESBL. Only 0.5%–0.7% of *K. pneumoniae* and *E. coli* isolates from the United States, Latin America, the Western Pacific region, and Canada were resistant to imipenem (table 7). Although *P. mirabilis* isolates expressing an ESBL phenotype were less common, up to 4% of these isolates were resistant to imipenem.

DISCUSSION

This study was designed to analyze the importance of ESBLs in the resistance against third-generation cephalosporins and other β-lactam antibiotics in different species of Enterobacteriaceae throughout the world. As the results show, the ESBL phenotype appears to be more frequent in Latin America and the Western Pacific region, whereas the United States and Canada have significantly fewer isolates with an ESBL phenotype. The selective pressure created by the use of third-generation cephalosporins has been described as one of the most important factors in the appearance of these strains [2, 3], so it is possible to speculate that such antimicrobials are used more frequently in these areas than in the other regions. In addition, molecular epidemiological studies have shown that isolates expressing an ESBL often disseminate within particular hospitals. Our data support the theory that intrahospital clonal spread is an important factor in the prevalence of ESBLs.

In general, ceftazidime appeared to be a better reagent for detecting an ESBL than ceftriaxone or aztreonam. On first analysis, however, ceftriaxone appeared to identify ESBL-expressing *P. mirabilis* in Latin America better than the other 2 antimicrobial agents. During this study period, an epidemic strain of *P. mirabilis* was identified in a single hospital in Brazil. The β-lactamases present in this clonal isolate hydrolyzed ceftriaxone better than the other antimicrobial agents [22]. Although ceftriaxone does not represent the best substrate for detection

Table 6. Data concerning *Klebsiella pneumoniae* (26 strains) and *Escherichia coli* (9 strains) isolated in cultures of blood samples from hospitalized patients in Latin American medical centers.

Organism, isolate no.	Medical center no. (country)	ESBL Etest[a]	Molecular typing results		pI results
			Ribotype	PFGE	
K. pneumoniae					
1161	39 (Argentina)	Pos	622-1		5.4, 6.8, 7.4, 8.0
3463	39 (Argentina)	Neg	614-1	G	5.4, 6.8, 7.6
3468	39 (Argentina)	Neg	614-1	G1	5.4, 6.8, 7.6
3477	39 (Argentina)	Pos	614-1	G	5.4, 6.8, 7.6
4863	39 (Argentina)	Pos	621-1	C	5.4, 6.5, 6.8, 7.6, 8.0
6156	39 (Argentina)	Pos	621-1	C	5.4, 6.5, 6.8, 7.6, 8.0
5418	40 (Argentina)	Pos	614-1	F	5.4, 7.6
6216	41 (Brazil)	Pos	204-1	D	5.4, 7.6, 8.2
6217	41 (Brazil)	Pos	204-1	D	5.4, 7.6, 8.2
6236	41 (Brazil)	Pos	204-1	D	5.4, 7.6
6241	41 (Brazil)	Pos	646-7		5.4, 7.0
3443	42 (Chile)	Pos	614-4	H	5.4, 7.6
3446	42 (Chile)	Pos	614-4	H	5.4, 7.6
3451	42 (Chile)	Pos	204-1	I	5.4, 6.8, 7.6, 8.2
3453	42 (Chile)	Pos	756-6		5.4, 7.6
6038	43 (Chile)	Pos	204-1	E	5.4, 5.6, 6.8, 7.6, 8.2
6176	44 (Colombia)	Pos	746-5		5.4, 7.6, 8.2
6189	44 (Colombia)	Pos	746-6		5.4, 7.6, 8.2
2225	44 (Colombia)	Pos	324-2		7.0, 8.2
3483	45 (Mexico)	Pos	756-7		5.4, 7.0
6090	46 (Brazil)	Pos	638-4		5.4, 8.0
609	48 (Brazil)	Pos	447-4		5.4, 7.6
1143	48 (Brazil)	Pos	497-1		5.4, 6.8, 7.6, 8.2
1597	48 (Brazil)	Pos	324-4	A	5.4, 8.2
2120	48 (Brazil)	Pos	324-4	B	5.4, 7.0
6115	48 (Brazil)	Pos	638.6		7.0, 8.2
E. coli					
3523	40 (Argentina)	Pos	252-1		5.4, 7.0, 7.6
6237	41 (Brazil)	Neg	257-2		5.4
1102	42 (Chile)	Pos	241-4		5.4, 7.0
1105	42 (Chile)	Neg	242-5		5.4
1308	44 (Colombia)	Neg	225-4		5.4, 8.0
1114	47 (Uruguay)	Pos	755-3		5.4, 8.0
572	48 (Brazil)	Pos	182-2	W	5.4, 7.0, 7.6
595	48 (Brazil)	Pos	182-2	W	5.4, 7.0, 8.2
2131	48 (Brazil)	Pos	595-4		5.4, 8.0

NOTE. Each strain was classified as having an ESBL according to National Committee for Clinical Laboratory Standards criteria (ceftriaxone and/or ceftazidime and/or aztreonam MIC ≥ 2 μg/mL). ESBL, extended-spectrum β-lactamase; Neg, negative; PFGE, pulsed-field gel electrophoresis; pI, isoelectric point; Pos, positive. Modified from [24].

[a] Criteria: >4-fold reduction in MIC with clavulanic acid/ceftazidime vs. ceftazidime alone was defined as a positive result.

Table 7. In vitro broth microdilution susceptibility results for *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis* with extended-spectrum β-lactamase phenotypes.

Organism, antimicrobial agent	Canada		Europe		Latin America		United States		Western Pacific	
	MIC_{50}/MIC_{90}	Susc, %	MIC_{50}/MIC_{90}	Susc, %	MIC_{50}/MIC_{90}	Susc, %	MIC_{50}/MIC_{90}	Susc, %	MIC_{50}/MIC_{90}	Susc, %
K. pneumoniae										
Imipenem	0.5/2	100.0	0.25/1	100.0	0.25/1	99.5	0.25/0.5	99.3	0.25/1	99.3
Cefepime	0.5/4	94.4	4/>16	63.6	16/>16	49.6	1/16	87.6	2/>16	76.1
Ceftazidime	2/>16	72.2	>16/>16	14.5	>16/>16	27.0	>16/>16	39.9	>16/>16	22.5
Aztreonam	4/>16	55.6	>16/>16	16.4	>16/>16	20.9	>16/>16	43.8	>16/>16	18.1
Ceftriaxone	1/>32	72.2	>32/>32	23.8	>32/>32	17.9	8/32	59.5	32/>32	17.4
Cefoxitin	16/>32	38.9	4/16	84.6	8/>32	71.0	8/>32	51.6	16/>32	43.5
Ciprofloxacin	0.25/>2	77.8	0.25/>2	75.7	0.25/>2	76.9	1/>2	65.4	1/>2	55.8
Tobramycin	1/>16	83.3	16/>16	19.2	16/>16	17.9	8/>16	45.8	16/>16	27.5
Gentamicin	1/>16	83.3	16/>16	35	16/>16	33.7	4/>16	51.0	16/>16	41.3
Amikacin	2/4	94.4	16/32	70.6	16/>32	60.9	2/16	92.2	8/32	84.1
E. coli										
Imipenem	0.25/0.5	100.0	0.25/1	100.0	0.25/0.5	99.4	0.25/0.5	100.0	0.25/0.5	100.0
Cefepime	0.25/8	94.1	0.5/>16	83.2	4/>16	59.0	0.25/4	94.6	2/>16	71.3
Ceftazidime	4/>16	80.4	4/>16	63.4	16/>16	46.2	4/>16	70.5	4/>16	59.8
Aztreonam	2/16	86.3	4/>16	59.9	>16/>16	32.9	4/>16	69.9	8/>16	57.5
Ceftriaxone	1/32	82.4	4/>32	66.8	>32/>32	28.9	1/32	78.9	16/>32	42.5
Cefoxitin	16/>32	39.2	16/>32	47.5	8/>32	54.9	16/>32	37.3	32/>32	25.3
Ciprofloxacin	0.03/>2	86.3	0.25/>2	65.8	>2/>2	47.4	0.12/>2	80.7	>2/>2	34.5
Tobramycin	2/8	86.3	2/>16	67.8	>16/>16	31.2	2/16	78.3	8/>16	31.0
Gentamicin	1/>8	84.3	1/>16	74.3	16/>16	42.2	1/>8	78.9	8/>16	24.1
Amikacin	4/8	96.1	4/32	89.1	8/>32	69.9	4/8	95.2	2/32	86.5
P. mirabilis										
Imipenem			2/4	95.9	2/4	100.0	2/4	96.6		
Cefepime			4/>16	65.3	>16/>16	22.7	2/>16	82.8		
Ceftazidime			8/>16	55.1	2/16	84.1	8/>16	62.1		
Aztreonam			1/>16	79.6	2/>16	68.2	8/>16	58.6		
Ceftriaxone			16/>32	42.9	>32/>32	18.2	1/>32	72.4		
Piperacillin			8/>32	53.1	8/16	88.6	4/>32	79.3		
Ciprofloxacin			2/>2	38.8	>2/>2	11.4	0.12/>2	65.5		
Tobramycin			8/16	40.8	16/>16	15.9	2/16	75.9		
Gentamicin			16/>16	38.8	>16/>16	9.1	4/>16	65.5		
Amikacin			4/>32	81.6	8/>32	68.2	4/8	96.6		

NOTE. MICs are μg/mL. Susc, susceptible.

of *P. mirabilis* encoding an ESBL throughout the world, these data highlight the need for individualizing ESBL detection for a particular hospital or, on occasion, a particular organism.

This study has evaluated organisms for which the MIC of extended-spectrum cephalosporins or monobactams was ⩾2 μg/mL and has identified these organisms as having an ESBL phenotype. These criteria are not restricted to organisms expressing an ESBL. Resistance to an extended-spectrum cephalosporin can also be explained by expression of an ampC-like β-lactamase or expression of TEM-1 or SHV-1 β-lactamase in association with alterations in the outer membrane permeability or even by the presence of a unique inhibitor-resistant β-lactamase [1–3].

In each of these cases, elevated MICs of extended-spectrum cephalosporins are unaffected by clavulanic acid. When selected isolates were tested for enhanced cephalosporin resistance in the presence of clavulanic acid, the predictive value of an ESBL phenotype was highest in high-prevalence regions, such as Latin America or the Western Pacific region. The likelihood that an elevated MIC of ceftazidime, ceftriaxone, or aztreonam was attributed to an ESBL was significantly lower in the United States (43%). However, a 43% confirmation rate in low-prev-

alence regions suggests that the NCCLS criteria do identify a significant number of isolates that express an ESBL. The numbers of isolates tested were relatively low, and more studies must be performed to confirm this finding.

Organisms that express an ESBL are frequently resistant to other antimicrobial agents, as many of these additional resistance genes are encoded on the ESBL-associated plasmid [16]. In our study, high rates of resistance were detected against aminoglycosides, tetracycline, trimethoprim-sulfamethoxazole, and the quinolones. Quinolone resistance, unlike the other co-resistances, is typically encoded chromosomally, although rare reports have identified low-level plasmid-mediated quinolone resistance [23]. The fact that quinolone resistance was quite prevalent in our study may reflect significant antibiotic pressure in the environment rather than co-carriage of this resistance gene on plasmid DNA.

As has been previously noted, molecular analysis of selected ESBL strains provided evidence of intrahospital spread of these organisms [24, 25]. However, clonal spread did not explain the overall prevalence of isolates expressing an ESBL in each hospital, since unrelated ESBL organisms were also identified. In addition, there was no clear evidence of significant clonal spread from hospital to hospital or country to country, although previous studies have identified more widespread transmission [26]. These data did identify distinct isolates expressing similar β-lactamases with more unusual pI values. Although the possibility was not addressed in this study, these isolates may encode similar plasmids that have been disseminated throughout the geographic regions.

In conclusion, β-lactam resistance due to ESBL is common throughout the world, with clear differences in prevalence between and within particular geographic regions. An elevated extended-spectrum cephalosporin or monobactam MIC is not definitive evidence of the presence of an ESBL. However, screening for the ESBL phenotype is an important tool, even in countries in which the prevalence of ESBLs is low. ESBLs provide an important mechanism of resistance in gram-negative organisms. Clinical microbiology laboratories, hospital epidemiologists, and clinicians must learn to recognize organisms that likely are encoding an ESBL, so that proper antimicrobial management and infection control can be instituted.

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