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First Report of the Emergence of CTX-M-Type Extended-Spectrum β-Lactamases (ESBLs) as the Predominant ESBL Isolated in a U.S. Health Care System▽

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CTX-M-type extended-spectrum β-lactamases (ESBLs) have become increasingly common worldwide, with the notable exception of the United States, where TEM- and SHV-type ESBLs have appeared to predominate. We have noted the emergence of ESBLs in our health care system (the University Health System in San Antonio, TX), especially in *Escherichia coli* isolates, that preferentially hydrolyze cefotaxime rather than ceftazidime, suggesting the possibility of CTX-M-type enzymes. Microbiology laboratory records were reviewed to identify ESBL-producing isolates and to compare the diameters of ceftazidime disk diffusion zones of inhibition to cefotaxime zone diameters. All isolates had been initially detected and confirmed using the procedures recommended by the Clinical and Laboratory Standards Institute. A total of 94 stored ESBL-producing isolates recovered between January 2000 and June 2006 (predominately from blood and normally sterile fluids) were retrieved for further study and screened using PCR primers specific for the presence of CTX-M, TEM, and SHV ESBLs. Only small numbers of retained ESBL-producing isolates were available for study in 2000 and 2002. The percentages of available ESBL-producing organisms in the following years were found to produce CTX-M enzymes: 2000, 25%; 2001, 10%; 2002, 0%; 2003, 60%; 2004, 69%; 2005, 89%; and 2006, 70%. The most common CTX-M-type ESBL was CTX-M-15, followed by CTX-M-16, CTX-M-8, and CTX-M-14. Comparing the disk diffusion zone diameters of cefotaxime and ceftazidime was helpful with the initial recognition of CTX-M-producing *E. coli*, which had an average cefotaxime zone diameter 7 mm smaller than the ceftazidime zone. However, comparing ceftazidime and cefotaxime zones for CTX-M-producing *Klebsiella* spp. was not helpful with initial recognition. CTX-M enzymes were also identified in *Proteus mirabilis*, *Enterobacter* spp., and *Morganella morganii*. Based on pulsed-field gel electrophoresis typing of the *E. coli* isolates, the CTX-M-producing isolates did not represent the spread of a single clone in the institution or in the community. In conclusion, CTX-M-type ESBLs are now the most common ESBL type isolated from patients in our health care system and may also be present but unrecognized in other U.S. locales.

Extended-spectrum-β-lactamase (ESBL)-producing gram-negative rods represent a significant challenge to the antibiotic armamentarium worldwide. Historically, and especially in the United States, the largest threat from ESBLs has come in the form of hospital-based outbreaks of ESBL-producing organisms, especially *Klebsiella* spp. or *Escherichia coli* producing either TEM- or SHV-type enzymes (4, 16). However, in recent years, CTX-M-type ESBLs have emerged as the predominant type of ESBL found in many regions of the world, including South America, Europe, and, more recently, parts of Canada (2, 10, 16, 20). A worrisome trend with the emergence of these enzymes has been an increasing frequency of *E. coli* isolates from outpatients or patients hospitalized for a very brief period, suggesting community acquisition of these strains. It has been suggested that this may be analogous to the emergence of community-associated methicillin-resistant *Staphylococcus aureus* (9). Though the existence of CTX-M enzymes has been recognized in the United States, it has been reported for only nine isolates that were geographically scattered (14). In the clinical microbiology laboratory at the University Health System, San Antonio, TX, in early 2004, we began to observe the emergence of ESBLs that appeared to preferentially hydrolyze cefotaxime rather than ceftazidime when the Clinical and Laboratory Standards Institute (CLSI) ESBL disk diffusion confirmatory test was performed. We noted that most of these ESBLs were found in *E. coli*, which is consistent with reports of increasing rates of CTX-M enzymes in *E. coli* in many locales outside the United States (1, 4, 10, 20, 21). This recognition of a change in ESBL hydrolysis phenotype and an increasing recognition of ESBLs in *E. coli* among outpatients prompted our current investigation.

MATERIALS AND METHODS

Test isolates. The database of the University Health System Clinical Microbiology Laboratory was searched for *Enterobacteriaceae* isolates that were reported as producers of ESBLs between 1 January 2000 and 30 June 2006. The resulting list of isolates was cross-indexed with the laboratory's list of retained frozen isolates. Isolates from blood, sterile body fluids, and tissues were routinely retained and frozen during this period. In addition, some highly drug-resistant isolates were retained periodically for infection control or research purposes, especially in 2005 and 2006. All available isolates were subcultured from the freezer vials, and the species, date of isolation, and source or site of isolation were recorded. Only one isolate per patient was included in this study. Patient identifiers were removed and the isolates transferred to our research laboratories for characterization. The isolates had been initially detected as possible ESBL

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TABLE 1. Primers used in PCRs

Primer	Primer sequence	Reference
Global CTX primers		
PANCTX-M.F	5′-TTTGCGATGTGCAGTACCAGTAA-3′	8
PANCTX-M.R	5′-CGATATCGTTGGTGGTGCCATA-3′	8
CTX-M group-specific primers		
CTXM.groupI.F3	5′-GACGATGTCACTGGCTGAGC-3′	19
CTXM.groupI.R2	5′-AGCCGCCGACGCTAATACA-3′	19
CTXM.groupII.TOHO1.2F	5′-GCGACCTGGTTAACTACAATCC-3′	19
CTXM.groupII.TOHO1.1R	5′-CGGTAGTATTGCCCTTAAGCC-3′	19
CTXM8.WSAgroupIII.F	5′-AGACCTGATTAACTACAATCCCATTA-3′	This study
CTXM8.WSAgroupIII.R	5′-ACTTTCTGCCTTCTGCTCTGGC-3′	This study
CTXM914.groupIV.F	5′-GCTGGAGAAAAGCAGCGGAG-3′	19
CTXM914.groupIV.R	5′-GTAAGCTGACGCAACGTCTG-3′	19
Miscellaneous primers		
TEM.F	5′-ATAAAATTCTTGAAGACGAAA-3′	12
TEM.R	5′-GACAGTTACCAATGCTTAATCA-3′	12
SHV.F	5′-GGGTTATTCTTATTTGTCGC-3′	12
SHV.R	5′-TTAGCGTTGCCAGTGCTC-3′	12
CTX.F	5′-AAAAATGATTGAAAGGTGGTTGT-3′	12
CTX.R	5′-TTACAGCCCTTCGGCGATGA-3′	12
OXA-1A	5′-CCA AAG ACG TGG ATG-3′	23
OXA-1R	5′-GTT AAA TTC GAC CCC AAG TT-3′	23

producers based upon testing with cefpodoxime (10 μg) disks as described by the CLSI (6, 7) or noting elevated cefotaxime, ceftazidime, or aztreonam MICs (i.e., ≥2 μg) when the isolates were tested with the VITEK 2 instrument (bioMérieux, Durham, NC) or when it was suggested by the VITEK 2 Advanced Expert system software. ESBL production was confirmed phenotypically in each isolate by disk testing with disks containing cefotaxime (30 μg), cefotaxime plus clavulanic acid (30 μg plus 10 μg, respectively), ceftazidime (30 μg), and ceftazidime plus clavulanic acid (30 μg plus 10 μg) (BD Microbiology Systems, Cockeysville, MD) on Mueller-Hinton agar (BD Microbiology). Strains were considered to produce an ESBL if there was an increase of ≥5 mm in zone diameter on either of the clavulanate-containing disks compared with the zone diameters on the disks containing cephalosporin alone, as recommended by the CLSI (7).

Genotypic identification of ESBLs. The test isolates were grown initially on 5% sheep blood agar (BD Microbiology) and then lysed to recover their DNA using Prepman Ultra (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Five microliters of this supernatant was used as a template in a 50-μl PCR mixture. Standard PCRs consisted of 2.5 units of *Taq* polymerase, 5 μl of the template DNA, 10 μM of each primer, 10 mM deoxynucleoside triphosphate, buffer, and distilled water to achieve a final volume of 50 μl. Screening PCRs (nonsequenced) were prepared using *Taq* polymerase from Invitrogen (Carlsbad, CA). Amplicons to be sequenced were prepared using Triple Master *Taq* polymerase (Eppendorf, Westbury, NY). Reactions were run in an MJ mini thermocycler (Bio-Rad Laboratories, Hercules, CA), using a basic cycling program of 94°C for 2 min (first cycle only), 94°C for 15 s, the primer-specific annealing temperature for 30 s, 72°C for 30 s (30 cycles), and 72°C for 3 min. The primers are shown in Table 1 and were synthesized at the Advanced Nucleic Acids Core Facility at the University of Texas Health Science Center at San Antonio. The primers for CTX-M group 3 were redesigned (CTX-M8.WSAgroupIII.F and CTX-M8.WSAgroupIII.R) using sequences from GenBank (accession numbers X92506, AF189721, X92507, and AF252622). The PCRs were standardized using control strains MISC336 (*E. coli*), MISC182 (*Proteus mirabilis*), MISC253 (*Enterobacter aerogenes*), and MISC419 (*E. coli*), which were previously classified as belonging to CTX-M groups 1, 2, 3, and 4, respectively (kindly provided by Kenneth S. Thomson). ESBL identities were assigned by first amplifying each strain with the PAN-CTX primer pair, followed by the group-specific primers. The isolates were also examined for TEM and SHV ESBLs by amplification with the primer pairs listed in Table 1 as previously described (12). The products resulting from TEM or SHV amplifications were also submitted for sequencing. All sequencing was performed by the Advanced Nucleic Acids Core Facility. Primer design and sequence alignment were performed using MacVector software (Accelrys, San Diego, CA).

Detection of OXA-1/OXA-30. Because OXA-1/OXA-30 has previously been reported in association with the presence of CTX-M-15 (20) in *E. coli*, we selected a convenience sample of CTX-M-15-producing isolates collected in 2005 to 2006 for OXA-1/OXA-30 PCR and sequencing. Total DNA was extracted with Prepman (ABI), and 2 μl was used as a template in the PCR assay. Oligonucleotide primers used for the PCR are presented in Table 1. The predicted PCR product was a 540-bp intragenic fragment of $bla_{\text{OXA-1}}$. Reactions were performed in a DNA thermal cycler (Eppendorf). Mixtures contained 2.5 U of *Taq* polymerase (Triple Master; Eppendorf); 10× buffer consisting of 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, and 2.5 mM deoxynucleoside triphosphate; and 2 mM of each oligonucleotide primer. Thirty-five cycles were performed for each reaction using the following temperature profile: 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min. All PCR products from the isolates were sequenced. The sequence of the gene $bla_{\text{OXA-30}}$ was GenBank accession number AF255921.

PFGE typing. Pulsed-field gel electrophoresis (PFGE) typing of the ESBL-producing isolates was performed as described previously (13). Briefly, a single colony from an 18- to 24-h subculture plate was inoculated into a tube containing 5 ml of brain heart infusion broth and incubated without shaking overnight at 37°C. Cell lysates were prepared and subjected to restriction endonuclease digestion with SpeI (Roche Applied Biosciences, Indianapolis, IN). DNA plug slices were run on a 0.8% PFGE agarose gel in 0.5× Tris-borate-EDTA buffer (Sigma-Aldrich, St. Louis, MO). PFGE was performed with a CHEF-DRII system (Bio-Rad). The pulse time was 5 to 60 s over 16 h at 200 V. Lambda concatemers (Cambrex, Rockland, ME) were used as the size standard. Gels were stained for 20 min in 3 μg/ml ethidium bromide, decolorized in distilled water for 10 min, and photographed by filtered UV illumination. To facilitate comparisons of restriction patterns, dendrograms were prepared using Bio-Rad Molecular Analyst software version 1.12 in order to calculate Dice similarity coefficients and clusters by the unweighted-pair group method using average linkages.

RESULTS

Ninety-four isolates recovered from individual patients between January 2000 and June 2006 and previously identified as ESBL producers by phenotypic tests were retrieved from the laboratory's frozen stocks and evaluated using the molecular techniques described above. Only one isolate per patient was counted in this study. Eighty-eight of the 94 (93.6%) stored isolates with an ESBL phenotype were shown to produce one or more ESBLs based upon molecular characterization and

TABLE 2. Extended-spectrum β-lactamases produced by the study isolates according to genus and species

ESBL produced	Organism	Total no. of isolates	No. of isolates also producing[a]:		
			SHV	TEM	CTX-M
CTX-M-15	*E. coli*	32	3	0	NA
	K. pneumoniae	4	3	0	NA
	K. oxytoca	1	1	0	NA
	Morganella spp.	1	0	0	NA
	Proteus mirabilis	1	0	0	NA
CTX-M-16	*E. coli*	1	0	0	NA
	K. oxytoca	5	0	0	NA
	Enterobacter cloacae	1	0	0	NA
CTX-M-8	*K. oxytoca*	2	0	0	NA
	K. pneumoniae	1	0	0	NA
	Enterobacter spp.	1	0	0	NA
CTX-M-14	*E. coli*	1	0	0	NA
	K. oxytoca	1	0	0	NA
SHV-12	*E. coli*	7	NA	0	2
	K. pneumoniae	7	NA	0	1
	K. oxytoca	5	NA	0	1
	E. cloacae	2	NA	0	0
SHV-5	*E. coli*	2	NA	0	1
	K. pneumoniae	7	NA	0	0
	K. oxytoca	1	NA	0	0
SHV-2	*E. coli*	2	NA	0	0
	K. pneumoniae	7	NA	0	1
SHV-11	*K. pneumoniae*	1	NA	0	1
TEM-15	*K. oxytoca*	1	0	NA	0
TEM-10	*E. coli*	1	0	NA	0

[a] NA, not applicable.

TABLE 3. Number of ESBL-producing isolates by year of isolation, organism, and type of enzyme

Yr and organism	No. of isolates producing:			
	CTX-M	TEM	SHV	CTX-M + SHV
2000				
E. coli	0	0	0	1
K. pneumoniae	0	0	1	0
K. oxytoca	0	1	1	0
2001				
E. coli	0	0	2	1
K. pneumoniae	0	0	4	0
K. oxytoca	0	0	3	0
2002				
E. coli	0	0	2	0
K. pneumoniae	0	0	3	0
E. cloacae	0	0	1	0
2003				
E. coli	3	1	0	0
K. pneumoniae	0	0	3	0
K. oxytoca	3	0	0	0
2004				
E. coli	8	0	2	0
K. pneumoniae	0	0	1	1
E. cloacae	0	0	1	0
2005				
E. coli	8	0	0	0
K. pneumoniae	1	0	1	0
K. oxytoca	4	0	1	1
Enterobacter spp.	2	0	0	0
2006 (first 6 mo)[a]				
E. coli	12	0	2	1
K. pneumoniae	1	0	6	2
K. oxytoca	1	0	0	0
M. morganii	1	0	0	0
P. mirabilis	1	0	0	0

[a] 27 isolates were recovered in this time.

isoelectric focusing of the various β-lactamases produced by each isolate (isoelectric focusing profiles kindly performed by A. M. Queenan). The numbers of ESBL-producing isolates available for study relative to the numbers reported for each year were as follows: 2000, 4/11 (36%); 2001, 10/15 (67%); 2002, 6/10 (60%); 2003, 10/31 (32%); 2004, 13/35 (37%); 2005, 18/31 (58%); and the first 6 months of 2006, 27/28 (96%). A total of 65 ESBL-producing isolates were reported during the entire year of 2006. The increasing numbers of ESBL-producing isolates detected annually in the clinical microbiology laboratory (especially in 2006) occurred despite no changes being made in our screening criteria or methods of phenotypic confirmation.

The most common ESBLs identified among the isolates were members of the CTX-M group (52), followed by SHV ESBLs (41), with only two TEM ESBLs found. Table 2 lists the ESBLs detected among the study isolates as well as the species that produced the various enzymes. It was uncommon for an isolate that produced a CTX-M ESBL to concomitantly produce an SHV or TEM enzyme; in fact, more than one ESBL was found in only 7 of 88 (8%) isolates.

In the years 2000 and 2001, only one CTX-M ESBL was detected each year and was produced in conjunction with an SHV enzyme. In 2002, only SHV ESBLs were found (Table 3). The most common enzymes produced from 2000 to 2002 were SHV-12 (12 isolates), followed by SHV-5, SHV-2, and one TEM-15 (Table 3). ESBL producers were almost equally represented by *E. coli*, *Klebsiella oxytoca*, and *K. pneumoniae* isolates from 2000 to 2003 (Table 3). Thereafter, *E. coli* was the most common ESBL producer (between 2004 and 2006).

While CTX-M ESBLs appear to have first entered our population in 2000, they did not increase in number until mid-2003 and represented 60% (6/10) of all ESBL isolates characterized that year (Table 3). The earliest CTX-M-producing isolates to appear produced CTX-M-15, but isolates producing CTX-M-14 and CTX-M-16 also occurred early in the evolution of CTX-M-producing isolates in our population. Two *K. oxytoca* isolates produced CTX-M-16, and the third produced CTX-M-14. The first four CTX-M-15-producing isolates collected between 2000 and 2003 were *E. coli*. A fifth *E. coli* isolate (from 2003) produced CTX-M-16. The number and proportion of CTX-M producers continued to increase steadily through 2006 (Table 3). Between January 2003 and June 2006, 50 bacterial isolates were determined to be positive for CTX-M-type ESBLs, which represented 72% of the total isolates genotypically confirmed as ESBL producers during that period (Table 3). *E. coli* was the most common CTX-M-producing organism isolated in our clinical laboratory during this time period. By mid-2006, 95% (18 of 19) of the CTX-M-containing

TABLE 4. Disk diffusion zone diameters resulting from CTX-M-producing isolates

Organism (no. of isolates)	Mean zone size (mm)	
	Cefotaxime	Ceftazidime
Escherichia coli (30)	8.8	15.9
Klebsiella pneumoniae (4)	10.8	9.8
Klebiella oxytoca (9)	16.8	9.2

isolates were found to produce CTX-M-15; of these, 68% (13 of 19) were *E. coli*. Other organisms containing CTX-M-type enzymes in this study are shown in Tables 2 and 3 and include *Enterobacter* spp., *Proteus mirabilis*, and *Morganella morganii*.

Twelve of the 2005 and 2006 CTX-M-15-producing isolates were further examined to determine the possible presence of OXA-1/OXA-30. Ten of these isolates were *E. coli*, one was *K. pneumoniae*, and one was *P. mirabilis* (data not further depicted). All of the *E. coli* isolates screened and the sole *P. mirabilis* isolate examined were positive for OXA-1/OXA-30; however, the *K. pneumoniae* isolate did not produce this enzyme.

Using the CLSI-recommended double-disk diffusion methodology for phenotypic ESBL confirmation, zone sizes for cefotaxime or cefotaxime plus clavulanic acid and ceftazidime or ceftazidime plus clavulanic acid were found to be useful predictors of the presence of CTX-M in *E. coli*. The zone diameters of cefotaxime (without clavulanate) with *E. coli* that produced CTX-M enzymes were noticeably and consistently smaller (mean, 7.1 mm) than the zone diameters of ceftazidime (without clavulanate) (Table 4). However, this observation did not hold for *Klebsiella* spp. producing CTX-M enzymes. *Klebsiella pneumoniae* isolates containing CTX-M enzymes produced similar zone diameters for cefotaxime and ceftazidime, while CTX-M-producing *K. oxytoca* isolates produced smaller zones for ceftazidime than for cefotaxime (Table 4). By comparison, non-CTX-M-producing ESBL *E. coli* isolates were found to produce average zone diameters of 21.0 mm for cefotaxime and 17.3 mm for ceftazidime.

The sources of the CTX-M-producing isolates are listed in Table 5. Sixty percent of CTX-M-producing isolates were recovered from urinary sources, 21% from blood cultures, 10% from respiratory cultures, and the remaining 9% from wound, catheter, or body fluid cultures. This compares to 40% of SHV and TEM isolates recovered from urine, 33% from blood cultures, 16% from respiratory specimens, and 12% from wound, catheter, or body fluid cultures.

PFGE typing performed on the CTX-M-producing *E. coli* isolates revealed six different groups of highly related isolates with Dice coefficients of ≥85%, two closely related groups (70% to 84%), and four possibly related groups (50% to 69%) (Fig. 1). One of the highly related groups contained seven isolates, one contained four isolates, and the remainder included three or fewer isolates. There was not a clear temporal relationship among the highly related isolates. Based on these data, the evolution of CTX-M in *E. coli* did not represent the spread of one or a few clones within our institution or community.

Susceptibility to gentamicin and trimethoprim-sulfamethoxazole was more common among CTX-M-producing isolates than among isolates producing either SHV or TEM enzymes (Table 6). Piperacillin-tazobactam was found to be active in vitro against 73% of the isolates that produced only a CTX-M ESBL and 64% of the isolates that produced only SHV- or TEM-type enzymes. Fluoroquinolone susceptibility among CTX-M-producing isolates (16%) was less common than among isolates producing either SHV or TEM enzymes (36%). When the seven isolates that produced a CTX-M and an SHV enzyme were analyzed, they were found to be rarely susceptible to any of the four agents noted above (Table 6).

TABLE 5. Sources of CTX-M-producing isolates

Organism	Total no. of isolates	Source(s) (no. of isolates)
Escherichia coli	34	Blood (7), urine (23), respiratory sample (1), other (3)
Klebsiella pneumoniae	5	Urine (3), respiratory sample (1), other (1)
Klebsiella oxytoca	9	Blood (3), urine (3), respiratory sample (2), other (1)
Enterobacter spp.	2	Urine (1), respiratory sample (1)
Morganella morganii	1	Urine (1)
Proteus mirabilis	1	Blood (1)

DISCUSSION

CTX-M-type ESBLs are a global concern for infectious disease clinicians and clinical microbiologists. Over the past decade, these enzymes have become the most common ESBLs in many widely dispersed geographic areas, including many parts of Europe and South America and, most recently, regions of Canada (10, 12, 15, 16, 20). This is in marked contrast to the epidemiology of ESBLs in the United States which has historically been associated with TEM- and SHV-type enzymes primarily found in patients with *Klebsiella* sp. infections and prolonged hospital stays (16, 17). Indeed, *Klebsiella oxytoca* was previously the most common ESBL producer in our institution prior to the initiation of the present study (4). The rapid emergence of CTX-M enzymes and the apparent success of CTX-M-15-producing *E. coli* in becoming the predominant ESBL-producing strain in our health care system were unexpected. However, the predominance of CTX-M-15 in our health care system is not a consistent finding worldwide, as different CTX-M-type enzymes have emerged as the predominant ESBLs in other locations (10).

Until this investigation, there had been only rare reports of CTX-M-type enzymes in the United States. A prior report demonstrated that CTX-M-producing isolates were present but only in widely dispersed areas of the United States and with only nine CTX-M-containing isolates previously identified (14). The authors of the prior report remarked that these organisms continue to be sent sporadically to their reference laboratory for characterization (Kenneth S. Thomson, personal communication). This is in contrast to certain other β-lactamases such as the KPC group of carbapenemases that after starting in the northeastern United States have appeared to spread to several locales (3, 11). Findings similar to ours were reported recently from Canada: initially CTX-M enzymes other than CTX-M-15 were present but appeared to be rapidly

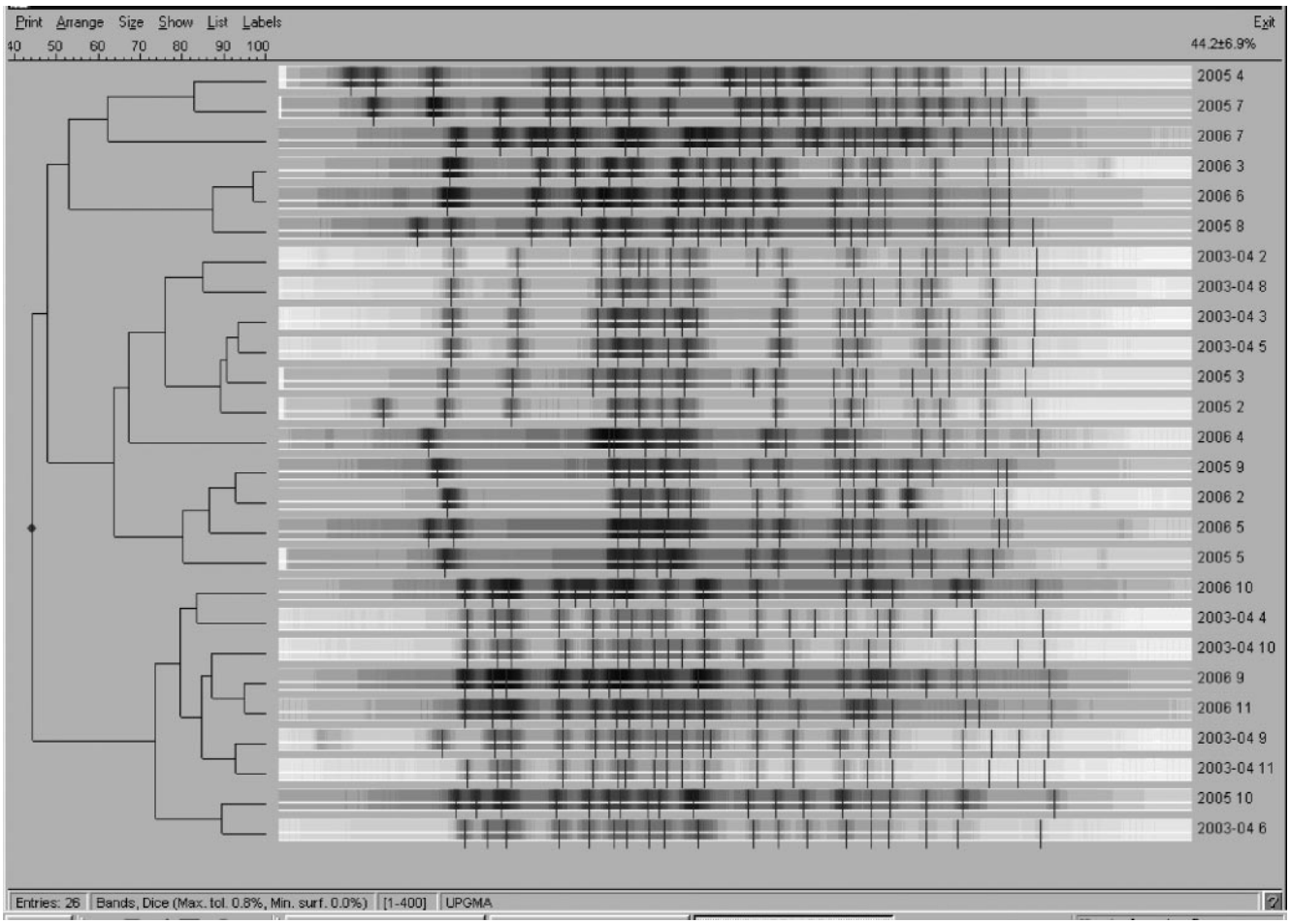

FIG. 1. Dendrogram with Dice coefficients of pulsed-field gel electrophoresis patterns of CTX-M-15-producing *E. coli* isolates included in this study. The year of isolation is indicated in the far right column.

displaced by CTX-M-15 produced by *E. coli* (10, 20). Our data also confirm the frequent association of OXA-1/OXA-30 and CTX-M-15 in *E. coli* noted previously (20). Many features of the CTX-M evolution in our health care system are strikingly similar to the emergence of these enzymes in Canada and the United Kingdom (15, 20). CTX-M-type ESBLs are the most common ESBL isolated in our tertiary referral health care system, which in all likelihood reflects that these enzymes have become the most prevalent ESBLs in San Antonio, since susceptibility phenotypes similar to ours have been observed at other local hospitals (personal communications; data not shown). This is further supported by the fact that many of our isolates have been recovered from outpatient sources such as patients with urinary tract infections.

TABLE 6. Susceptibility to other antimicrobial agent classes according to the type of ESBL produced

Enzyme(s) produced	No. of isolates susceptible to indicated drug(s)/total no. tested (%)			
	Piperacillin-tazobactam	Gentamicin	TMP-SMX[b]	Fluoroquinolones
CTX-M-15	25/32 (78)	12/32 (38)	13/32 (41)	4/32 (13)
CTX-M-16	5/7	1/7	0/7	1/7
CTX-M-8	3/4	3/4	2/4	2/4
CTX-M-14	0/2	0/2	0/2	0/2
SHV-12	13/17 (76)	2/17 (12)	0/17 (0)	7/17 (42)
SHV-5	6/9	6/9	3/9	4/9
SHV-2	2/8	2/8	1/8	2/8
TEM-15	1/1	0/1	0/1	0/1
TEM-10	1/1	0/1	0/1	0/1
CTX-M-15 + SHV[a]	1/7	2/7	1/7	1/7

[a] SHV enzymes included are SHV-2, -5, -11, and -12.
[b] TMP-SMX, trimethoprim-sulfamethoxazole.

Clinical microbiology laboratorians, hospital infection control practitioners, and clinicians must recognize the presence of ESBLs in outpatients, for whom routine laboratory screening of urinary samples may be limited, as well as in organisms other than *Klebsiella* spp. and *E. coli*. The common practice in many U.S. clinical microbiology laboratories of not screening urinary isolates for ESBL production may be problematic given the frequent isolation of CTX-M-producing *E. coli* from urinary sources in our study. Previous authors have clearly shown that these urinary isolates are very capable of causing invasive diseases such as bacteremia and could represent an unrecognized reservoir for spreading infection to other patients within a health care institution (21, 22). While the CLSI methodology for ESBL screening now includes *P. mirabilis*,

one of which was found among our isolates, many other organisms that are potentially important are not addressed by the current CLSI screening and confirmation methods (5, 6, 7). In fact, *Enterobacter* spp. and *Morganella* spp., both of which were found to be capable of producing ESBLs in our study, represent a challenge for clinical microbiology laboratories because of the common presence of high levels of chromosomal AmpC β-lactamase in those organisms. One of the three *Enterobacter* sp. isolates in our study was found to produce an ESBL by the use of a non-CLSI-approved methodology of testing cefepime disks with and without clavulanic acid and noting an increase in the cefepime zone when clavulanate was added, similar to the standard confirmatory tests using cefotaxime and ceftazidime. If CTX-M enzymes become common in organisms other than those with currently approved CLSI detection methods, therapeutic failures might result from the inability of clinical laboratories to routinely detect the presence of these enzymes in those genera. Although in our experience cefpodoxime works well to screen for the presence of CTX-M enzymes, the emergence of these ESBLs underscores the need for clinical laboratories using commercial devices that perform initial detection with clavulanate combinations to include both ceftazidime and cefotaxime in their testing, since the CTX-M enzymes often more efficiently hydrolyze cefotaxime.

CTX-M ESBLs have become a worldwide problem, perhaps further illustrated by their arrival in our area of the United States. It is impressive to note how quickly these organisms appear to have become the globally predominant ESBL. As authors from several other countries have noted, we found that the majority of our isolates were *E. coli* and were from patients with urinary tract infections, many of whom appeared to have developed their infections as outpatients or after short hospital stays (10, 15, 20, 21). It is important that clinical microbiology laboratories be aware that CTX-M enzymes are also present in *Klebsiella* spp., although preferential hydrolysis of cefotaxime may not be evident based upon testing with cefotaxime and ceftazidime. Our limited data showed almost no difference in cefotaxime and ceftazidime zone diameters with *K. pneumoniae* isolates, perhaps because they also produced an SHV ESBL. Furthermore, ceftazidime zones were much smaller than the cefotaxime zones with isolates of *K. oxytoca* containing CTX-M enzymes, perhaps due to the concomitant presence of the native K1 β-lactamase.

Carbapenems have been suggested as the drug class of choice for serious infections due to ESBL-producing strains (18). A noteworthy difference between isolates that produced SHV and TEM ESBLs and those producing CTX-M enzymes in our study was the susceptibility to agents other than carbapenems. The isolates that produced only CTX-M ESBLs were more likely to be susceptible in vitro to piperacillin-tazobactam (73%) but were more likely to be resistant to fluoroquinolones (16% susceptible). Though its use remains controversial, piperacillin-tazobactam might represent an alternative to carbapenem therapy in some types of infections. These data also underscore the decline in fluoroquinolone susceptibility of these drug-resistant pathogens. Interestingly, we noted lower rates of gentamicin and trimethoprim-sulfamethoxazole resistance among isolates that produced only CTX-M enzymes than those that produced only an SHV or TEM ESBL. This may provide other noncarbapenem therapy options, e.g., trimethoprim-sulfamethoxazole for urinary tract infections due to susceptible strains. In strains that produced both a CTX-M and an SHV ESBL, resistance to all four agents was more likely.

The limitations of our study include its retrospective design and selective retention of many but not all ESBL isolates recovered by our clinical microbiology laboratory in the early part of the study period. Historically, we did not retain ESBL-producing urinary isolates, which limited our ability to analyze those potentially important strains. It was not until early 2004, when we began noticing increasing numbers of *E. coli* isolates and a shift toward strains that more efficiently hydrolyzed cefotaxime in the disk diffusion test, that we began saving some of the urinary isolates for further study. Saving all ESBL-producing urinary isolates was not emphasized until 2006, when we undertook the molecular characterization of isolates as described herein. Upon review of the microbiology laboratory database, many urinary isolates from late 2003 to 2005 that were not retained corresponded to the CTX-M phenotype (*E. coli* and small or nonexistent cefotaxime disk diffusion zone).

In summary, CTX-M-type ESBLs, primarily CTX-M-15, have emerged as the predominant type of ESBL produced by common gram-negative rods in our health care system and, in all likelihood, represent an unrecognized new type of ESBL in other health care systems in the United States. The preexisting belief that screening urine cultures for ESBL-producing organisms is unnecessary requires reevaluation in light of the fact that many of the infections due to these ESBL producers originated from urinary sources. The long-expected arrival and dissemination of CTX-M ESBLs in the United States should raise concern among clinicians, infection control practitioners, and clinical microbiologists.

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AUTHOR'S CORRECTION

First Report of the Emergence of CTX-M-Type Extended-Spectrum β-Lactamases (ESBLs) as the Predominant ESBL Isolated in a U.S. Health Care System

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Vol. 51, no. 11, p. 4015–4021, 2007. Subsequent to the publication of our article, it was recognized that some details of our PCR and sequencing procedures were not thoroughly described.

Page 4016, column 1: Lines 17–30 should read as follows. "Amplicons to be sequenced (high-fidelity PCRs) were prepared using Triple Master *Taq* polymerase (Eppendorf, Westbury, NY) according to the manufacturer's instructions. The high-fidelity PCRs consisted of a final concentration of 2.5 units of *Taq* polymerase, 5 μl of the template DNA, 200 nM of each primer, 200 μM dNTP, 1× buffer with magnesium, and distilled water to achieve a final volume of 50 μl. Screening PCRs (nonsequenced) were prepared with *Taq* polymerase from Invitrogen (Carlsbad, CA) using component concentrations recommended by the manufacturer for the basic PCR protocol. Reactions were run in an MJ mini thermocycler (Bio-Rad Laboratories, Hercules, CA), using a basic cycling program of 94°C for 2 min (first cycle only), 94°C for 15 s, the primer-specific annealing temperature (see references from Table 1) for 30 s, 72°C for 30 s (30 cycles), and 72°C for 3 min. The primers are shown in Table 1 and were synthesized at the Advanced Nucleic Acids Core Facility at the University of Texas Health Science Center at San Antonio. The primers for CTX-M group 3 were redesigned (CTX-M8.WSAgroupIII.F and CTX-M8.WSAgroupIII.R; annealing temperature, 65°C) using sequences from GenBank (accession numbers X92506, AF189721, X92507, and AF252622)."

Page 4016, column 2: Lines 4–13 should read as follows. "The predicted PCR product was a 540-bp intragenic fragment of $bla_{\text{OXA-1}}$. PCRs were performed in a DNA thermal cycler (Eppendorf) and prepared as described above using Triple Master *Taq* polymerase (Eppendorf). Thirty-five cycles were performed for each reaction using the following temperature profile: 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min. All PCR products from the isolates were sequenced. The sequence of the $bla_{\text{OXA-30}}$ gene was deposited under Genbank accession number AF255921."