C1-113
ICAAC/IDSA 2008
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Emergence of *bla*$_{CTX-M}$ among Enterobacteriaceae isolates in USA Hospitals: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: Spread of *bla*$_{CTX-M}$ among community and hospital Enterobacteriaceae (ENT) isolates have changed the ESBL scenario worldwide. Until recently the reports of isolates carrying *bla*$_{CTX-M}$ in USA were limited and this gene was considered rare. We report the escalated prevalence and distribution of *bla*$_{CTX-M}$ among ENT isolates recovered in several USA centers.

Methods: 2,843 ENT isolates were collected from 26 USA hospitals during the 2007 SENTRY Program and tested by the CLSI broth microdilution method. All strains displaying CLSI criteria for ESBL were tested for clavulanic acid inhibition with Etest strips. Isolates were further tested for *bla*$_{CTX-M}$ and other ESBL genes by multiplex PCR. Amplicons were sequenced and results analyzed.

Results: 206 (7.3%) ENT isolates displayed the CLSI criteria for ESBL, and 124 (60.6%) isolates showed clavulanate inhibition. *bla*$_{CTX-M}$ was detected in 55 (26.7% of 206) strains. These isolates were collected in 16 medical centers, located in 13 cities and 10 states (KY, NY, NJ, TX, MI, WI, MA, HI, OH, WA). *bla*$_{CTX-M-15}$ was the most prevalent (33 strains; 60.0%; 23 *E. coli*, 10 *K. pneumoniae*). *bla*$_{CTX-M-14}$ (21, 38.2%) was detected in 21 strains (3 species; *E. coli*, *K. pneumoniae*, and *P. mirabilis*) in 11 hospitals. *bla*$_{CTX-M-2}$ was found in one *E. coli* strain from Seattle (1.8%). Six medical centers had multiple *bla*$_{CTX-M}$-types. One isolate showing negative clavulanate inhibition was positive for *bla*$_{CTX-M-15}$.

Conclusions: CTX-M-15 and -14 producing isolates appear to be rapidly disseminating in USA hospitals. More than half of the hospitals had more than one *bla*$_{CTX-M}$-type, and one West Coast hospital had three *bla*$_{CTX-M}$-types detected in the study. Although CTX-M-15 was more prevalent; CTX-M-14 was detected in a greater diversity of bacterial species and hospitals. These results suggest that *bla*$_{CTX-M-14}$ is probably carried by a genetic structure with higher potential for mobilization and spread.

INTRODUCTION

Several types of acquired extended spectrum ß-lactamases (ESBLs) other than TEM and SHV types have been described in Enterobacteriaceae, including CTX-M, VEB, GES/IBC, PER and others. Among these, CTX-M-type ESBLs are by far the most successful in terms of spread, and their impact is currently comparable or even greater than that previously attributed to TEM- and SHV-enzymes.

CTX-M-type enzymes encode resistance to penicillins, oxyiminocephalosporins and monobactams, conferring cefotaxime/ceftriaxone MIC values usually higher than those for ceftazidime. The numerous CTX-M variants reported to date can be divided in five groups according to the similarity of the amino acid sequences: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25. The origin of these genes has been recently discovered and CTX-M genetic determinants apprear to have evolved from the chromosome of *Kluyvera* species, *K. ascorbata* for groups 1 and 2 and *K. georgiana* for groups 8 and 9.

CTX-M ß-lactamases are considered highly prevalent in Europe, Asia and Latin America where CTX-M carrying isolates have been reported for more than a decade; however, only recently have these ESBLs been detected in the United States (USA). In this study, we evaluated the prevalence of CTX-M producing isolates in USA medical centers participating in the SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Bacterial isolates. A total of 2,843 Enterobacteriaceae isolates were collected from 26 USA hospitals during the SENTRY Program (2007). Only one isolate per patient from documented infections were included in the study. Isolates were collected from bloodstream, respiratory tract and skin structures infections according to a common protocol. Species identification was confirmed by standard biochemical tests and the Vitek System (bioMerieux, Hazelwood, MO), when necessary.

Antimicrobial susceptibility testing. All isolates were tested for antimicrobial susceptibility using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS). Cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). Categorical interpretations for all antimicrobials were those found in M100-S18 and quality control (QC) was performed using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents.

ESBL confirmatory test. Enterobacteriaceae isolates displaying the CLSI MIC criteria for ESBL production were confirmed with the clavulanate inhibition Etest (AB BIODISK, Solna, Sweden). Isolates showing elevated carbapenem MIC values were not included in the study analysis, probable serine-carbapenemases.

Genotypic detection of resistance and sequencing analysis. Custom *bla*$_{CTX-M}$ primers (CTX-MGenF 5'-CAG CAC CAG TAA RGT KAT-3' and CTX-MGenR 5'-GG CAC CGC TGC CGG TRT TAT C-3') were designed based on alignments of multiple sequences of CTX-M encoding genes obtained from genetic databases. These primers were used, combined with an internal control set of primers for 16S ribosomal RNA gene. PCR amplicons were sequenced on both strands and the nucleotide sequences and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI). Sequences were compared to others available via internet sources (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS

- Among 206 Enterobacteriaceae isolates displaying the CLSI MIC criteria for ESBL collected in USA during 2007, 124 (60.6%) isolates showed positive results by the clavulanate inhibition test. All 206 isolates were tested for the presence of *bla*$_{CTX-M}$, which was detected in 55 (26.7%) strains.

Table 1. Occurrence of CTX-M-types among different bacterial species collected during 2007 in USA medical centers as part of the SENTRY Program.

Bacterial Species (no. of isolates)	CTX-M-type (no. of isolates)
Escherichia coli (39)	CTX-M-15 (23)
	CTX-M-14 (15)
	CTX-M-2 (1)
Klebsiella pneumoniae (15)	CTX-M-15 (10)
	CTX-M-14 (5)
Proteus mirabilis (1)	CTX-M-14 (1)

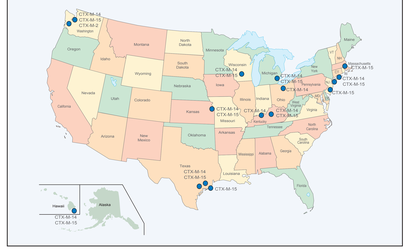
- *bla*$_{CTX-M}$ was dominantly detected among *E. coli* strains (39). However, *Klebsiella pneumoniae* and *Proteus mirabilis* were also observed to harbor these ESBL genes (Table 1).
- CTX-M-producing strains were recovered in 16 of the 26 (61.5%) USA medical centers evaluated. These institutions were located in 13 cities (11 states; Figure 1).
- Although more frequently detected, *bla*$_{CTX-M-15}$ (60.0%) was restricted to *E. coli* and *K. pneumoniae* (23 and 10 strains respectively). In contrast, *bla*$_{CTX-M-14}$ was detected in 21 strains of 3 different species (*E. coli*, *K. pneumoniae* and *P. mirabilis*).
- CTX-M-15-producing strains were detected in 13 hospitals while CTX-M-14 was detected in 11 medical centers. Eight institutions had multiple *bla*$_{CTX-M}$-types.
- One isolate showing negative clavulanate inhibition test was positive for *bla*$_{CTX-M-15}$.
- CTX-M-producing isolates showed higher resistance rates to fluoroquinolones (levofloxacin; 78.0%) and tetracycline (76.3%) when compared to other strains displaying the CLSI ESBL criteria but not possessing these enzymes (44.3 and 29.9%, respectively; Table 2).
- CTX-M producing isolates had lower MIC values for the combination piperacillin/tazobactam (MIC$_{50}$, 8 µg/ml; 67.8% susceptible), compared to the non-CTX-M ESBL strains (MIC$_{50}$, 64 µg/ml; 35.7% susceptible Table 2).

Table 2. Comparison of in vitro activity of selected antimicrobial agents tested against 206 Enterobacteriaceae strains collected in USA medical centers in 2007 during the SENTRY Program.

	CTX-M-producing isolates (55 isolates)			non-CTX-M ESBL positive isolates[a] (70 isolates)			ESBL negative isolates[b] (81 isolates)		
Antimicrobial agent	MIC$_{50}$	MIC$_{90}$	% susceptible/resistant	MIC$_{50}$	MIC$_{90}$	% susceptible/resistant	MIC$_{50}$	MIC$_{90}$	% susceptible/resistant
Ceftriaxone	>32	>32	6.8 / 86.4	16	>32	22.9 / 24.3	32	>32	36.4 / 32.5
Ceftazidime	16	>16	44.1 / 39.0	>16	>16	25.7 / 67.1	>16	>16	40.3 / 51.9
Cefepime	>16	>16	37.3 / 52.5	2	16	87.1 / 5.7	2	16	81.8 / 7.8
Cefoxitin	8	>16	67.8 / 11.9	8	>16	52.9 / 30.0	>16	>16	7.8 / 83.1
Piperacillin/tazobactam	8	>64	67.8 / 18.6	64	>64	35.7 / 48.6	32	>64	41.6 / 40.3
Amikacin	2	16	94.9 / 3.4	1	16	91.4 / 0.0	1	8	93.5 / 0.0
Gentamicin	≤2	>8	64.4 / 33.9	8	>8	48.6 / 34.3	≤2	>8	61.0 / 26.0
Levofloxacin	>4	>4	22.0 / 78.0	4	>4	47.1 / 44.3	>4	>4	48.1 / 50.6
Tetracycline	>8	>8	22.0 / 76.3	4	>8	64.3 / 22.9	8	>8	45.5 / 39.0
Trimethoprim/sulfamethoxazole	>2	>2	27.1 / 72.9	>2	>2	34.3 / 65.7	>2	>2	41.6 / 58.4

a. Isolates showing CLSI MIC ESBL criteria and positive confirmatory clavulanate inhibition test.
b. Isolates showing CLSI MIC ESBL criteria and negative confirmatory clavulanate inhibition test.

Figure 1. Distribution of the CTX-M-producing isolates in 13 cities among participating USA medical centers in the SENTRY Program (2007).



CONCLUSIONS

- CTX-M-14 and CTX-M-15 appear to be rapidly disseminating among USA hospitals.
- CTX-M-encoding genes were recently found to be more common among isolates of the *E. coli* ST131 clone. Strains demonstrating this multilocus sequence typing (MLST) profile, similar to the isolates tested in this study, were shown to have elevated fluoroquinolone MIC values. The correlation among *E. coli* ST131, resistance to fluoroquinolones and the presence of CTX-M enzymes should be further investigated.
- Overall, this study highlights the increasing prevalence of CTX-M-producing strains that were previously considered rare in the USA. These isolates seem to be emerging as an important and prevalent resistance mechanism among Enterobacteriaceae isolates in USA hospitals.

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