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RAPID EMERGENCE AND DISSEMINATION OF *bla*$_{CTX-M}$ AMONG ENTEROBACTERIACEAE IN USA MEDICAL CENTERS: REPORT FROM THE MYSTIC PROGRAM (2007)

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MYSTIC
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AMENDED ABSTRACT

Objective: To characterize extended-spectrum beta-lactamase (ESBL)- and plasmid-mediated AmpC (pAmpC)-encoding genes among Enterobacteriaceae (ENT) isolates collected from USA medical centers, and to identify plasmid-mediated fluoroquinolone resistance (pFQ-R)-encoding genes among ß-lactamase-producing isolates.

Methods: 1,392 ENT isolates were collected from 15 USA medical centers through the MYSTIC Program during 2007 and tested by the CLSI broth microdilution method. ESBL-positive (clavulanate inhibited) strains were tested for the presence of PER, GES, VEB, CTX-M and OXA enzymes with a multiplex PCR approach. ESBL screen positive but not confirmed isolates were screened for pAmpC genes. Isolates were also tested for *qnr*-type, *qepA* and *aac(6')*-Ib-cr. Primers annealing on genetic structures flanking the resistance encoding genes were used to obtain complete DNA sequences. Amplicons were sequenced in both strands and results analyzed.

Results: Among 70 (5.0% of the total) ENT isolates with an ESBL phenotype, *bla*$_{CTX-M}$ was detected in 28 (38.8%; 25 *E. coli*, 2 *K. pneumoniae* and 1 *P. vulgaris*). CTX-M-15 (17 strains, 60.7%) and CTX-M-14 (10, 35.7%) were most prevalent; CTX-M-3 was observed in one strain (3.6%). These isolates were collected in 12 centers (80.0% of the participating sites). OXA-encoding genes with ESBL spectrums were identified in 9 isolates, one co-producing CTX-M. Six isolates harbored *bla*$_{CMY-2}$ (5 *E. coli* and 1 *K. pneumoniae*), while 4 isolates carried *bla*$_{FOX-5}$ (3 *K. pneumoniae* and 1 *K. oxytoca*). Nine ESBL-positive (9.8%) isolates carried *qnr* genes (2 *qnrA*, 6 *qnrB* and 1 *qnrS*). The *qnr* genes were found in one isolate carrying each of the beta-lactamases: *bla*$_{FOX-5}$, *bla*$_{CMY-2}$, *bla*$_{CTX-M-15}$, *bla*$_{OXA-2}$ and *bla*$_{OXA-10}$. Only one (1.1%) CTX-M-producing strain carried *aac(6')*-Ib-cr. This isolate harbored *bla*$_{OXA-10}$ and *bla*$_{CTX-M-14}$. Meropenem was active against all *bla*$_{CTX-M}$ and ESBL-producing strains.

Conclusions: For over a decade CTX-M-producing isolates have been reported as the highly prevalent ESBL resistance mechanism in Europe and Asia. In contrast, these enzymes were considered unusual in the USA. In this 2007 collection of ENT from USA medical centers, at least 2 distinct CTX-M-type enzymes have disseminated among different species and institutions. In addition, the prevalence of pFQ-R encoding genes among beta-lactamase-producing isolates was higher than that observed in ENT not carrying these resistance determinants.

INTRODUCTION

The increasing number of CTX-M extended spectrum ß-lactamase (ESBL) types and recognition of multiple clones carrying these enzymes has increased the complexity of the ESBL-threat in numerous geographic locations. In recent years, CTX-M has become the most prevalent ESBL globally in both community and hospital settings. Moreover, plasmid-mediated cephalosporinase (pAmpC) enzymes have also arisen through the mobilization of chromosomal genes of inducible AmpC ß-lactamases onto plasmids. When transferred into other organisms such as *Escherichia coli and Klebsiella pneumoniae,* these cephalosporinases have similar substrate profiles to the parent chromosomal enzymes but differ in having constitutively expressed enzyme activity. Failure to differentiate these enzymes from ESBLs by routine in vitro susceptibility test methods has contributed to their uncontrolled spread and associated therapeutic failures.

During the MYSTIC Program in 2007, a total of 92 isolates collected from United States (USA) medical centers displaying MIC criteria for ESBL production were selected for further molecular characterization. These isolates were screened for the presence of ESBL and pAmpC mechanisms. In addition, these isolates were also screened for transferable quinolone resistance genes to evaluate their association with troublesome ß-lactamase producing strains.

MATERIALS AND METHODS

Bacterial isolates. A total of 1,392 Enterobacteriaceae isolates collected from 15 medical centers geographically dispersed across the USA were evaluated during the MYSTIC Program 2007. These strains were recovered from serious infections in hospitalized patients and each participating institution contributed 200 isolates, including Gram-positive and Gram-negative species. Only one isolate per patient from documented infections were included in the study. 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 *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI documents. Enterobacteriaceae isolates displaying the CLSI criteria for ESBL production were confirmed with the clavulanate inhibition Etest (AB BIODISK, Solna, Sweden).

Genotypic detection of resistance. A multiplex PCR approach to detect genes encoding ESBL types was developed for this study. Custom primers designed for PER, GES, VEB, CTX-M and oxacillinases (OXA-ESBL) were used combined with an internal control set of primers. A multiplex PCR protocol was also used to detect pAmpC, as previously described. Isolates were also screened for the plasmid encoded quinolone resistance genes: *qnrA*, *qnrB*, *qnrS*, *qepA* and *aac(6')*-Ib-cr.

Sequencing Analysis. Primers annealing to genetic structures flanking the resistance encoding genes were used to obtain complete DNA sequences. 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

- The presence of ESBL phenotypes was confirmed using clavulanate inhibition in 70 of 92 (76.1%; 5.0% overall Enterobacteriaceae) strains displaying the CLSI criteria for ESBL production.
- *bla*$_{CTX-M}$ was detected in 28 of 70 (38.8%) ESBL confirmed strains including: 25 *E. coli*, 2 *K. pneumoniae* and 1 *Proteus vulgaris* (Table 1); isolates were collected from 12 of 15 (80.0%) medical centers participating in the MYSTIC Program (2007).
- Among CTX-M-producers, 17 strains (9 medical centers) produced CTX-M-15 (60.7%) and 10 strains (8 medical centers) produced CTX-M-14 (35.7%). CTX-M-3 was observed in only one (3.6%) strain (Table 1).
- Oxacillinase encoding genes were identified in 9 isolates (12.8% of ESBL-producers). OXA-2 was detected in 7 isolates and OXA-10 was detected in 2 strains, one of them co-producing CTX-M-14.
- pAmpC genes were detected in 10 of the 22 (45.5%) isolates showing negative clavulanate inhibition: 6 isolates carried *bla*$_{CMY-2}$, and 4 harbored *bla*$_{FOX-5}$.
- Nine (9.8% of 92 strains) *qnr*-carrying isolates were observed among the ß-lactamase-producing strains and three types of *qnr* genes were identified: *qnrA*, *qnrB* and *qnrS* (Table 2).
- *aac(6')*-Ib-cr, the plasmid encoded fluoroquinolone and aminoglycoside modifying gene, was found in one isolate harboring both *bla*$_{CTX-M-14}$ and *bla*$_{OXA-10}$ (Table 2); *qepA*-carrying strains were not detected in this study.

Table 1. Distribution of the characterized ß-lactamases in USA medical centers (MYSTIC Program, 2007).

ß-lactamase (no. of isolates)	Medical center location (no. of isolates)	Bacterial species (no. of isolates)
CTX-M-15 (17)	Texas (4) Washington (3) California (3) Nebraska (2) New York (1) Ohio (1) Hawaii (1) New Jersey(1) Utah (1)	*E. coli* (16), *K. pneumoniae* (1)
CTX-M-14 (10)	Hawaii (2) California (2) Colorado (1) Kentucky (1) New York (1) Arkansas (1) Washington (1) Ohio (1)	*E. coli* (8), *K. pneumoniae* (1), *P. vulgaris* (1)
CTX-M-3 (1)	Washington (1)	*E. coli* (1)
CMY-2 (6)	Iowa (2) Texas (1) New York (1) Louisiana (1) California (1)	*E. coli* (5), *K. pneumoniae* (1)
FOX-5 (4)	Arkansas (1) Louisiana (1) Iowa (1) New Jersey (1)	*K. pneumoniae* (3), *K. oxytoca* (1)
OXA-2 (7)	Iowa (4) New Jersey (2) Colorado (1)	*K. oxytoca* (5)[a], *E. cloacae* (1), *K. pneumoniae* (1)
OXA-10 (2)	New York (1) California (1)	*K. pneumoniae* (2)

a. Clonal dissemination within medical centers were identified.

Table 2. Presence of transferable quinolone-resistance genes among Enterobacteriaceae strains from USA medical centers and associated ß-lactamases (MYSTIC Program, 2007).

Plasmid-mediated fluoroquinolone resistance gene (no. of isolates)[a]		Bacterial species	ß-lactamase
qnrA (2)	*qnrA1*	*K. pneumoniae*	FOX-5
	qnrA1	*K. pneumoniae*	ESBL positive[b]
qnrB (6)	*qnrB2*	*E. coli*	CTX-M-15
	qnrB2	*E. cloacae*	OXA-2
	qnrB2	*E. aerogenes*	ESBL positive[b]
	qnrB2	*K. oxytoca*	ESBL positive[b]
	qnrB4	*K. pneumoniae*	OXA-10
	qnrB5	*K. pneumoniae*	ESBL positive[b]
qnrS (1)	*qnrS1*	*K. pneumoniae*	CMY-2
aacA(6')-Ib-cr (1)		*E. coli*	CTX-M-14 and OXA-10

a. *qepA* was screened, but no PCR-positive strains were observed.
b. ESBL positive: isolates displaying the CLSI criteria for ESBL production and showing inhibition with clavulanate.

CONCLUSIONS

- CTX-M-encoding genes, previously considered rare in the USA, were detected in nearly 40% of ESBL positive isolates and were observed in 80% of the participating medical centers.
- The prevalence of *qnr* genes among ß-lactamase-producing strains was higher in the present study when compared to reports for collections showing elevated fluoroquinolone MIC values (9.8% versus 0.5 to 2.0%).
- Overall, ß-lactamase (ESBL and pAmpC)-mediated resistance in the USA MYSTIC Program (2007) was not as alarming as observed in other geographic regions, such as Europe, Latin America and Asia (data not shown).
- Continued surveillance of the MYSTIC Program sites having recent CTX-M emergence appears prudent along with local infection control interventions to limit the dissemination of these worrisome pathogens and their resistance mechanisms.

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