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Prevalence of ß-lactamase-encoding Genes in *Escherichia coli* Strains Collected in the USA: Report of the SENTRY Antimicrobial Susceptibility Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: Multidrug-resistant *E. coli* are of great concern in both the nosocomial and community setting. Acquired ß-lactamase-encoding genes are often associated with resistance to aminoglycosides, fluoroquinolones, and other antimicrobial agents, limiting the treatment options for infections caused by organisms carrying these resistance determinants. We evaluated the distribution of ß-lactamases present among 60 ESBL-phenotype *E. coli* isolates from USA hospitals during 2011.

Methods: Among 1,680 *E. coli* isolates that were susceptibility tested by reference broth microdilution methods during 2011, 60 ESBL-phenotype (CLSI criteria) isolates were randomly selected and evaluated using Check-MDR CT101 kit (CP) and custom PCR reactions to detect PER, VEB, GES, PSE, OXA-2, OXA-10, OXA-18/-45, and OXA-1/30. Positive CP results were re-amplified using specific primers. PCR amplicons were sequenced.

Results: The 60 ESBL-phenotype *E. coli* isolates were from 30 USA states representing all 9 USA census regions. CTX-M-encoding genes were detected alone or combined with other ß-lactamases in 36 isolates. CTX-M-15, the dominant ESBL variant (n = 24), was identified in 21 hospitals within 15 states. Other CTX-M variants included CTX-M-14 (n = 7), CTX-M-27 (n = 4) and (new) CTX-M-134 (n = 1) that differed from CTX-M-14 by one amino acid substitution (G242S). Nine isolates carried CMY-2, including one also harboring $bla_{CTX-M-15}$ and $bla_{OXA-1/30}$. TEM-12 and TEM-19 (both, group 2be) were also detected. Two isolates were resistant to imipenem and carried KPC-2 or -3. These isolates were collected in different hospitals from New York. The KPC-2-producer also carried OXA-10, the KPC-3-producer OXA-1/30 and TEM-1. Ten isolates were negative for ESBL genes (five carried TEM-1 and/or SHV-1); for eight of these only one of the ESBL screening markers (ceftriaxone, ceftazidime, and aztreonam) was slightly elevated (MIC, ≤4 µg/ml). Nine different combinations of ß-lactamases were detected, among which CTX-M-15 + OXA-1/30 was most common (n = 11).

Conclusions: CTX-M-producing *E. coli* now predominate among ESBL producing *E. coli* in the USA, as in other geographic regions. Further study is needed to identify the virulence genotypes and genomic profiles of these isolates, which display a variety of enzymes and combinations of ß-lactamases.

INTRODUCTION

Extended-spectrum ß-lactamases (ESBLs) are plasmid-mediated resistance determinants that confer elevated MIC values to penicillins, cephalosporins and aztreonam. The genes encoding these enzymes are often encountered among members of the Enterobacteriaceae family and strains carrying ESBL-encoding genes are frequently resistant to other antimicrobial agents, such as trimethoprim/sulfamethoxazole, aminoglycosides, tetracyclines and fluoroquinolones. Among ESBLs, CTX-M enzymes with or without other ß-lactamases are very prevalent in various Enterobacteriaceae species, but mainly *Escherichia coli* and our collaborative investigations show that the majority of the CTX-M-15-producing *E. coli* strains from our past surveys were ST131 that represent a clinical threat due to its potential associated virulence.

In this study, we evaluated the presence of ESBLs, acquired cephalosporinases (plasmid-mediated AmpC), other broad-spectrum ß-lactamases and carbapenemases among 60 ESBL-phenotype *E. coli* collected from clinical specimens in United States (USA) hospitals during 2011 as part of the SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Bacterial isolates and antimicrobial susceptibility testing. Sixty *E. coli* isolates were selected from 49 hospitals located in 30 USA states during 2011 as part of the SENTRY Program. One or two initial isolates per hospital were tested that met the Clinical and Laboratory Standards Institute (CLSI) ESBL criteria. All isolates were susceptibility tested using the broth microdilution method as described by the CLSI document M07-A9. Categorical interpretations for all antimicrobials were those found in M100-S23 (2013) and quality control (QC) was performed using *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within specified ranges as published in CLSI M100-S23 (2013) document.

Genotypic detection of ß-lactamases. Isolates were initially screened using the Check-MDR CT101 kit (Check-points, Wageningen, Netherlands). The assay was performed according to the manufacturer's instructions. This kit has the capabilities to detect CTX-M Groups 1, 2, 8+25 and 9, TEM wild-type (WT) and ESBL, SHV WT and ESBL, ACC, ACT/MIR, CMYII, DHA, FOX, KPC and NDM-1. PCR screening was performed for bla_{GES}, bla_{VEB}, bla_{PER}, bla_{PSE} and oxacillinases with ESBL spectrum (bla_{OXA-2}-, bla_{OXA-10}- and bla_{OXA-30}-group, bla_{OXA-18} and bla_{OXA-45}). Positive microarray results were amplified using specific primers. All 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 60 ESBL-phenotype *E. coli*, CTX-M-encoding genes were detected alone or combined with other ß-lactamases in 36 isolates (60.0%; Table 1).
- CTX-M-15 was the dominant ESBL variant, being detected in 24 isolates (40.0%) that were collected from 21 hospitals located in 15 states (Table 2).
- Other CTX-M variants included CTX-M-14 (seven isolates), CTX-M-27 (four isolates) and a new variant was in a single strain (Table 1).
- The new CTX-M, CTX-M-134, differed from CTX-M-14 by one amino acid substitution (G242S; Figure 1) and the isolate carrying this enzyme was collected in Lexington, Kentucky from a 70 y/o patient that underwent gastric surgery.
- Nine isolates carried CMY-2, including one also harbouring $bla_{CTX-M-15}$ and $bla_{OXA-1/30}$. TEM-12 and TEM-19 (both, ESBLs/group 2be) were also detected.
- Two isolates were resistant to imipenem (MIC, >2 µg/ml) and carried KPC-2 and OXA-10 or KPC-3-with OXA-1/30 and TEM-1. These isolates were collected in New York hospitals.
- Ten isolates were negative for ESBL/carbapenemase genes and five carried TEM-1 and/or SHV-1; for eight of these only one of the ESBL screening markers (ceftriaxone, ceftazidime, and aztreonam) was slightly elevated (MIC, ≤4 µg/ml) eg. false-positive.
- Nine different combinations of ß-lactamases were detected, among which CTX-M-15 + OXA-1/30 was most common (11 strains; Table 1).

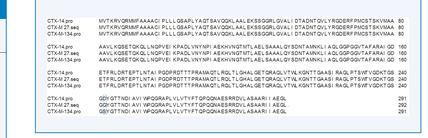
Table 1. ß-lactamases and combinations detected among *E. coli* isolates collected in USA hospitals.

ß-Lactamases/combinations	No. of isolates
CTX-M-15-like, OXA-1/30	11
CMY-2, TEM-1	6
CTX-M-15-like, TEM-1	6
CTX-M-14, TEM-1	4
CTX-M-15-like, OXA-1/30, TEM-1	4
CTX-M-27	4
TEM-1	4
Negative	4
CTX-M-14	3
CMY-2	2
CTX-M-15-like	2
OXA-1/30	2
CMY-2, CTX-M-15-like, OXA-1/30	1
CTX-M-134	1
KPC-2, OXA-10	1
KPC-3, OXA-1/30, TEM-1	1
OXA-1/30, TEM-19	1
SHV-1	1
SHV-1, TEM-1	1
TEM-12	1

Table 2. Distribution of CTX-M-15 positive *E. coli* in USA states.

	No. by State:															
ß-Lactamases/combinations	CA	FL	IA	IL	IN	MA	MI	MN	NC	NJ	OR	PA	TX	VA	WI	Total
All CTX-M-15-positive	4	1	1	2	1	1	2	1	1	2	1	2	2	1	1	23
CTX-M-15-like		1							1							2
CTX-M-15-like, OXA-1/30	2			2	1			1		1			2	1	1	11
CTX-M-15-like, OXA-1/30, TEM-1	2									1		1				4
CTX-M-15-like, TEM-1			1			1	2				1	1				6

Figure 1. Protein alignment of CTX-M-134 (new ESBL discussed in this survey) compared with closest ancestors CTX-M-14 and CTX-M-27.



CONCLUSION

- An increasing prevalence of CTX-M-producing *E. coli* isolates have been detected in USA hospitals. These strains, that in several instances belong to sequence type (ST) 131, generally harbor virulence genes and can lead to severe infections.
- Surveillance and further genetic analysis of these USA isolates must be performed regularly to guide interventions and antimicrobial stewardship.

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