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Changing prevalence of *Escherichia coli* with CTX-M–type extended-spectrum β-lactamases in outpatient urinary *E. coli* between 2003 and 2008

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Abstract

One hundred ninety-three single-patient isolates of *Escherichia coli* harboring extended-spectrum β-lactamases (ESBL) were identified among 11 407 *E. coli* urine isolates recovered from single-patient outpatient urine cultures from 2003 to 2008. The percentage of ESBL-producing *E. coli* among community-onset *E. coli* urine isolates increased from 0.21% in 2003 to 2.99% in 2008. One hundred seven of the ESBL producers were positive for the presence of $bla_{CTX\text{-}M}$ genes. The percentage of CTX-M–producing *E. coli* rose from 0.07% in 2003 to 1.66% in 2008. The annual percentage of ESBL *E. coli* producing CTX-Ms changed from 35% in 2003 to 64% in 2008. Genes belonging to 3 $bla_{CTX\text{-}M}$ groups: $bla_{CTX\text{-}M\text{-}1}$ group, $bla_{CTX\text{-}M\text{-}2}$ group, and $bla_{CTX\text{-}M\text{-}9}$ group, were detected. In addition, resistance to commonly used antimicrobial agents for community-acquired urinary tract infections was found common among CTX-M–producing *E. coli* isolates. Ertapenem and nitrofurantoin showed good in vitro activity against CTX-M producers.
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1. Introduction

Extended-spectrum β-lactamase (ESBL)-producing isolates are commonly resistant to the multiple antibiotics used for treating infections caused by Gram-negative bacteria (Paterson and Bonomo, 2005). Most ESBLs can be divided into 3 groups: TEM, SHV, and CTX types based on the amino acid sequence homology (Pitout and Laupland, 2008). Variants of SHV and TEM ESBLs are most commonly associated with nosocomial pathogens, such as *Klebsiella* spp, and are often recovered from respiratory tract, intra-abdominal, and bloodstream infections (Paterson, 2006). Different from the TEM and SHV types of ESBLs, CTX-M enzymes are most often carried by *Escherichia coli* associated with community-onset urinary tract infections (UTIs) (Pitout et al., 2005). The CTX-M enzymes originated from the *Kluyvera* spp. of environmental bacteria and are associated with mobile elements (Olson et al., 2005; Rodriguez et al., 2004). Mobile genetic elements directly linked to $bla_{CTX\text{-}M}$ genes include the *ISEcp*-1–like insertion sequences, the CR1 (common region 1) element, phage-related sequences, and a putative transposase (Canton and Coque, 2006). The mobile elements not only are responsible for the dissemination of $bla_{CTX\text{-}M}$ genes but also may contribute to the high-level expression of the genes by providing additional promoters (Saladin et al., 2002). Transposons and plasmid-mediated transmission of $bla_{CTX\text{-}M}$ genes among genetic distinct strains have been shown in many different regions (Canton

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and Coque, 2006). Based on the observations that *E. coli* isolates with CTX-M are not clonal and bla_{CTX-M} genes are carried on different genetic elements, spreading of CTX-Ms has been attributed to transmission of bla_{CTX-M} genes through mobile genetic elements rather than dissemination of particular clones (Lewis et al., 2007; Livermore et al., 2007).

The CTX-M β-lactamase family consists of more than 50 members and is divided into 5 groups, including CTX-M-1 group, CTX-M-2 group, CTX-M-8 group, CTX-M-9 group, and CTX-M-25 group, with members of each group sharing >94% identity, whereas members from different groups share <90% similarity (Pitout and Laupland, 2008). Most CTX-M enzymes have greater activity against cefotaxime than ceftazidime and are inhibited by β-lactamase inhibitors (Walther-Rasmussen and Hoiby, 2004). Coresistance to other classes of antimicrobial agents are common among CTX-M–producing strains (Pitout et al., 2007; Pournaras et al., 2004; Woodford et al., 2004). Like TEM and SHV types of ESBLs, which are commonly resistant to aminoglycosides, tetracycline, and sulfonamides, a large numbers of CTX-M producers also show resistance to the fluoroquinolones.

CTX-M–type ESBLs are rapidly disseminating because they are detected in every populated continent and are responsible for major outbreaks in Europe (Canton and Coque, 2006). In the United States, the emergence of CTX-M–type ESBLs was first reported in 2003 (Moland et al., 2003). CTX-Ms were detected in isolates of several Enterobacteriaceae species in the United States, including *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter cloacae*, *Morganella morganii*, *Proteus mirabilis*, and *Proteus vulgaris* (Castanheira et al., 2008; Lewis et al., 2007). Following Lewis's study, 2 other reports from the United States revealed high prevalence of CTX-Ms among the ESBL-positive isolates recovered in medical centers and the appearance of CTX-Ms in the *E. coli* isolates from the community (Castanheira et al., 2008; Hanson et al., 2008). However, the prevalence of CTX-M producers among community-onset *E. coli* causing UTIs in the United States has not been studied.

This study aims to evaluate the prevalence of CTX-M–type ESBLs among the *E. coli* isolates from patients with suspected community-onset UTIs from 2003 to 2008 and to assess the susceptibility of these isolates to common antibiotics important for treating community-acquired UTIs.

2. Materials and methods

2.1. Isolate identification and collection

Isolates of *E. coli* used for the study were selected from the frozen isolates maintained by the clinical microbiology laboratory at Northwestern Memorial Hospital (NMH) in Chicago, IL. All isolates were recovered from voided urines from patients with suspected UTIs seen in outpatient clinics and the emergency room (ER) between January 2003 and December 2008. The colony count of each *E. coli* isolate in the original specimen was $\geq 10^5$ CFU/mL. Isolates tested positive for ESBLs by Clinical and Laboratory Standards Institute (CLSI)-recommended double-disk diffusion method were collected and stored frozen. One isolate per individual patient per year was included. Species identification was performed with the Vitek 2 system (bioMérieux, Balmes-les-Grottes, France). Manual biochemical reactions were used when identification by the Vitek 2 system was inconclusive.

2.2. Phenotypic identification of ESBLs

Antimicrobial susceptibility was determined with the Vitek 2 system using the AST-GN09 card according to manufacturer's instruction and was qualitatively reported as susceptible, intermediate, or resistant according to CLSI guidelines (Institute, 2007). Isolates with elevated MIC (MIC ≥2 μg/mL) for any 1 of the 3 drugs, aztreonam, ceftazidime, and ceftriaxone, were tested for ESBLs by CLSI-recommended double-disk method with disks containing cefotaxime (30 μg), cefotaxime plus clavulanic acid (30 μg plus 10 μg), ceftazidime (30 μg), and ceftazidime plus clavulanic acid (30 μg plus 10 μg) (BD Microbiology System, Cockeysville, MD). Isolates were considered as ESBL positive when the addition of clavulanic acid resulted in a ≥5-mm increase in a zone diameter for either antimicrobial agent.

2.3. Susceptibility testing

Susceptibility of ertapenem, ciprofloxacin, trimethoprim–sulfamethoxazole (TMP–SMX), gentamicin, tetracycline, and nitrofurantoin was determined by the disk diffusion method in accordance with CLSI standards using the disks obtained from BD Microbiology System (Institute, 2006).

2.4. Detection of bla_{CTX-M} genes by polymerase chain reaction

To prepare DNA template for polymerase chain reaction (PCR), 2 to 3 bacterial colonies were suspended in 50 μL of water, boiled for 5 min, and centrifuged. One microliter of the template DNA was used for 50-μL PCR reaction. PANCTX-M.F and PANCTX-M.R were used to detect for the presence of bla_{CTX-M} genes. Group-specific primers were used for identifying the bla_{CTX-M} genes to the group level (Table 1). Previous studies suggested that members of CTX-M belonging to CTX-M-1 and CTX-M-9 are the predominant CTX-Ms in United States. In our study subgrouping of bla_{CTX-M} genes was first performed with primers specific for $bla_{CTX-M-9}$ genes. Isolates negative for $bla_{CTX-M-9}$ genes were further tested with primers specific for $bla_{CTX-M-1}$ genes. Only the isolates negative for both were tested with primers targeting CTX-M-2 group and CTX-M-8 group. PCR product from 5 isolates was sequenced to confirm the specificity of the PCR reaction. PCR amplification was performed with GeneAmp rTthPCR kit (Applied Biosytems,

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