CTX-M β-Lactamase–producing Klebsiella pneumoniae in Suburban New York, New York, USA

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CTX-M extended-spectrum β-lactamase (ESBL)–producing Klebsiella pneumoniae isolates are infrequently reported in the United States. In this study, we analyzed non-duplicate ESBL-producing K. pneumoniae and Escherichia coli clinical isolates collected during 2005–2012 at a tertiary care medical center in suburban New York City, USA, for the presence of blaCTX-M, blaSHV, blaTEM, and blabpo genes. Despite a high prevalence of blaCTX-M genes in ESBL-producing E. coli since 2005, blaCTX-M genes were not detected in K. pneumoniae until 2009. The prevalence of CTX-M–producing K. pneumoniae increased significantly over time from 1.7% during 2005–2009 to 26.4% during 2010–2012 (p<0.0001). CTX-M-15 was the dominant CTX-M genotype. Pulsed-field gel electrophoresis and multilocus sequence typing revealed high genetic heterogeneities in CTX-M–producing K. pneumoniae isolates. This study demonstrates the recent emergence and polyclonal spread of multidrug-resistant CTX-M–producing K. pneumoniae isolates among patients in a hospital setting in the United States.

CTX-M enzymes are a group of class A extended-spectrum β-lactamases (ESBLs) that are rapidly spreading among Enterobacteriaceae worldwide (1). Since the initial isolation of CTX-M-1 from a European patient in the late 1980s (2), >130 CTX-M allelic variants have been described (http://www.lahey.org/Studies/other.asp#table1). These CTX-M variants have been divided into 5 major phylogenetic groups, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, or CTX-M-25 on the basis of their amino acid sequences (I,2).

During the past decade, CTX-M enzymes have become the most prevalent ESBL enzymes in clinical Enterobacteriaceae isolates, especially in ESBL-producing Escherichia coli in Europe, Asia, and South America (1,3). By contrast, SHV- and TEM-type ESBL enzymes are primarily found in ESBL-producing K. pneumoniae and E. coli clinical isolates in North America (3). In the United States, CTX-M–like ESBL-producing Enterobacteriaceae was first reported in 2003, when CTX-M enzymes were detected in 9 E. coli clinical isolates from 5 US states (4). The spread of CTX-M type ESBL in Enterobacteriaceae, however, was not appreciated until 2007 when a Texas study showed a high prevalence of CTX-M ESBL in E. coli clinical isolates recovered during 2000–2005 (5). Since then, CTX-M–producing E. coli isolates have been documented in dispersed US geographic regions (3,6,7). CTX-M enzymes are now the predominant ESBL type in E. coli clinical isolates in Texas (5), Pennsylvania (6), Illinois (8), and New York (9,10).

CTX-M–type ESBL enzymes have also been reported in the United States in some non–E. coli Enterobacteriaceae species, such as Klebsiella spp. (5,11,12), Proteus mirabilis (5,11), Enterobacter spp. (5), Salmonella spp. (13), Shigella spp. (14), and Morganella morganii (5). Nevertheless, CTX-M–type ESBL have been principally detected and reported in E. coli clinical isolates. To date, <50 CTX-M–producing K. pneumoniae isolates have been described in the United States, and the epidemiologic and microbiological data provided have been limited (5,11,12,15–18). The implications of CTX-M–producing K. pneumoniae for laboratory detection, patient care, and public health in the United States remain to be elucidated.

In this study, we investigated the prevalence of SHV-, TEM-, and CTX-M–encoding genes in a large collection of ESBL-producing K. pneumoniae and E. coli clinical isolates from a tertiary care medical center in suburban New York City in Westchester County, New York, over an

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of CTX-M ESBL-producing \textit{K. pneumoniae} isolates were examined, and certain clinical/epidemiologic features of patients with these isolates were analyzed.

**Materials and Methods**

**Bacterial Isolates and Phenotypic Detection of ESBLs**

Nonduplicate \textit{K. pneumoniae} clinical isolates were recovered from patient specimens during January 2005–July 2012 at the clinical microbiology laboratory of Westchester Medical Center. These included 208 \textit{bla}_{KPC}-negative non-\textit{K. pneumoniae} carbapenemase (non-KPC) ESBL-producing or third-generation cephalosporin-resistant \textit{K. pneumoniae} isolates and 228 KPC (\textit{bla}_{KPC}-positive)–producing \textit{K. pneumoniae} isolates. In addition, 163 nonduplicate ESBL-producing \textit{E. coli} clinical isolates from the same period were also analyzed for comparison. Isolates were randomly selected to span the entire study year with an approximately equal number of isolates per quarter; only 1 isolate from each patient was chosen and tested. The center is a 643-bed academic tertiary-care medical center in Westchester County, New York. The Institutional Review Board of New York Medical College approved this study.

The bacterial isolates were identified and evaluated for antimicrobial drug susceptibility with the MicroScan WalkAway 96 system (Siemens, Sacramento, CA, USA). ESBL production was phenotypically confirmed by a double-disc or broth microdilution method for suspected ESBL isolates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (20). The antimicrobial drug susceptibility of CTX-M–producing \textit{K. pneumoniae} isolates against selected antimicrobial drugs was also assessed with standardized CLSI disk diffusion and Etest methods. Bacterial isolates were refrigerated on nutrient agar slants or were frozen (-80°C) in MicroBank cryovials containing 20% glycerol (Pro-Lab Diagnostics, Round Rock, TX, USA). For antimicrobial drug susceptibility testing of frozen isolates, fresh subcultures were used from CLSI guidelines.

**PCR Detection of \textit{bla}_{CTX-M}, \textit{bla}_{SHV}, \textit{bla}_{TEM}, \textit{bla}_{KPC} Genes**

For PCR, bacterial genomic DNA was extracted directly from colonies on nutrient slants or from fresh subcultures grown on Trypticase soy agar with 5% sheep blood (TSA II, BBL, Sparks, MD, USA) by boiling a dense suspension of an approximately no. 1 McFarland standard in sterile distilled water. As the DNA template in the PCR assays, 2–3 μL of the boiled cell suspension was used. PCR amplification of \textit{bla}_{CTX-M}, \textit{bla}_{SHV}, \textit{bla}_{TEM}, and \textit{bla}_{KPC} genes in \textit{K. pneumoniae} and \textit{E. coli} clinical isolates was performed by using a consensus primer pair specific to each type of β-lactamase as described (20–22). A multiplex PCR was developed and used for simultaneous detection of \textit{bla}_{CTX-M} (551 bp) and \textit{bla}_{TEM} (972 bp) genes. Two PCRs were performed for \textit{bla}_{SHV}-ESBL and \textit{bla}_{KPC}, respectively. PCRs were carried out by using the HotStart DNA polymerase master mix (QIA-GEN, Germantown, MD, USA) with 30–35 cycles at an annealing temperature of 50°C for \textit{bla}_{CTX-M}, and 52°C for \textit{bla}_{SHV} and \textit{bla}_{KPC}. PCR products were analyzed by agarose gel electrophoresis or by using the QIAxcel system (QIAGEN). The specificity of PCR amplicons on representative isolates was confirmed by DNA sequencing.

**DNA Sequencing**

For DNA sequencing, PCR products were purified by using the PCR Purification kit (QIAGEN) or the ExoSAP-IT PCR Clean-up kit (Affymetrix, Cleveland, OH, USA), according to the manufacturer’s instructions. The purified DNA amplicons were sequenced by using an ABI Prism BigDye Terminator (version 1.1) cycle sequencing ready reaction kit on the ABI Prism 3730xl or ABI 3500xl DNA Analyzers (Applied Biosystems, Foster City, CA, USA) in-house, or by a commercial facility (GeneWiz, South Plainfield, NJ, USA). The CTX-M, TEM, and SHV gene sequences were compared with sequences in GenBank by using the NCBI basic local alignment search tool (www.ncbi.nlm.nih.gov/BLAST).

**Multilocus Sequence Typing**

Multilocus sequence typing (MLST) was performed by using primers and conditions as described by Diancourt et al. (23). PCR products from MLST were sequenced as described above. Allelic profiling and sequence types (STs) were determined by querying the \textit{K. pneumoniae} MLST database maintained by the Pasteur Institute (www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).

**Pulsed-field Gel Electrophoresis (PFGE)**

Pulsed-field gel electrophoresis (PFGE) on CTX-M ESBL–producing \textit{K. pneumoniae} isolates representing each CTX-M genotype was performed as described (24). The GelCompare II software, (version 2.0; Applied Maths, Austin, TX, USA) was used to calculate the Dice similarity coefficients and generate dendrograms by cluster analysis with the unweighted-pair group method using average linkages. Pulsoftype designations were assigned at the ≥80% profile similarity level.

**Results**

**CTX-M in ESBL-producing, non-KPC \textit{K. pneumoniae} Clinical Isolates**

Of the 121 ESBL–producing \textit{K. pneumoniae} isolates originally recovered during 2005–2009, \textit{bla}_{SHV} and \textit{bla}_{TEM} genes were detected in 102 (84.3%) and 61 (50.4%) of 121 isolates, respectively (Table 1). Overall, 25 CTX-M-type
ESBL *K. pneumoniae* were identified. However, none of the 81 *K. pneumoniae* isolates from 2005 through 2008 was positive for $bla_{CTX-M}$ genes. CTX-M–type ESBL was first detected in 2 (5.0%) of 40 *K. pneumoniae* isolates from 2009. The prevalence of *K. pneumoniae* isolates carrying the CTX-M–encoding genes increased to 6 (17.6%) of 34 in 2010 and 12 (34.3%) of 35 in 2011. The level remained high (27.8%, 5/18) in the first 7 months of 2012. Overall, only 2 (1.7%) of 121 ESBL-producing *K. pneumoniae* isolates from 2005 through 2009 carried the $bla_{CTX-M}$ genes, compared with 23 (26.4%) of 87 isolates from 2010 through 2012 ($p<0.0001$, Fisher exact test), indicating the rapid emergence and spread of CTX-M enzymes among ESBL-producing *K. pneumoniae* clinical isolates since 2009.

**CTX-M in ESBL-producing *E. coli* Clinical Isolates**

One hundred sixty-three ESBL-producing *E. coli* clinical isolates from 2005 through 2012 were analyzed by PCR for detection of $bla_{ESBL}$ genes of the SHV, TEM, and CTX-M types (Table 2). Unlike the situation with *K. pneumoniae*, $bla_{CTX-M}$ genes were detected in ESBL-producing *E. coli* isolated as early as 2005. Overall, 89 (54.6%) of 163 ESBL *E. coli* isolates from the 8-year period carried $bla_{CTX-M}$ genes. CTX-M was the leading ESBL type in all years examined except 2008. The $bla_{CTX-M}$ genes from 47 (52.8%) of 89 CTX-M–producing *E. coli* isolates were sequenced. CTX-M-15 was determined in 45 (95.7%) of 47 CTX-M–producing *E. coli* isolates analyzed. CTX-M-1 and CTX-M-3 genotypes were each found in 1 ESBL *E. coli* isolate.

**CTX-M in KPC-producing *K. pneumoniae* Clinical Isolates**

Two hundred twenty-eight KPC-producing *K. pneumoniae* isolates from 2005 to 2012 were examined by PCR for detection of $bla_{KPC}$ gene. All *K. pneumoniae* isolates were positive for the $bla_{KPC}$ gene by PCR as described (22). None was positive for the $bla_{CTX-M}$ gene.

**Clinical and Microbiological Characteristics of CTX-M–producing *K. pneumoniae***

Selected clinical/epidemiologic features of the 25 patients with CTX-M–producing *K. pneumoniae* and certain microbiological characteristics of the isolates are shown in Table 3, Appendix (wwwnc.cdc.gov/EID/article/19/11/12-1470-T3.htm). Mean patient age was 56 years, and 13 (52%) of the patients were male. Sixteen patients (64%) had bacteriuria. CTX-M–producing *K. pneumoniae* isolates were recovered from 13 (52%) patients within 72 hours of hospital admission; however, 18 (72%) of these patients had been hospitalized in the 8 months before the current admission.

The $bla_{CTX-M}$ genes from all 25 CTX-M–producing *K. pneumoniae* isolates from 2009 through 2012 were sequenced. CTX-M-15 was identified in 19 (76.0%) and was the dominant CTX-M genotype. The remaining 6 isolates were determined to be CTX-M-3 (n = 4), CTX-M-1 (n = 1), and CTX-M-2 (n = 1), respectively. Twenty-four (96.0%) had coexisting $bla_{SHV}$ β-lactamases, which were predominantly non-ESBL $bla_{SHV-11}$ (n = 15) and $bla_{SHV-1}$ (n = 5) Four additional *K. pneumoniae* carried ESBL-type $bla_{SHV}$ β-lactamases, including $bla_{SHV-12}$ (n = 1), $bla_{SHV-21}$ (n = 1), and $bla_{SHV-22}$ (n = 2). Seventeen (68.0%) were positive for TEM-type β-lactamases, and all were confirmed to be $bla_{TEM-1}$.

The antimicrobial drug susceptibilities of CTX-M–producing *K. pneumoniae* isolates are summarized in Table 4. Of the 25 CTX-M–producing *K. pneumoniae* isolates examined in this study, only 12% (n = 3) and 36% (n = 8) of isolates were susceptible to ciprofloxacin and gentamicin, respectively. Low susceptibility rates were also observed for piperacillin/tazobactam (36%), tetracycline (20%) and trimethoprim/sulfamethoxazole (4%). Twenty-three of the 25 (92%) isolates tested were susceptible to carbapenems. Notably, the 2 carbapenem-resistant *K. pneumoniae* isolates (PK30 and PK107) carried $bla_{CTX-M-3}$, $bla_{SHV-11}$, and $bla_{TEM-1}$. One of these *K. pneumoniae* isolates also showed resistance to colistin with an MIC of 64µg/mL. Both patients died of complications associated with bloodstream and respiratory tract infections. Three of 22 CTX-M–producing *K. pneumoniae* isolates examined by Etest were nonsusceptible to tigecycline (MICs 3 µg/mL, 3 µg/mL, and 8 µg/mL). All 25 CTX-M–producing *K. pneumoniae* isolates examined were resistant to cefotaxime, and all but 1 isolate...
showed higher MICs of cefotaxime than of ceftazidime. The 50% minimum inhibitory concentration (MIC<sub>50</sub>) for cefotaxime among these isolates was >256 µg/mL. By contrast, the MIC<sub>50</sub> and 90% inhibitory concentration for ceftazidime were 16 µg/mL and 128 µg/mL, respectively. Two CTX-M–producing K. pneumoniae isolates (8.0%) were susceptible (MIC ≤4 µg/mL) and 5 isolates (20%) were intermediate in susceptibility (8 µg/mL) to ceftazidime according to the 2010 revised CLSI breakpoints (Figure 1). In addition, we determined the susceptibilities of 22 CTX-M–producing K. pneumoniae isolates against cefotaxime and ceftazidime by using the standard disk diffusion method. All CTX-M–producing K. pneumoniae isolates examined were resistant to cefotaxime by disk diffusion (mean inhibitory zone size 8.3 mm; range 6–13 mm). Two of these isolates were susceptible (≥21 mm) and 5 had intermediate (18–20 mm) susceptibility to ceftazidime by disk diffusion (Table 3, Appendix). The disk diffusion results showed a category agreement with the Etest MIC of 100% for cefotaxime and 90.9% for ceftazidime with 2 minor errors.

**PFGE and MLST Analysis of CTX-M–producing K. pneumoniae**

Of 17 representative CTX-M–producing K. pneumoniae isolates analyzed by PFGE, 8 different pulsotypes (PF1–8) were identified with Dice coefficients of ≥80% similarity (Figure 2). Ten of 17 K. pneumoniae isolates examined belonged to 3 major groups (PF3, PF4, PF5) with 3–4 isolates in each group. The remaining pulsotypes contained only 1 or 2 K. pneumoniae isolates. No clear temporal relationship was shown among the highly related isolates.

MLST was performed on 18 CTX-M–producing K. pneumoniae isolates. These isolates were selected to represent different CTX-M genotypes, pulsotypes, antimicrobial susceptibility profiles, and years of isolation. Twelve STs were recognized for the K. pneumoniae isolates examined (Table 3, Appendix). Notably, all 3 CTX-M group 1, non–CTX-M-15 K. pneumoniae isolates analyzed (KP38, PK107, and PK135) had ST11, whereas 10 different STs (ST15, ST16, ST17, ST48, ST147, ST252, ST258, ST280, ST392, and ST437) were identified for the 14 CTX-M-15 K. pneumoniae isolates. Isolate F351 was the only non–CTX-M-1 group K. pneumoniae isolate identified in this study and was determined to be a separate group (ST792) by MLST. Of the 14 CTX-M–producing K. pneumoniae isolates evaluated simultaneously by DNA sequencing, PFGE and MLST, a high genetic divergence was demonstrated by the detection of 4 CTX-M genotypes (CTX-M-1, CTX-M-2, CTX-M-3, and CTX-M-15), 8 pulsotypes (PF1–8) and 11 STs (ST11, ST15, ST16, ST17, ST18, ST47, ST252, ST280, ST392, ST437, and ST792) (Figure 2).

**Discussion**

CTX-M ESBL–producing E. coli, especially ST131 strains, have emerged in recent years in several US states (5–7,25,26). In this study, we detected bla<sub>CTX-M</sub> genes in ESBL-producing E. coli strains isolated from patients at a tertiary care medical center in suburban New York City as early as 2005. Eighty-nine (54.6%) of 163 ESBL-producing E. coli isolates in the study period (2005–2012) carried bla<sub>CTX-M</sub>. Our findings confirm the emergence and dominance of CTX-M enzymes in ESBL-producing E. coli since the mid-2000s in the New York City metropolitan area (9,10).

Despite this high prevalence of CTX-M in ESBL-producing E. coli since 2005, none of 81 ESBL-producing K. pneumoniae isolates recovered from patients at the same tertiary care medical center from 2005 through 2008 was positive for bla<sub>CTX-M</sub>. CTX-M–type ESBL was first detected in K. pneumoniae isolates from our institution in 2009. The percentage of K. pneumoniae isolates carrying bla<sub>CTX-M</sub> has increased significantly since then. During 2010–2012, bla<sub>CTX-M</sub> genes were identified in 23 of 87 (26.4%) ESBL-producing K. pneumoniae isolates. These data demonstrate the rapid emergence and spread of CTX-M ESBL–producing K. pneumoniae in our patients. To date, CTX-M–producing K. pneumoniae has been recognized in several US states, including Texas (2004–2007, n = 11) (5,12), Nebraska (2005, n = 1)
particularly group 1 CTX-M was detected in 47 (100%) of 47 (n = 19), CTX-M-1 (n = 1), and CTX-M-3 (n = 4). Similarly, group 1 CTX-M, including isolates encoding CTX-M-15 and 2009–2010 isolates recovered during 2008–2009 (16) a few CTX-M-12 New York, Washington, and Wisconsin (18). Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 19, No. 11, November 2013 1807

In clinical strains, CTX-M–encoding genes have common lineages. This hypothesis contrasts with findings regarding lineage was observed by us (34). Our data, in combination with findings from other backgrounds. This hypothesis contrasts with findings regarding KPC-producing K. pneumoniae: a clonal spread of KPC-producing K. pneumoniae isolates belonging to the ST258 lineage was observed by us (33) and Pournaras et al. (34).

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genetic elements has been described (2,35,36). Given the dominance of CTX-M-15 genotypes among genetically heterogeneous K. pneumoniae isolates, our study also implies the probable horizontal transfer of a genetic element carrying blaCTX-M among K. pneumoniae isolates.

Of the 12 STs determined for the CTX-M ESBL–producing K. pneumoniae isolates, ST11, ST15, ST17, ST48, ST147, and ST258 have been reported in CTX-M–positive K. pneumoniae in Spain, Hungary, or Korea (28,37,38). Among these, only ST17 was reported among CTX-M–producing K. pneumoniae isolates in Canada (39). In this study, we determined the STs among CTX-M–producing K. pneumoniae isolates in the United States and documented the existence of 6 STs (ST16, ST252, ST280, ST392, ST437, ST792) in CTX-M–producing K. pneumoniae not previously described.

The CTX-M–producing K. pneumoniae isolates evaluated in this study showed several notable epidemiologic, clinical, and microbiological features. First, most CTX-M–producing isolates were recovered from patients with bacteriuria, which is similar to that observed for infections caused by CTX-M–producing E. coli in New York, NY, (9,10). Although CTX-M–producing K. pneumoniae was isolated in clinical specimens collected within 72 hours of hospitalization in about half of the patients, 18 (72%) of 25 patients had been hospitalized in the prior 8 months. This factor highlights the potential for acquiring CTX-M–producing K. pneumoniae in health care settings and differs from the experience with CTX-M–producing E. coli that are associated with infections arising in the community setting unrelated to exposure to health care facilities (26). Second, the CTX-M–producing K. pneumoniae study isolates exhibited high rates of resistance to gentamicin (68%), trimethoprim-sulfamethoxazole (96%), and tetracycline (80%), in addition to resistance to ciprofloxacin (88%) and pipercillin-tazobactam (64%) as described previously in Europe and Asia (27,28,37). Whether such high rates of resistance are associated with the dominant spread of CTX-M-15–producing, rather than CTX-M-14–producing, K. pneumoniae, in these patients is not known. The coexistence of CTX-M ESBL and TEM-1 and SHV-type β-lactamases in these isolates may have also contributed to the observed high rate of antimicrobial drug resistance. All except 1 of our CTX-M–positive K.
*pneumoniae* isolates produced SHV- and CTX-M–type ESBLs. These findings have clinical implications for selecting empiric antimicrobial drug therapy when infection caused by ESBL-producing *K. pneumoniae* is suspected. The rapid emergence of such CTX-M–producing *K. pneumoniae* isolates, mainly in US hospitals, is also raising new concerns for public health and infection control practice. Third, none of the 228 KPC-producing *K. pneumoniae* isolates examined carried bla<sub>KPC</sub>. Coexistence of bla<sub>KPC</sub> and bla<sub>CTX-M</sub> has only been reported in KPC-producing *K. pneumoniae* in China (40). Whether certain genetic mechanisms prevent KPC-producing *K. pneumoniae* from acquiring bla<sub>CTX-M</sub> is unclear.

This study reveals the rapid emergence and polyclonal spread of CTX-M–producing *K. pneumoniae* in patients in Westchester County, New York. A limitation of our study is that the clinical isolates were collected from patients at a single tertiary-care medical center. Investigations of CTX-M–producing *K. pneumoniae* isolates from a variety of geographic regions should be undertaken to clarify the epidemiology and clinical and public health effects of the emergence of CTX-M–producing *K. pneumoniae* in the United States.

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**References**


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