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CTX-M-12 β-Lactamase in a *Klebsiella pneumoniae* Clinical Isolate in Colombia

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We describe the detection of the CTX-M-12 β-lactamase from a clinical isolate of *Klebsiella pneumoniae* in Colombia. Screening of nosocomial *Klebsiella* spp. and *Escherichia coli* isolates from a network of teaching hospitals revealed the presence of CTX-M enzymes in multiple cities. This is the first description of CTX-M in Colombia.

Extended-spectrum β-lactamases (ESBLs) appear to be more common than South America than in North America (19). In addition, the type of enzymes that are prevalent is different, because the CTX-M class appears to be the dominant class in some countries, notably Argentina (2, 10, 17). CTX-M class enzymes have not been reported previously in Colombia. The CTX-M enzymes are a new, rapidly growing family of Ambler class A (Bush group 2be) ESBLs that are plasmid mediated (1, 9) and have been reported worldwide. These enzymes may be even more common than current reports suggest due to inadequate detection, because some laboratories may not be screening correctly. CTX-M enzymes hydrolyze cefotaxime and ceftriaxone more efficiently than ceftazidime, so that screening with ceftazidime alone may easily miss these enzymes (10, 17). Nevertheless, the activity of these enzymes against ceftazidime is highly variable; some strains appear susceptible, while others are frankly resistant.

In 2002, we formed a network of nine teaching hospitals in three cities (Bogotá, Medellín, and Cali) in Colombia. During the first 6 months of 2002, all nosocomial isolates of *Escherichia coli* and *Klebsiella* spp. that showed an ESBL phenotype (resistance to any extended-spectrum cephalosporin or aztreonam) were sent to our central laboratory at the Centro Internacional de Entrenamiento e Investigaciones Médicas (CIDEIM). Bacterial identification was confirmed and antibiotic susceptibility testing was performed on each of the isolates received at CIDEIM by using the VITEK system (BioMerieux, Lyon, France) according to the manufacturer's instructions. Pure strains with no more than 24 h of growth were used. The oxidase test was performed, and a 1.0 McFarland suspension was prepared in 0.85% saline solution, which was used to inoculate the VITEK cards. GNI+ cards were used for identification, and GNS-121 and GNS-118 cards were used to determine susceptibility. The GNS-121 card contained an ESBL confirmatory test that uses ceftazidime and cefotaxime with and without clavulanic acid. A growth reduction of ≥50% in the medium containing clavulanic acid confirms the production of ESBLs. *E. coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603 were used as internal quality control strains for MIC determinations and the ESBL confirmatory test, respectively.

ESBL characterization by isoelectric focusing (IEF) was done by the method described by Matthew et al. (13). Reference bacterial strains known to produce TEM-6, TEM-7, SHV-1, and TEM-9 were used as controls. Clinical isolates of ESBL-producing *Klebsiella* spp. and *E. coli* that were highly resistant to cefotaxime were subjected to IEF. Seven were found to have isoelectric points above 8, and those isolates were screened for CTX-M genes by a PCR method. Plasmid DNA was released from the cells by alkaline lysis (5). Five microliters of plasmid DNA was mixed with 2 μl (10 mM) of deoxynucleoside triphosphates (dNTPs) (Pharmacia Biotech, Peapack, N.J.), 16 μl of each primer, 10 μl of 10× reaction buffer, 44.5 μl of MilliQ sterile water, and 0.5 μl of *Taq* DNA polymerase 5 U/μl (Promega, Madison, Wis.). Primers derived from a representative enzyme of each group of cefotaximases were used. For the CTX-M-1 group (CTX-M-1, -3, -10, -11, -12, -15, and -22 and UOE-1), primers 5′CGCTTTGCGATG TGCAG 3′ and 5′ACCGCGATATCGTTGGT3′ were used. For the CTX-M-2 group (CTX-M-2, -4, -5, -6, and -7 and Toho-1), primers 5′TTAATGACTCAGAGCATTC3′ and 5′ GATACCTCGCTCCATTTATTG3′ (Gibco BRL, Gaithersburg, Md.) were used. For the CTX-M-8 group (CTX-M-8 and -25), primers 5′TGAATACTTCAGCCACACG3′ and 5′TAG AATTAATAACCGTCGGT3′ were used. Finally, for the CTX-M-9 group (CTX-M-9, -13, -14, -15, -16, -17, -18, -19, and

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-21 Toho-2 and -3), primer 5′CGCTTTATGCGCAGACGA3′ was used. A Mastercycler 5330 (Eppendorf, Hamburg, Germany) apparatus was used with the following parameters: DNA denaturation during 5 min at 95°C; 20 cycles of denaturation for 1 min at 95°C, annealing for 1 min at 55°C, and polymerization 1 min at 72°; and a final extension of 20 min at 72°C.

We used a clinical isolate of *K. pneumoniae* from a patient with a nosocomial surgical wound infection (our index isolate) for sequencing of CTX-M genes. The PCR product was cloned at the *Sma*I site of a pK19 vector (kanamycin resistance) and introduced by transformation into competent *E. coli* Top10 cells. Transformant clones were selected on Luria-Bertani agar plates, containing IPTG (isopropyl-β-D-thiogalactopyranoside; 1 mM), X-Gal (5-bromo-4-chloro-3-indolyl-β-D-thiogalactopyranoside; 40 μg/ml), and kanamycin (30 μg /ml). Sequencing of this gene was performed on both DNA strands, and these results were reproducible. The same plasmid DNA was used as templates in PCRs for SHV genes and PER-2 genes by using specific primers as previously described (4). Pulsed-field gel electrophoresis (PFGE) of chromosomal DNA was done by the method of Matushek et al. (14).

Susceptibility testing (by VITEK) showed that the MICs of cefotaxime and ceftriaxone for all seven isolates were >32 μg/ml and that the isolates had variable susceptibility to cefepime (MICs, 4 to 32 μg/ml), as well as ceftazidime (MICs for three isolates >32, while four isolates remained susceptible), and all isolates were susceptible to imipenem and meropenem (MICs, 2 to 4 μg/ml). Antibiotic susceptibility testing of the index isolate of *K. pneumoniae* was repeated by broth microdilution with the following MICs: ceftazidime, 128 μg/ml; cefotaxime, 32 μg/ml; and ceftriaxone, 32 μg/ml. This isolate had an intermediate level of susceptibility to cefepime (MIC, 16 μg/ml) and was susceptible to imipenem and meropenem (MIC of 2 μg/ml for both). Furthermore, by broth macrodilution, the MICs for the related transformant *E. coli* pK19-1338 were as follows: ceftazidime, <8 μg/ml; cefotaxime, >128 μg/ml; and ceftriaxone, >256 μg/ml.

Screening with PCR probes specific for different CTX-M genes confirmed the presence of CTX-M group 1 in our seven isolates. These isolates were collected in three different hospitals from Bogotá, Medellín, and Cali. The CTX-M enzymes detected were harbored by strains of *Klebsiella oxytoca*, *K. pneumoniae*, and *E. coli*. Two of the CTX-M-producing *K. pneumoniae* isolates came from the same hospital. Sequence data were obtained for the CTX-M gene from a transformant *E. coli* isolate by using our index patient's *K. pneumoniae* isolate as a donor. The results indicated the presence of CTX-M–12 (GenBank accession no. AF305837), an enzyme reported only once previously from an isolate from Kenya (12). PCR-based screening for PER-2 and SHV genes was negative in all these strains. PFGE of the four CTX-M-harboring *K. pneumoniae* isolates proved that these strains were not clonal (data not shown).

First described at the beginning of the 1990s (3), the CTX-M class of enzymes are a fast-growing family that comprises 19 enzymes and the related enzymes Toho-1 and Toho-2. CTX-M isolates have been found in various species of the family *Enterobacteriaceae* from different geographic areas: mainly during nosocomial outbreaks that occurred in Japan (11); Europe (7, 8, 15); South America (3, 17); and, more recently, Africa (12), China (18), and Korea (16). A few isolates have recently been described in the United States (E. S. Moland, J. A. Black, A. Hossain, N. D. Hanson, and K. S. Thomson, Letter, Antimicrob. Agents Chemother. **47:**2382-2383, 2003). The present study shows that CTX-M enzymes occur in Colombia as well.

It is very difficult to predict the origin of CTX-M enzymes because of the divergence in amino acid sequences among the subgroups (between 70 and 99% sequence identity) (6), as well as the geographical dispersion of the host strains. Efforts should be made to screen for them by always testing susceptibilities to cefotaxime, ceftriaxone, or cefepime on a routine basis, along with susceptibility to ceftazidime (17). Optimal therapy for organisms harboring these enzymes has not been defined. The carbapenems appear to be the most active compounds in vitro, and thus they may be the most reasonable agents until more clinical data are available.

In the present study, we report the first CTX-M-12 type β-lactamase found in Colombia and also, to our knowledge, the first isolation of this enzyme outside of Kenya. The CTX-M-12 type was previously isolated from *K. pneumoniae* isolates from an outbreak among six newborn babies in Kenya at the National Hospital in Nairobi (12). It is noteworthy that in the Kenya study, both the clinical *Klebsiella* isolates and their *E. coli* transconjugants were resistant to cefotaxime (MIC, 24 μg/ml by E-test) but susceptible (MIC, 1 μg/ml) to ceftazidime. In our index case, the parent *K. pneumoniae* isolate was ceftazidime, cefotaxime, and ceftriaxone resistant, while its *E. coli* transformant was susceptible to ceftazidime but highly resistant to cefotaxime and ceftriaxone. Our index isolate presumably owes its high-grade resistance to ceftazidime to the presence of additional ESBLs of the TEM and/or SHV families. IEF studies of this isolate revealed multiple bands with pIs of 5.2, 7.3, 8.4, and 8.9. A more comprehensive review of the organisms from the network and further studies on the identity of the other CTX-M enzymes we detected will be the subject of subsequent reports.

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