Detection of Five Novel CTX-M-Type Extended Spectrum 
Beta-Lactamases with One to Three CTX-M-14 Point 
Mutations in Isolates from Hefei, 
Anhui Province, China

Following the introduction of expanded spectrum cephalosporins into clinical usage, the production of extended-spectrum $\beta$-lactamases (ESBLs) by members of the Enterobacteriaceae has been documented (5, 6). One of these ESBLs, CTX-M, has become the predominant ESBL type in eastern Asia, South America, and Eastern Europe. And it also exhibits an overall preferential hydrolysis of cefotaxime and ceftriaxone over ceftazidime and a higher susceptibility to tazobactam than to clavulanic (2, 8, 9). In our study, we also found the dissemination of CTX-M-14 enzyme in our region. Furthermore, several point mutations were also found in the $bla_{\text{CTX-M-14}}$ gene that resulted in amino acid substitution.

A total of 45 strains of Escherichia coli and 53 strains of Klebsiella pneumoniae, which were confirmed to be producing ESBLs by inhibitor-potentiated broth dilution testing according to the CLSI (formerly NCCLS)-recommended criterion of 1999 (10), were isolated from patients in four hospitals in Hefei, Anhui province, between 1999 and 2000. Two of the hospitals are tertiary-care teaching hospitals with over 1,000 beds, and the other two are tertiary-care hospitals with more than 500 beds.

PCR and DNA sequencing methods were used in our study. A total of 30 K. pneumoniae and 24 E. coli isolates were identified as containing $bla_{\text{CTX-M}}$ genes by PCR with newly designed versatile primers for three-group $bla_{\text{CTX-M}}$ genes. And then the BLAST program of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) was used to search databases for similar nucleotides. The following primers were used in these reactions: for $bla_{\text{CTX-M-1}}$ group genes, primers CTX-M-1-A (5'-ACAGCGATAACGTGGCGATG-3') and CTX-M-1-B (5'-TCACCCAATGCTTTACCCAG-3') for $bla_{\text{CTX-M-14}}$ group genes; primers CTX-M-9-A (5'-CTAGCCTGTCGA-3') and CTX-M-9-B (5'-TCAGTGCGATCCGAATGTTGTAATTTG-3') and CTX-M-2 group genes, primers TOHO-1A (5'-TGGAAGCCCTGGAGAAAAGT-3') and TOHO-1B (5'-CTTATCGCTCTCGCTCTGTT-3'). All these primer pairs will amplify only part of the ORF of the target gene. Of these, approximately 59.2% (32/54) and 42.6% (23/54) produced CTX-M-9 and CTX-M-1 groups, respectively why they happened, and more epidemiology experiments need to be performed to reveal the relationship between these strains.

### REFERENCES


### TABLE 1. Point mutation site induced amino acid substitution and relevant data

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Point mutation(s)</th>
<th>Amino acid substitution(s)</th>
<th>Laboratory*</th>
<th>Designation</th>
<th>Genbank accession no.</th>
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| 105     | G                   | GAAAGCGAGTCATAATCTTCTGTAGAAATAG-3' and 9-B (5'-CGGGATCCCGGGCCAGTTGGTGTAATTTG-3') were used for amplification of a ca. 1,101-bp product containing the whole CTX-M-9 group ORF. Bacterial DNA was prepared by suspension of one or two fresh colonies in 50 µl of sterile distilled water and heating at 95°C for 10 min. PCR amplification was carried out under the following conditions: 94°C for 6 min for predenaturing, followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 15 min. All of the nucleotide sequences were determined by bidirectional sequencing of PCR production with an ABI Prism 377-96 automatic DNA sequencer. The results of DNA sequencing with the whole ORF primer indicated that production of the CTX-M-9 group was almost identical to that of CTX-M-14. One to three point mutations occurred in eight isolates, resulting in the amino acid substitutions shown in Table 1. These isolates were designated CTX-M-42, CTX-M-43, CTX-M-44, CTX-M-45, and CTX-M-46.

* 1, the First Affiliated Hospital of Anhui Medical University; 2, the 105 Hospital of the People’s Liberation Army, China; 3, the First People’s Hospital of Hefei.


Hui Li
Jia Bin Li
Department of Infectious Diseases
The First Affiliated Hospital
of Anhui Medical University
218 Ji Xi Rd.
Hefei, Anhui Province, 230022
People’s Republic of China

*Phone: (86) 551 2922281
Fax: (86) 551 2922281
E mail: lijijbin948@sohu.com