Molecular Characterization and Antimicrobial Susceptibility Testing of *Escherichia coli* Isolates from Patients with Urinary Tract Infections in 20 Chinese Hospitals

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A total of 222 urinary *Escherichia coli* isolates from 20 tertiary hospitals in 15 different provinces and 4 municipalities in mainland China were characterized by antimicrobial susceptibility, phylogrouping, and the presence of plasmid-mediated quinolone resistance genes. A subset of 138 suspected extended-spectrum cephaporphinase (ESC) producers were examined for genes encoding cephaporphin resin. Forty-three isolates harboring *blaCTX-M-14* or *blaCTX-M-15* were analyzed by pulsed-field gel electrophoresis (PFGE), and plasmids containing these genes were typed using PCR-based replicon typing (PBRT). Thirteen phylogroup B2 *blaCTX-M-14* and *blaCTX-M-15* positive isolates were analyzed by multilocus sequence typing (MLST). A frequent occurrence of resistance (>46%) was observed toward cephalosporins, gentamicin, and fluoroquinolones. Among the 222 isolates, 4 *qnrS1*, 4 *qepA*, and 16 *aac(6′)-Ib-cr* genes were confirmed. Four major phylogroups (A, B1, B2, and D) and nontypeable isolates (NTs) were found among the isolates, with phylogroup D (54%) being the most common phylogroup. A total of 110 (80%) of the 138 screened isolates harbored *blaCTX-M* genes, with *blaCTX-M-14* (71%) and *blaCTX-M-15* (24%) being the most prevalent of these genes. Nine of the 13 CTX-M-15- or CTX-M-14-containing B2 isolates belonged to ST131. PFGE typing showed a high level of diversity, and plasmid analysis indicated a very large pool of different resistance plasmids mediating the spread of *blaCTX-M* genes in mainland China. An equally very high frequency of resistance and equally high levels of diversity in phylogroups, PFGE types, and plasmids were observed among community- and hospital-acquired *E. coli* isolates, indicating the presence of a large reservoir in the community and a long-term spread of cephalosporin resistance in China.

*Escherichia coli* is among the most important human pathogens and is responsible for up to 90% of all community-acquired and almost 50% of nosocomial urinary tract infections (UTIs), with *E. coli* strains associated with UTIs among larger geographic regions in China, and no comparison of community- and hospital-acquired isolates has been performed. Furthermore, to our knowledge, no information is currently available on the phylogrouping of urinary *E. coli* isolates or the diversity of plasmids mediating cephaporphin resistance in China.

This study was conducted to provide information on antimicrobial susceptibility, phylogrouping, the prevalence of PMQRs and genes encoding extended-spectrum cephaporphinases (ESCs), including both extended-spectrum β-lactamases (ESBLs) and AmpC enzymes, clonal relatedness, and multilocus sequence typing (MLST) of *E. coli* strains causing UTIs in mainland China. The genetic location of the *blaCTX-M-14* and *blaCTX-M-15* genes and potential

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differences between hospital- and community-acquired isolates were also investigated.

MATERIALS AND METHODS

Bacterial isolates. A total of 945 nonreplicate clinical E. coli isolates were collected from 20 widely dispersed tertiary Chinese hospitals participating in the Ministry of Health National Antimicrobial Resistance Surveillance Network (Mohanmari) study during January 2007 to March 2008 and were sent to the Institute of Clinical Pharmacology, Beijing University First Hospital, where they were stored at −80°C until further analysis. A total of 305 E. coli clinical isolates associated with human UTIs, including cystitis, pyelonephritis, and bacteruria, were obtained, and 222 of these isolates were available for this study. The strains originated from the following 20 hospitals: Beijing University First Hospital (Beijing) (n = 12), Beijing Hospital (Beijing) (n = 22), Jilin University First Hospital (Jilin) (n = 4), Chinese Medical University First Hospital (Liaoning) (n = 4), General Hospital of Tianjin Medical University (Tianjin) (n = 18), the Second Hospital of Hebei Medical University (Hebei) (n = 3), the 3rd Affiliated Hospital of Nanjing Medical University (Jiangsu) (n = 16), Zhongshan Hospital of Fudan University (Shanghai) (n = 8), The First Affiliated Hospital of Zhejiang University (Zhejiang) (n = 2), Shenzhen People’s Hospital (Guangzhou) (n = 7), Wuhan University First Hospital (Hubei) (n = 25), Xiangya Hospital Affiliated with Central South University (Hunan) (n = 2), The People’s First Hospital of Kunming (Yunnan) (n = 6), Chengdu Children’s Hospital (Sichuan) (n = 1), Southwest Hospital of The Third Military University (Chongqing) (n = 6), Xijing Hospital of The Fourth Military University (Shanxi) (n = 21), Jinan City Center Hospital (Shandong) (n = 25), The First Hospital of Fujian Medical University (Fujian) (n = 15), Lanzhou University Second Hospital (Gansu) (n = 17), and The First Hospital of Xining Medical University (Xinjiang) (n = 10). These hospitals are located in 15 different provinces and 4 municipalities in mainland China, covering all 6 districts. Among the 222 isolates, 95 isolates originated from community-acquired infections, defined as the pathogen being isolated within 48 h after hospital admittance. Another 39 isolates were from hospital-acquired infections, where the pathogens were isolated more than 48 h after admittance. The onset of the remaining 88 isolates was unknown.

Antimicrobial susceptibility testing. Susceptibility to antimicrobial agents was determined using the agar serial dilution method according to CLSI standards. The following antimicrobial agents were tested: amoxicillin-clavulanic acid, piperacillin, piperacillin-tazobactam, cefazolin, cefuroxime, cefprozil, cefoperazone, ceftiraxone, cefazidime, cefpodoxime, cefepime, moxalactam, gentamicin, amikacin, imipenem, ciprofloxacin, levofloxacin, and moxifloxacin. Twenty-eight isolates were analyzed further by confirmatory phenotypic testing for the presence of ESC according to previously described methods (11). These strains included 16 blaCTX-M-negative isolates and 12 only-blaTEM-positive ones. E. coli ATCC 25922 was run in parallel for quality control, and the results were analyzed and interpreted according to CLSI guidelines. Due to the absence of a CLSI breakpoint for the interpretation of moxifloxacin results, the interpretation was made using the current European Committee on Antimicrobial Susceptibility Testing (EUCAST) (www.euCAST.org) guidelines; a cutoff MIC of ≥0.5 μg/ml was used as the breakpoint for susceptibility, and a cutoff MIC of >1 μg/ml was used as the resistance breakpoint.

Determination of phylogroups. A multiplex PCR phylotyping assay was implemented to determine the phylogroups to which the 222 isolates belonged, as previously described (10), and these isolates were assigned to the four major E. coli phylogenetic lineages or nontypeable isolates (NTs) according to the following criteria: A, yjaA positive; B1, tspe4.c2 positive; B2, chuA and yjaA positive or chuA, yjaA, and tspe4.c2 positive; D, chuA positive or chuA, yjaA, and tspe4.c2 positive; and NT, yjaA, chuA, and tspe4.c2 negative (13). E. coli strains ECOR 20 (yjaA positive), ECOR 48 (chuA positive), ECOR 58 (tspe4.c2 positive), and ECOR 62 (chuA, yjaA, and tspe4.c2 positive) were taken as positive controls, and E. coli strain ECOR 4 was the negative control (all of these controls were kindly provided by Statens Serum Institute, Denmark).

Characterization of resistance genes and mutations. The presence of PMQR genes [qnrA, qnrB, qnrC, qnrD, qnrS, qepA, and aac(6’)-ib-cr] was examined by PCR as previously described (8, 9, 33, 44). A total of 138 isolates nonsusceptible to cefepime or cefotaxime were suspected as ESC producers and were examined for the presence of bladTEM and bladCTX, (14). Nine isolates nonsusceptible to cefoxitin were screened for bladTEM and bladCTX (14). The chromosomal ampC-promoter of these isolates were also amplified and sequenced to detect mutations affecting the ampC expression level (41). Amplicons from all of the bladCTX-positive and only-bladTEM-positive isolates were sequenced. The nucleotide sequences were aligned by Vector NTI 10.0 (InforMax, Inc.) and further analyzed by comparison to sequences obtained from a catalogue of the β-lactamases (http://www.lahey.org/studies/). The DNA sequences of ampC gene amplicons from the nine isolates nonsusceptible to cefoxitin were compared to the same region from E. coli K-12 (GenBank accession number U00969) to identify known mutations in this region.

Microarray analysis. In order to determine the main genes mediating resistance to β-lactams in the 138 suspected ESC producers, a miniaturized microarray (IdentiBac Amp′ve array tubes; Identibac, New Haw, Addlestone, Surrey, United Kingdom) was implemented for five strains showing different levels of resistance to ceftriaxone for rapid detection of possible β-lactam resistance genes, including bladCTX-M-1, bladCTX-M-2, and a group, bladCTX-M-8, and a group, bladCTX-M-9, bladPER-1, bladAV-1, bladAV-2, bladPER-2, and bladKSO-1 (1). DNA for performing the microarray was extracted and purified using an Easy-DNA kit (Invitrogen), and further analysis was conducted according to the protocol provided by the manufacturer.

PFGE. Forty-three isolates containing the bladCTX-M-1 or bladCTX-M-8 genes were analyzed for genetic relatedness by pulsed-field gel electrophoresis (PFGE) using XbaI according to the U.S. CDC PulseNet protocol (35). Briefly, electrophoresis was performed using 1.5% SeaKem agarose in 0.5% Tris-borate-EDTA under the following running conditions: switch time from 2.2 to 54.2 s at a gradient of 6 V/cm and an included angle of 120° for 19 h. A Salmonella enterica serovar Braenderup universal marker (kindly provided by the Centers for Disease Control and Prevention, Atlanta, GA) was used as a molecular weight standard. The analysis of the PFGE profiles was performed using BioNumerics software v3.5 (Applied Maths, Sint-Martens-Latem, Belgium), using the Dice similarity coefficient on the basis of the unweighted-pair group method using average linkages (UPGMA), with a 1.5% band tolerance. Furthermore, cutoff lines at 85% and 60% were used to analyze genetic relatedness.

Plasmid analysis. The same 43 isolates were investigated further for the genetic relationships of the resistance plasmids carrying bladCTX-M genes. Plasmid DNA was purified with a Qiagen minikit (Qiagen, Hilden, Germany) and used to transform competent E. coli GeneHog cells. Selection of the transformants was performed on brain heart infusion (BHI) agar (BD, France) plates containing cefotaxime (2 μg/ml), and the presence of the plasmids in the transformants was confirmed by PCR detection of bladCTX-M-1 gene as described above. Plasmid size was determined by comparison to the known bands of Salmonella enterica serovar Braenderup by S1-PFGE, as described below. To locate the positions of small plasmids in isolates A012 and B233, Southern blot hybridization with CTX-M-1 and CTX-M-9 probes was carried out as previously described (27), with α DNA digested by HindIII as a marker. Thirty-eight transformants bearing only a single plasmid harboring bladCTX-M were analyzed further by PCR-based replicon typing (PBRT) (9). Multiplex PCR was implemented for detection of replicons of IncI1, IncX, IncH1, IncN, IncH2, and IncL/M, using DreamTag Green PCR master mix (Mermenta, Canada); other replicons, including less common ones such as IncX-like and IncN-like replicons and repA1 (FIA-like), were detected by simple PCR with the 38 resistance plasmids.

SI-PFGE. SI-PFGE was implemented according to previously described nomenclature to estimate the size of the replicons (38). This method differed from PFGE by the use of a higher bacterial density (0.83 to 0.87) of the bacterial suspension in the plugs, and the whole genomic DNA was digested by S1 nuclease (Promega, Madison, WI) for 45 min at 37°C.

MLST. Thirteen phylogroup B2 E. coli isolates, including 3 bladCTX-M-15 and 10 bladCTX-M-1-positive isolates, were analyzed by MLST. Seven housekeeping genes (adk, fumC, gpyr, icd, mdt, rnuA, and recA) were amplified and analyzed following protocols available at http://mlst.ucc.ie/mlst/dbs/Ecoli.

Statistical analysis. SPSS software (version 16.0) was used for statistical analysis. The differences in resistance rates and distributions of bladCTX-M-14 and bladCTX-M-15 genes, as well as E. coli phylogroups, among the community- and hospital-acquired isolates were analyzed by the chi-square test. Fisher’s exact test was used to analyze the prevalent relationship between the replicons of resistant plasmids and the carried bladCTX-M-14 or bladCTX-M-15 genes as well as the difference in prevalence of replicons among the analyzed community- and hospital-acquired isolates.

RESULTS

Antimicrobial susceptibility. All 222 E. coli isolates were susceptible to imipenem. A few isolates were resistant to piperacillin-tazobactam (0.45%) and moxalactam (1%), and some were resistant to amikacin (7%), cefepime (14%), and amoxicillin-clavulanate (25%). High frequencies of resistance were
observed toward moxifloxacin (85%), ciprofloxacin (75%), piperacillin (73%), levofloxacin (71%), cefeprozin (69%), cepodoxime (69%), cefazolin (68%), ceftazidine (67%), cefuroxime (67%), gentamicin (63%), cefoperazone (57%), and ceftiraxone (46%). Hospital-acquired isolates were slightly more resistant to the following agents than community-acquired isolates: amoxicillin-clavulanate (46%), moxalactam (3% and 5%) (P = 0.428), ceftazidime (10% and 9%) (P = 0.242), cefalotin (3% and 4%) (P = 0.87), and levofloxacin (97% and 93%) (P = 0.506), cefazidime (10% and 9%) (P = 1.000), cepodoxime (77% and 72%) (P = 0.526), cefepime (23% and 14%) (P = 0.182), gentamicin (71% and 61%) (P = 0.079), amikacin (10% and 6%) (P = 0.67), and ciprofloxacin (82% and 73%) (P = 0.25). Hospital-acquired isolates were slightly less resistant to the following agents than community-acquired isolates: amoxicillin-clavulanate (26% and 28%) (P = 0.744), cefoperazone (59% and 69%) (P = 0.242), moxalactam (3% and 5%) (P = 0.87), and levofloxacin (69% and 71%) (P = 0.882).

Among the 28 isolates further analyzed by confirmatory phenotypic testing for ESC, 9 isolates were resistant or intermediate to cefoxitin. The remaining 19 isolates were found to be sensitive to the tested antimicrobials, indicating no ESC production.

**Phylogenetic characterization.** All four major phylogenetic lineages (A, B1, B2, and D) and NTs were found among the 222 urinary E. coli isolates, and phylogroups D (54%) and B2 (19%) were the most common. The different phylogroups and NTs were distributed in both hospital and community settings, and no significant differences were observed (P = 0.489 for group A, P = 0.670 for group B1, P = 0.054 for group B2, P = 0.204 for group D, and P = 0.211 for NTs), although phylogroup D was more common among the hospital-acquired isolates (62%) than among the community-acquired isolates (49%). Phylogroup B2 was more common among the community-acquired isolates (27%) than among hospital-acquired isolates (10%). Phylogroups D (57%) and B2 (10%) were observed for most CTX-M-14-producing E. coli isolates, while phylogroups D (40%) and A (25%) were common for the CTX-M-15-producing E. coli isolates (Table 1).

**Identification of genes and mutations mediating cephalosporin resistance.** Four qnrS1, 4 qepA, and 16 aac(6’)-Iib-cr genes were confirmed among the 222 urinary isolates. A total of 110 of 138 (80%) ESC-suspected isolates showed positive PCRs for bla<sub>CTX</sub>-Seq. Sequencing of the bla<sub>CTX-M</sub> amplicons identified the presence of bla<sub>CTX-M-14</sub> in 70 isolates, bla<sub>CTX-M-15</sub> in 24 isolates, bla<sub>CTX-M-3</sub> in 8 isolates, bla<sub>CTX-M-65</sub> in 3 isolates, bla<sub>CTX-M-79</sub> in 2 isolates, bla<sub>CTX-M-27</sub> in 2 isolates, and bla<sub>CTX-M-22</sub> in 1 isolate. A total of 79 isolates were positive for <i>bla</i><sub>TEM</sub>, and the sequenced results indicated that <i>bla</i><sub>TEM-16</sub> was the major subtype. The <i>bla</i><sub>CTX-M-14</sub>-positive isolates originated from hospitals widely distributed in all the provinces involved except for Zhejiang. The <i>bla</i><sub>CTX-M-15</sub> gene was observed in isolates from all provinces except for Jilin, Sichuan, and Hunan, where only a few isolates were collected. Overall, <i>bla</i><sub>CTX-M</sub> genes were found in all districts and for both community- and hospital-acquired infections. No significance difference in the distributions of <i>bla</i><sub>CTX-M-14</sub> (n = 25 and 16 for community- and hospital-acquired isolates, respectively; P = 0.428) and <i>bla</i><sub>CTX-M-15</sub> (n = 11 and 5; P = 0.699) among the 95 community- and 39 hospital-acquired isolates was observed. Three <i>bla</i><sub>CMY-2</sub> and two <i>bla</i><sub>CMY-1</sub> genes were detected by PCR in the nine isolates nonsusceptible to cefoxitin. DNA sequencing identified only the three <i>bla</i><sub>CMY-2</sub> genes. Seven of these nine isolates showed multiple mutations in the <i>ampC</i> promoter region: –88 (C → T), –82 (A → T), –76 (G → A), –42 (C → T), –18 (G → T), –11 (C → T), –22 (C → T), +26 (TA → GT), +32 (G → A), +58 (C → T), and +70 (C → T). These were in accordance with previous reports as a cause of overexpression of chromosomal <i>ampC</i> (6, 7, 41, 45).

**Identification of resistance genes by microarray analysis.** Array analysis indicated that the <i>β</i>-lactamase gene groups <i>bla</i><sub>CTX-M-9</sub> and <i>bla</i><sub>TEM</sub> were the most common in mediating resistance to cephalosporins, in accordance with PCR results for <i>bla</i><sub>CTX-M</sub> and <i>bla</i><sub>TEM</sub>; the <i>bla</i><sub>CTX-M-9</sub> group genes were confirmed to be <i>bla</i><sub>CTX-M-14</sub> and the <i>bla</i><sub>TEM-1</sub> gene showed 100% similarity to strains carrying <i>bla</i><sub>TEM-1b</sub> in GenBank by PCR and DNA sequencing.

**Clonal relatedness.** There was no predominant PFGE type. The cutoff line at 69% showed diversity, with 7 distinct PFGE clusters, without 10 unique DNA patterns included in these clusters, and the cutoff line at 85% displayed high genetic diversity among the 43 <i>bla</i><sub>CTX-M-14</sub> or <i>bla</i><sub>CTX-M-15</sub>-containing urinary E. coli isolates acquired from both hospitals and communities (Fig. 1).

**Plasmid characterization.** Thirty-eight <i>bla</i><sub>CTX-M-14</sub> and <i>bla</i><sub>CTX-M-15</sub> genes were observed on plasmids of various sizes ranging from <10 kb to 137 kb (Fig. 1). Two of 38 plasmids were untypable, whereas the remaining could be assigned to different replicon types. Inc<sub>R</sub>RepB typing detected the presence of <i>IncFRepB</i>, <i>IncI1</i>, <i>IncN</i>, <i>IncFIA</i>, <i>IncFIB</i>, <i>IncIB/O</i>, and <i>IncQ</i> replicons and of <i>repA1l</i>, with <i>IncFRepB</i> and <i>IncI1</i> plasmids as the most prevalent ones (n = 17 and 15, respectively). The coexistence of more than one replicon in the same plasmid was also observed, including colocation of <i>IncFRepB</i> and <i>IncFIB</i>, <i>IncFRepB</i> and <i>IncI1</i>, and <i>IncI1</i> and <i>IncIB</i>. Moreover, three replicons of <i>IncFRepB</i>, <i>IncFIA</i>, and <i>IncFIB</i> were detected simultaneously on a single plasmid carrying <i>bla</i><sub>CTX-M-14</sub> (Fig. 1). No statistically significant relationships between the replicons of <i>IncFRepB</i> and <i>IncI1</i> and <i>bla</i><sub>CTX-M-14</sub> (P = 0.210) or <i>bla</i><sub>CTX-M-15</sub> (P = 1.0) were observed. There was no significant difference between the distributions of <i>IncI1</i> (n = 6 and 6, respectively; P = 0.707) and <i>IncFRepB</i> (n = 3 and 7; P = 0.424) among 12 community- and 15 hospital-acquired infections by Fisher’s exact test.

### Table 1. Phylogenetic groups for 222 urinary E. coli isolates

<table>
<thead>
<tr>
<th>Phylogroup</th>
<th>CTX-M-14 positive</th>
<th>CTX-M-15 positive</th>
<th>Other isolates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>6</td>
<td>14</td>
<td>27</td>
</tr>
<tr>
<td>B1</td>
<td>4</td>
<td>4</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>B2</td>
<td>10</td>
<td>3</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td>D</td>
<td>44</td>
<td>10</td>
<td>66</td>
<td>120</td>
</tr>
<tr>
<td>NTs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5</td>
<td>1</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>24</td>
<td>128</td>
<td>222</td>
</tr>
</tbody>
</table>

<sup>a</sup> Strains that were not assigned to a phylogroup.
Sequence types (STs). MLST indicated that all 3 CTX-M-15-containing isolates and 6 of 10 CTX-M-14-positive ones belonged to ST131; among the remaining four B2 group CTX-M-14-positive isolates, two ST73 complex and one ST127 isolate, as well as one ST10 complex isolate, were observed.

**DISCUSSION**

In this study, 222 E. coli isolates from UTIs were collected from clinical microbiological laboratories in 20 hospitals from 15 different provinces and municipalities covering all six districts in mainland China. The isolates originated from both community- and hospital-acquired infections. More than half of the 222 isolates were collected from five provinces, namely, Beijing, Hebei, Shaanxi, Hubei, and Shandong (n/H11022 20), while fewer isolates were collected from Jilin, Sichuan, Hunan, and Zhejiang (n/H11349 4). No marked differences in the occurrence of resistance or prevalence and diversity of ESC, PMQR genes, phylogroups, or replicons could be observed among the isolates in the different geographic areas involved. This suggests that resistance and resistant genes are distributed equally over several geographic areas of mainland China.

We found a very high frequency of resistance to most of the antimicrobial agents usually used to treat E. coli infections. Resistance to cephalosporins and fluoroquinolones was so high that these agents should not be recommended for empirical treatment. It is noteworthy that in contrast to most observations from Europe and North America, the levels of resistance and resistant genes were equally high among both hospital- and community-acquired isolates, as well as those originating from clinical and hospital-acquired infections.
quired infections, indicating a very large reservoir of resistance in the community all over China.

The diverse CTX-M enzymes detected in our study confirmed that the globally prevalent CTX-M-14 (22, 42) and CTX-M-15 (32, 37) enzymes are quite common in China, as reported previously (46). CTX-M-22, CTX-M-27, and CTX-M-65 have been reported on relatively few occasions (46), but mainly from China, and all have previously been observed among food animals in China (23, 25, 39). Thus, the detection of these enzymes in our isolates may indicate that food animals are an important reservoir for cephalosporin-resistant E. coli in China. CTX-M-79 has previously been observed among elderly people in the community in China (40). Such diversity in CTX-M enzymes in China may also indicate the rapid evolution of CTX-M enzymes.

To our knowledge, this study provides the first data on phylogroups, PFGE typing, and plasmid analysis for a large collection of urinary E. coli isolates from different geographic regions in mainland China. Uropathogenic E. coli isolates have previously been reported to belong mainly to phylogroups B2 and D (12, 19, 34). Our study showed that most isolates were of phylogroup D, followed by phylogroup B2. The presence of isolates of phylogroups A and B1, which have been reported as animal or human commensal E. coli strains (5, 9, 19), indicates that animals might also be a source of some of the UTI E. coli isolates observed in China (18, 26). Furthermore, the observation in our study of group D and B2 blaCTX-M-14-containing isolates, as well as group D and A blaCTX-M-15-positive ones, differs from a previous study in Spain (42) which showed that blaCTX-M-14 was harbored mainly by E. coli isolates belonging to phylogroups A, B1, and D (42), and another study showed that blaCTX-M-15 was found mainly in isolates of phylogroups B2 and D (12).

Although our study showed a high prevalence of the international clone ST131 (20, 32) among phylogroup B2 blaCTX-M-15* and blaCTX-M-14-positive isolates, the genetic diversity of the 43 UTI isolates examined supported previous reports showing diverse populations of E. coli carrying blaCTX-M genes (12, 15, 29). The further molecular typing of the resistance plasmids from these isolates showed that the dissemination of blaCTX-M-14 and blaCTX-M-15 was associated mainly with IncI1 and IncF<sub>repB</sub> plasmids of widely different sizes, which confirmed that different IncF plasmids are the most common mediators of CTX-M enzymes (12, 16). To our knowledge, such a high degree of diversity as that observed in our study has not previously been observed. Moreover, we provide the first report of multireplicon plasmids in China, including two replicons coexisting on the same plasmid carrying the blaCTX-M-14 or blaCTX-M-15 gene and the colocation of three replicons in one plasmid harboring blaCTX-M-15, which might indicate the rapid evolution of plasmids under antimicrobial pressure (30). This has previously been reported in the United Kingdom and France (16, 28).

In conclusion, our data showed equally distributed high prevalence rates of cephalosporin and fluoroquinolone resistance among both community- and hospital-acquired UTI E. coli isolates, which were closely associated with phylogroups D and B2, in China. Cephalosporin resistance was mediated mainly by blaCTX-M-14 and blaCTX-M-15 genes harbored by diverse plasmids among a highly diverse population of E. coli strains, which suggests that cephalosporin resistance has evolved for some time in China and that a huge reservoir for resistance is maintained in the community. Thus, it is difficult to prevent the dissemination of resistance clones and plasmids, and interventions aiming at reducing cephalosporin resistance may be quite necessary.

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