The emergence of \( \text{bla}_{\text{CTX-M-15}} \)-carrying *Escherichia coli* of ST131 and new sequence types in Western China

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**Abstract**

**Background:** \( \text{bla}_{\text{CTX-M-15}} \), the most widely distributed gene encoding extended-spectrum β-lactamases globally, was not common in China. This study was performed to characterize \( \text{bla}_{\text{CTX-M-15}} \)-carrying *Escherichia coli* in western China.

**Findings:** Out of 144 *Escherichia coli* isolates from 20 hospitals in western China, 8 were found carrying \( \text{bla}_{\text{CTX-M-15}} \). \( \text{bla}_{\text{CTX-M-15}} \) was carried by isolates of ST131 and 5 new STs (ST3342, ST3513, ST3516, ST3517 and ST3518). The 5 new STs shared 5 identical alleles out of 7 but only had up to 2 alleles identical to ST131. \( \text{bla}_{\text{CTX-M-15}} \) was located on plasmids of IncI1 (ST16) or IncFII-related group (four replicon types). The co-transfer of a few antimicrobial resistance genes including \( \text{qnrA} \), \( \text{qnrB} \), \( \text{qnrS} \), \( \text{qepA} \), \( \text{aac (6')-Ib-cr} \), \( \text{aac (3)-II} \), \( \text{tetA} \), \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{OXA-1}} \) with \( \text{bla}_{\text{CTX-M-15}} \) were examined but only \( \text{bla}_{\text{TEM}} \) was found co-transferring with \( \text{bla}_{\text{CTX-M-15}} \).

**Conclusions:** Five new STs of *E. coli* and three new types of IncFII-related plasmids carrying \( \text{bla}_{\text{CTX-M-15}} \) were identified. This study together with several reports suggested that \( \text{bla}_{\text{CTX-M-15}} \) has emerged in China and the interruption of both vertical and horizontal transmission of \( \text{bla}_{\text{CTX-M-15}} \) is required to hurdle its further spread.

**Keywords:** *Escherichia coli*, Plasmids, MLST, Beta-lactamases, Antimicrobial resistance
typing (MLST) [9], the 8 isolates carrying \( \text{bla}_{\text{CTX-M-15}} \) were assigned to 6 sequence types (ST) including ST131 and 5 new STs (Table 1). Of note, \( E. \ coli \) of ST131 was found carrying \( \text{bla}_{\text{CTX-M-3}} \) except for \( \text{bla}_{\text{CTX-M-15}} \) in our local settings previously [10]. This study demonstrated the emergence of the globally-spread ST131 carrying \( \text{bla}_{\text{CTX-M-15}} \) in our region. The 5 new STs (ST3342, ST3513, ST3516, ST3517 and ST3518) shared 5 identical alleles (\( \text{gyrB}, \text{idc}, \text{mdh}, \text{purA} \) and \( \text{recA} \)) out of 7 and these STs might therefore belong to a common clonal complex. In contrast, the 5 STs had only up to 2 alleles identical to ST131, suggesting the 5 STs and ST131 had different clonal origins.

Self-transmissible plasmids carrying \( \text{bla}_{\text{CTX-M-15}} \) were obtained from 7 out of the 8 isolates using mating as described previously [4]. The remaining isolate V12 did not yield transconjugants carrying \( \text{bla}_{\text{CTX-M-15}} \) despite repeated attempts but transformants carrying \( \text{bla}_{\text{CTX-M-15}} \) were obtained from V12 by electroporation with plasmids prepared using alkaline lysis [11]. This suggests that \( \text{bla}_{\text{CTX-M-15}} \) was carried by a non-conjugative plasmid in isolate V12. Plasmids carrying \( \text{bla}_{\text{CTX-M-15}} \) were prepared using alkaline lysis and were subjected to PCR-based replicon typing [12]. Four plasmids were of IncI1 and the other four belonged to the IncFII-related group, two of which contained replicons of IncFIA and IncFIB in addition to the IncFII-related replicon. IncF plasmid replicons sequence typing (RST) [13] and IncI1 plasmid MLST (pMLST) [14] were employed to investigate the relatedness of these plasmids carrying \( \text{bla}_{\text{CTX-M-15}} \) (Table 1). All of the IncI1 plasmids except the non-conjugative one from isolate V12 were of ST16 (1-5-10-8-6). According to the IncI1 pMLST database (http://pubmlst.org/plasmid/), ST16 IncI1 plasmids have been found carrying \( \text{bla}_{\text{CTX-M-15}} \) in isolates from cattle in the UK but were not found in human isolates carrying \( \text{bla}_{\text{CTX-M-15}} \) before. The identification of ST16 IncI1 plasmids in both cattle and human isolates suggested the transfer of \( \text{bla}_{\text{CTX-M-15}} \) between animal and human, which implicates that the control of the transmission of \( \text{bla}_{\text{CTX-M-15}} \) should address sources beyond human. Two alleles, \( \text{ardA} \) and \( \text{sogS} \), both of which are associated with conjugation, were unable to be amplified from the non-conjugative plasmid (pV12) carrying \( \text{bla}_{\text{CTX-M-15}} \) from isolate V12. Therefore, the ST could not be assigned for pV12 but the remaining three alleles of pV12 were identical to those of ST16, suggesting that pV12 might be derived from a ST16 IncI1 plasmid. Four different RST profiles were present for the four IncF plasmids carrying \( \text{bla}_{\text{CTX-M-15}} \), suggesting that the spread of \( \text{bla}_{\text{CTX-M-15}} \) in our local settings might have been mediated by multiple IncF plasmids. Among the four RST types of IncF plasmids identified here, F2:A: B- plasmids carrying \( \text{bla}_{\text{CTX-M-15}} \) appeared to be widely distributed and have been found in Canada, France, Italy and the UK (http://pubmlst.org/plasmid/), while the remaining three types, F1:A2:B1, F1:A2:B20 and F31:A-B- have not been deposited in the RST database, representing new IncF RST types.

The co-transfer of a few antimicrobial resistance genes including \( \text{qnrA}, \text{qnrB}, \text{qnrS}, \text{qepA}, \text{aac(6)-Ib-cr}, \text{aac(3)-II}\), \( \text{tet}A \), \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{OXA-1}} \) with \( \text{bla}_{\text{CTX-M-15}} \) were examined for transconjugants and transformants by PCR. Unlike previous studies, the co-transfer of resistance genes with \( \text{bla}_{\text{CTX-M-15}} \) except for \( \text{bla}_{\text{TEM-1}} \) was not common in this study (Table 1).

In summary, although \( \text{bla}_{\text{CTX-M-14}} \) was the most common \( \text{bla}_{\text{CTX-M}} \) variant in China, the present study together with recent reports [6,7] suggested that \( \text{bla}_{\text{CTX-M-15}} \) has emerged in China. The prevalence of \( \text{bla}_{\text{CTX-M-15}} \)-carrying isolates would compromise the efficacy of the widely-used broad-spectrum cephalosporins and therefore represents a serious challenge for clinical treatment and public health. The spread of \( \text{bla}_{\text{CTX-M-15}} \) in our local settings is mediated by two clonal complexes and by self-transmissible

### Table 1 Molecular characteristics of 8 \( E. \ coli \) isolates carrying \( \text{bla}_{\text{CTX-M-15}} \)

| Isolate | Source | ST    | Phylogenetic group | Inc group | pMLST/RST | Additional resistance genes |
|---------|--------|-------|--------------------|-----------|-----------|-----------------------------|
| A63     | urine  | 3513  | A                  | IncIA     | F1A:B-    | \( \text{bla}_{\text{TEM}}, \text{aac(3)-II} \) |
| H9      | urine  | 3342  | D                  | IncIA FIA FIB | F1:A2:B1 | \( \text{bla}_{\text{TEM}}, \text{aac(3-II)}, \text{aac(6)-Ib-cr} \) |
| D57     | urine  | 3516  | B2                 | IncIA FIA FIB | F1:A2:B20 | \( \text{bla}_{\text{TEM}}, \text{aac(3-II)} \) |
| W19     | urine  | 131   | B2                 | IncIA     | F2:A-B    | \( \text{bla}_{\text{TEM}}, \text{qnrS} \) |
| V12     | sputum | 3518  | B2                 | IncI1     | ST16 (1-5-10-8-6) | \( \text{bla}_{\text{TEM}}, \text{aac(3-II)} \) |
| I10     | sputum | 3517  | B2                 | IncI1     | ST16 (1-5-10-8-6) | \( \text{bla}_{\text{TEM}}, \text{qnrS} \) |
| U35     | urine  | 131   | B2                 | IncI1     | ST16 (1-5-10-8-6) | \( \text{ bla }_{\text{TEM}}, \text{aac(3-II)} \) |
| I20     | ascites| 131   | B2                 | IncI1     | ST16 (1-5-10-8-6) | \( \text{bla}_{\text{TEM}}, \text{qnrS} \) |

1 Inc, incompatibility; Inc group and pMLST/RST results are for plasmids carrying \( \text{bla}_{\text{CTX-M-15}} \).

2 ND, not determined.

3 Resistance genes that were co-transferred with \( \text{bla}_{\text{CTX-M-15}} \) are in bold.
plasmids of IncI1 or IncF. The spread of isolates carrying bla\textsubscript{CTX-M-15} in China warrants more studies. The interruption of both vertical and horizontal transmission of bla\textsubscript{CTX-M-15} using infection control measures such as standard precautions and contact precautions appear to be the key to hurdle the further spread of this antimicrobial resistance determinant.

Availability of supporting data
The data set supporting the results of this article is included within the article.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
ZL carried out the study, participated in the sequence alignment and helped to draft the manuscript. LX participated in the design of the study and helped to draft the manuscript. ZZ conceived of the study, participated in the sequence alignment and helped draft the manuscript. All authors read and approved the final manuscript.

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