ANNALS OF CLINICAL MICROBIOLOGY AND ANTIMICROBIALS

SHORT REPORT Open Access

The emergence of $bla_{CTX-M-15}$-carrying *Escherichia coli* of ST131 and new sequence types in Western China

Lei Zhang[1,2], Xiaoju Lü[1,2] and Zhiyong Zong[1,2*]

Abstract

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

Findings: Out of 144 *Escherichia coli* isolates from 20 hospitals in western China, 8 were found carrying $bla_{CTX-M-15}$. $bla_{CTX-M-15}$ was carried by isolates of ST131and 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. $bla_{CTX-M-15}$ was located on plasmids of IncI1 (ST16) or IncFII-related group (four replicon types). The co-transfer of a few antimicrobial resistance genes including *qnrA*, *qnrB*, *qnrS*, *qepA*, *aac (6')-Ib-cr*, *aac (3)-II*, *tetA*, bla_{TEM} and bla_{OXA-1} with $bla_{CTX-M-15}$ were examined but only bla_{TEM-1} was found co-transferring with $bla_{CTX-M-15}$.

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

Keywords: *Escherichia coli*, Plasmids, MLST, Beta-lactamases, Antimicrobial resistance

Findings

Escherichia coli producing extended-spectrum β-lactamases (ESBLs) is a challenge for clinical treatment and infection control. $bla_{CTX-M-15}$ has emerged as the most widely distributed gene encoding ESBLs globally, but it was not common in China [1]. We performed a snapshot survey on the molecular epidemiology of *E. coli* carrying $bla_{CTX-M-15}$ in Sichuan province, western China.

All non-duplicated *E. coli* clinical isolates (n = 144) that were recovered in 20 hospitals in Sichuan, western China, from June 22 to 25, 2011 were collected regardless of their antimicrobial susceptibility profiles and types of infections. The presence of bla_{CTX-M} genes were detected using both a single PCR with universal primers CTX-U1/U2 and a multiplex PCR [2,3] and 25.7% (37/144) isolates were found carrying bla_{CTX-M}. A total of 17 isolates had a bla_{CTX-M} gene belonging to the $bla_{CTX-M-1}$ group and the complete sequence of the $bla_{CTX-M-1}$-like gene in all 17 isolates was obtained using PCR with primers ISEcp1IR-F and orf477-R [4]. Sequencing revealed $bla_{CTX-M-15}$ in 8 isolates, corresponding to 21.6% of bla_{CTX-M} variants, and the remaining 9 isolates had either $bla_{CTX-M-55}$ (n = 8) or $bla_{CTX-M-57}$ (n = 1). The 8 isolates carrying $bla_{CTX-M-15}$ were from urine (n = 5), sputum (n = 2) and ascites (Table 1). Although $bla_{CTX-M-15}$ has become the dominant gene encoding ESBLs in many countries and *E. coli* of ST131 carrying $bla_{CTX-M-15}$ was widely distributed [5], $bla_{CTX-M-15}$ had only been occasionally found in isolates recovered before 2007 in China [1]. However, several studies on *E. coli* isolates obtained in 2007 and afterwards in China have found that $bla_{CTX-M-15}$ accounted to 14% to 24% of bla_{CTX-M} variants detected from human [6,7]. The present study and previous reports therefore suggested the emergence of *E. coli* carrying $bla_{CTX-M-15}$ in China in recent 5 years.

Six of the isolates carrying $bla_{CTX-M-15}$ belonged to the phylogenetic group B2 and the remaining two were of group A1 and D, respectively, determined as described previously [8] (Table 1). Using multi-locus sequence

* Correspondence: zongzhiy@scu.edu.cn
[1]Center of Infectious Diseases, West China Hospital, Sichuan University, Guoxuexiang 37, Chengdu 610041, China
[2]Division of Infectious Diseases, the State Key Laboratory of Biotherapy, Chengdu, China

Table 1 Molecular characteristics of 8 *E. coli* isolates carrying $bla_{CTX-M-15}$

Isolate	Source	ST	Phylogenetic group	Inc group[1]	pMLST/RST[1]	Additional resistance genes[3]
A63	urine	3513	A	FIIA	F31:A-:B-	**bla_{TEM}**, *aac (3)-II*
H9	urine	3342	D	FIIA FIA FIB	F1:A2:B1	**bla_{TEM}**, *aac (3)-II*, *aac (6')-Ib*-cr
D57	urine	3516	B2	FIIA FIA FIB	F1:A2:B20	**bla_{TEM}**, *aac (3)-II*
W19	urine	131	B2	FIIA	F2:A-:B-	**bla_{TEM}**, *qnrS*
V12	sputum	3518	B2	I1	1-ND[2]-10-ND[2]-6	
I10	sputum	3517	B2	I1	ST16 (1-5-10-8-6)	**bla_{TEM}**, *aac (3)-II*
U35	urine	131	B2	I1	ST16 (1-5-10-8-6)	*qnrS*
I20	ascites	131	B2	I1	ST16 (1-5-10-8-6)	*aac (3)-II*

[1]Inc, incompatibility; Inc group and pMLST/RST results are for plasmids carrying $bla_{CTX-M-15}$.
The allele number of the IncI1 pMLST profile (*repI*, *ardA*, *trbA*, *sogS* and *pilI*) is indicated.
[2]ND, not determined.
[3]Resistance genes that were co-transferred with $bla_{CTX-M-15}$ are in bold.

typing (MLST) [9], the 8 isolates carrying $bla_{CTX-M-15}$ were assigned to 6 sequence types (ST) including ST131 and 5 new STs (Table 1). Of note, *E. coli* of ST131 was found carrying $bla_{CTX-M-3}$, $_{-14}$ and $_{-65}$ but not $_{-15}$ in our local settings previously [10]. This study demonstrated the emergence of the globally-spread ST131 carrying $bla_{CTX-M-15}$ in our region. The 5 new STs (ST3342, ST3513, ST3516, ST3517 and ST3518) shared 5 identical alleles (*gyrB*, *icd*, *mdh*, *purA* and *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 $bla_{CTX-M-15}$ were obtained from 7 out of the 8 isolates using mating as described previously [4]. The remaining isolate V12 did not yield transconjugants carrying $bla_{CTX-M-15}$ despite repeated attempts but transformants carrying $bla_{CTX-M-15}$ were obtained from V12 by electroporation with plasmids prepared using alkaline lysis [11]. This suggests that $bla_{CTX-M-15}$ was carried by a non-conjugative plasmid in isolate V12. Plasmids carrying $bla_{CTX-M-15}$ 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 $bla_{CTX-M-15}$ (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 $bla_{CTX-M-15}$ in isolates from cattle in the UK but were not found in human isolates carrying $bla_{CTX-M-15}$ before. The identification of ST16 IncI1 plasmids in both cattle and human isolates suggested the transfer of $bla_{CTX-M-15}$ between animal and human, which implicates that the control of the transmission of $bla_{CTX-M-15}$ should address sources beyond human. Two alleles, *ardA* and *sogS*, both of which are associated with conjugation, were unable to be amplified from the non-conjugative plasmid (pV12) carrying $bla_{CTX-M-15}$ 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 $bla_{CTX-M-15}$, suggesting that the spread of $bla_{CTX-M-15}$ 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 $bla_{CTX-M-15}$ 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 *qnrA*, *qnrB*, *qnrS*, *qepA*, *aac(6′)-Ib*-cr, *aac(3)-II*, *tetA*, bla_{TEM} and bla_{OXA-1} with $bla_{CTX-M-15}$ were examined for transconjugants and transformants by PCR. Unlike previous studies, the co-transfer of resistance genes with $bla_{CTX-M-15}$ except for bla_{TEM-1} was not common in this study (Table 1).

In summary, although $bla_{CTX-M-14}$ was the most common bla_{CTX-M} variant in China, the present study together with recent reports [6,7] suggested that $bla_{CTX-M-15}$ has emerged in China. The prevalence of $bla_{CTX-M-15}$-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 $bla_{CTX-M-15}$ in our local settings is mediated by two clonal complexes and by self-transmissible

plasmids of IncI1 or IncF. The spread of isolates carrying $bla_{CTX\text{-}M\text{-}15}$ in China warrants more studies. The interruption of both vertical and horizontal transmission of $bla_{CTX\text{-}M\text{-}15}$ 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 coordination and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors are grateful for Yanyu Gao for the technical assistance. This work was supported by a grant from the National Natural Science Foundation of China (project no. 30900052) and was also partially supported by a grant from the US-China Collaborative on Emerging and Re-emerging Infectious Diseases.

Received: 21 August 2013 Accepted: 24 November 2013
Published: 27 November 2013

References

1. Yu Y, Ji S, Chen Y, Zhou W, Wei Z, Li L, Ma Y: **Resistance of strains producing extended-spectrum β-lactamases and genotype distribution in China.** *J Infect* 2007, **54:**53–57.
2. Mulvey MR, Soule G, Boyd D, Demczuk W, Ahmed R: **Characterization of the first extended-spectrum β-lactamase-producing *Salmonella* isolate identified in Canada.** *J Clin Microbiol* 2003, **41:**460–462.
3. Woodford N, Fagan EJ, Ellington MJ: **Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β-lactamases.** *J Antimicrob Chemother* 2006, **57:**154–155.
4. Zong Z, Partridge SR, Thomas L, Iredell JR: **Dominance of $bla_{CTX\text{-}M}$ within an Australian extended-spectrum β-lactamase gene pool.** *Antimicrob Agents Chemother* 2008, **52:**4198–4202.
5. Rogers BA, Sidjabat HE, Paterson DL: ***Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain.** *J Antimicrob Chemother* 2011, **66:**1–14.
6. Cao X, Cavaco LM, Lv Y, Li Y, Zheng B, Wang P, Hasman H, Liu Y, Aarestrup FM: **Molecular characterization and antimicrobial susceptibility testing of *Escherichia coli* isolates from patients with urinary tract infections in 20 Chinese hospitals.** *J Clin Microbiol* 2011, **49:**2496–2501.
7. Li B, Sun JY, Liu QZ, Han LZ, Huang XH, Ni YX: **High prevalence of CTX-M β-lactamases in faecal *Escherichia coli* strains from healthy humans in Fuzhou, China.** *Scand J Infect Dis* 2011, **43:**170–174.
8. Clermont O, Bonacorsi S, Bingen E: **Rapid and simple determination of the *Escherichia coli* phylogenetic group.** *Appl Environ Microbiol* 2000, **66:**4555–4558.
9. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, *et al*: **Sex and virulence in *Escherichia coli*: an evolutionary perspective.** *Mol Microbiol* 2006, **60:**1136–1151.
10. Zong Z, Yu R: **$bla_{CTX\text{-}M}$-carrying *Escherichia coli* of the O25b ST131 clonal group have emerged in China.** *Diagn Microbiol Infect Dis* 2011, **69:**228–231.
11. Sambrook J, Russell DW: *Molecular Cloning. A laboratory manual, vol. 1.* Cold Spring Harbour, NY: Cold Spring Harbour Laboratory Press; 2001.
12. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ: **Identification of plasmids by PCR-based replicon typing.** *J Microbiol Methods* 2005, **63:**219–228.
13. Villa L, Garcia-Fernandez A, Fortini D, Carattoli A: **Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants.** *J Antimicrob Chemother* 2010, **65:**2518–2529.
14. Garcia-Fernandez A, Chiaretto G, Bertini A, Villa L, Fortini D, Ricci A, Carattoli A: **Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum β-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin.** *J Antimicrob Chemother* 2008, **61:**1229–1233.

doi:10.1186/1476-0711-12-35
Cite this article as: Zhang *et al.*: The emergence of $bla_{CTX\text{-}M\text{-}15}$-carrying *Escherichia coli* of ST131 and new sequence types in Western China. *Annals of Clinical Microbiology and Antimicrobials* 2013 **12**:35.