Molecular Characteristics of ST1193 Clone among Phylogenetic Group B2 Non-ST131 Fluoroquinolone-Resistant Escherichia coli

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Objectives: Sequence type 1193 is emerging as a new, virulent and resistant lineage among fluoroquinolone resistant Escherichia coli (FQr E. coli). In this study, we investigated the prevalence and molecular characteristics of this clone isolated from a Chinese university hospital.

Methods: 73 phylogenetic group B2-FQr-non-ST131 isolates were collected from August 2014 and August 2015 at a Chinese university hospital. Isolates were screened for ST1193 by multilocus sequence typing. E. coli ST1193 then underwent lactose fermentation determination, susceptibility testing, virulence genotyping, PCR-based O typing, pulsed-field gel electrophoresis (PFGE) and FQr mechanism analysis.

Results: Of 73 B2-FQr-non ST131 E. coli isolates, 69.9% (n = 51) were ST1193. 90.2% (46/51) of ST1193 isolates were O75 serotype and 96.1% (49/51) of the ST1193 isolates were lactose non-fermenters. 35 clusters were identified by PFGE. ST1193 isolates exhibited a set of 3 conserved mutations defining quinolone-resistance determining region substitutions (gyrA S83L, D87N, and parC S80I). The most frequent VF genes detected in these E. coli ST1193 isolates were fyuA (yersiniabactin, 96.1%), fimH (type 1 fimbriae, 94.1%), iutA (iron uptake gene, 90.2%), kpsMT II (group II capsule, 90.2%), kpsK1 (K1 capsule, 86.3%) and PAI.

Conclusion: ST1193 lineage accounts for the majority of group B2-FQr-non-ST131 E. coli clinical isolates. Most of the ST1193 are serotype O75 and lactose non-fermenting. Strategic surveillance and control schemes are needed in the future for this newly emerging clone of E. coli: B2-FQr-ST1193.

Keywords: Escherichia coli, ST1193, fluoroquinolone resistance, phylogenetic group B2, O75

INTRODUCTION

Escherichia coli (E. coli) is commonly found in the gut flora of humans and can cause many different kinds of extra-intestinal infections, such as septicemias, meningitis and urinary tract infections (Pitout, 2012; Poolman and Wacker, 2016). Many methods can be used for typing E. coli, including serotyping, pulsed-field gel electrophoresis (PFGE) and various PCR-based molecular methods.
Multilocus sequence typing (MLST) is a PCR-based molecular typing method and is considered the “gold standard” subtyping technique that can usually be used to detect the genetic relatedness between strains and identify a specific clone with high discriminatory ability for various bacterial pathogens (Maiden et al., 2000; Cespedes et al., 2017; Roer et al., 2017). Successful MLST clones of E. coli have been reported in various regions of the world (Maiden et al., 2000; Dautzenberg et al., 2016). E. coli ST131 is a classic clonal group from phylogenetic group B2. It typically possesses a wide variety of virulence factors (VFs) and is strongly associated with resistance to fluoroquinolone and extended spectrum cephalosporins (Maiden et al., 2000; Dautzenberg et al., 2016).

Simultaneously, ST1193 has been reported recently, as a new, virulent and resistant E. coli that commonly causes extra-intestinal infections (Platell et al., 2012; Chang et al., 2014; Matsumura et al., 2017). E. coli ST1193 shares many common features, including fluoroquinolone resistance (FQr), lactose non-fermenting and phylogenetic group B2. It typically possesses a wide variety of virulence factors (VFs) and is strongly associated with resistance to fluoroquinolone and extended spectrum cephalosporins (Maiden et al., 2000; Dautzenberg et al., 2016).

Virulence Gene Profiling
The presence of 26 known VFs genes was screened by a multiplex PCR method (Johnson and Stell, 2000). For each isolate, the virulence score (VF score) was calculated as the sum of all virulence-associated genes detected in this study. The sum of all the VF scores of the isolates was then calculated, and finally this sum was divided by the number of isolates to give the mean VF score.

Detection of Fluoroquinolone Resistance Genes and blaCTX-M Gene
Escherichia coli ST1193 isolates resistant to cefotaxime and/or ceftazidime underwent detection of blaCTX-M using PCR and sequencing (Li et al., 2011). As to all ST1193 isolates, mutations in the quinolone-resistance determining region (QRDR) in chromosomal gyrA, parC and parE genes were determined by PCR and sequencing (Everett et al., 1996). The presence of plasmid mediated quinolone resistance determinants [PMQRs; qnrA, qnrB, qnrC, qnrD, qnrS, and aac(6’)-Ib-cr] were detected by PCR as previously described (Chen et al., 2012).

RESULTS
Bacterial Isolates
One hundred and thirty one B2-FQr E. coli isolates were collected in this study, including 58 ST131 and 73 non-ST131
strains. 73 B2-FQ3-based ST131 E. coli isolates were grouped into 10 distinct STs, with the predominant ST being ST1193 (n = 51, 69.9%), followed by ST95 (n = 4, 5.5%), ST140 (n = 3, 4.1%), ST648 (n = 3, 4.1%), ST73 (n = 3, 4.1%), ST92 (n = 2, 2.7%), ST117 (n = 2, 2.7%), ST354 (n = 2, 2.7%), ST1318 (n = 1, 1.4%), ST2558 (n = 1, 1.4%), and ST3177 (n = 1, 1.4%). The majority of E. coli ST1193 strains (34, 66.7%) were isolated from urine, followed by blood (n = 7, 13.7%), secretions (n = 6, 11.8%), fluids (n = 3, 5.9%), and pus (n = 1, 2.0%). Females accounted for 72.5% (37/51) of the ST1193 isolates, and the average age was 65 years (range: 10–91 years). E. coli ST1193 strains were isolated from different wards (Supplementary Table S1).

**O Typing and Lactose Fermentation Determination**

90.2% (46/51) of the E. coli ST1193 were classified as O-type O75. The other five isolates could not be typed. 96.1% (49/51) of the ST1193 isolates were lactose non-fermenters, including 46 O75 and 3 non-O typeable isolates.

**Antimicrobial Susceptibility Testing**

Escherichia coli ST1193 isolates showed high resistance against trimethoprim–sulfamethoxazole (64.7%). Resistance rates were low than 50% to cefotaxime (45.1%), aztreonam (21.6%), ceftapepine (19.6%), ceftazidime (9.8%), and amikacin (7.8%), respectively. All the strains were susceptible to imipenem and cefepime (19.6%), ceftazidime (9.8%), and amikacin (7.8%). Resistance rates were high with ST131 among group B2-FQr. Meanwhile, two isolates harbored blacTX-M variants and none – Ib-cr variants found in our B2-FQr isolates.

**Fluoroquinolone Resistance Characteristics and blacCTX-M Gene**

All of the 51 E. coli ST1193 isolates contained a set of 3 conserved mutations defining QRDR substitutions (gyrA S83L, D87N, and parC S80I). Two ST1193 isolates had additional mutations in the QRDR of parC (N167Y). No mutation was found in parE. Meanwhile, two isolates harbored aac(6′)-Ib-cr variant and none of other PMQR determinants (qnrA, qnrB, qnrC, qnrD, qnrS, oqxAB, and qepA) were detected in this study.

Among 23 ceftoxatime and/or ceftazidime E. coli ST1193 isolates, 22 (95.7%) contained blacCTX-M genes. Of these blacCTX-M positive isolates, 12 isolates carried blacCTX-M-14, 6 isolates carried blacCTX-M-15 and 2 isolates carried blacCTX-M-123. The remaining 2 isolates co-harbored blacCTX-M-14 and blacCTX-M-15. blacCTX-M-2, blacCTX-M-8 and blacCTX-M-25 groups were not detected in these isolates.

**Virulence Gene Profiling**

The most frequent VF genes were fyuA (yersiniabactin, 96.1%), fimH (type 1 fimbriae, 94.1%), iutA (iron uptake gene, 90.2%), kpsMTII (group II capsule, 90.2%), kpsK1 (K1 capsule, 86.3%) and PAI (86.3%), each of which was detected in ≥80% of the isolates. Four genes were identified in less than 10% of isolates, including kpsK5 (7.8%), sfa/focDE (7.8%), traT (5.9%), and papG allele II (2.0%). kpsMTIII, papAH, papDE, papC, papG alleleI, rfaE, rfc, papGII/III, hlyA, sfaS, gspD, cvaC, cdtB, focG, cnf1, and afa/draBC were not detected in this study.

**DISCUSSION**

Fluoroquinolones are the most widely used antimicrobial agents for the treatment of different infections in China. The high frequency of fluoroquinolone resistance in E. coli is considered as a feature of clinical bacteriology in the last 10 years in China according to a CHINET report (Hu et al., 2016). Fluoroquinolone resistance usually occurred in low-virulence E. coli phylogenetic group A isolates rather than in isolates from groups B2 and D. However, the recent worldwide emergence of B2-FQr-E. coli has occurred primarily through clonal expansion of E. coli ST131 (Nicolas-Chanoine et al., 2014; Dautzenberg et al., 2016). Our study confirmed that while ST131 is still the predominant subclone among B2-FQr-E. coli clinical isolates in China, ST1193 is emerging as an important subgroup and accounts for a similar percentage with ST131 among group B2-FQr-E. coli. The reasons for the expansion of such subclone are unclear and it is worth investigating in future study to explain its dissemination in China.

Historically, E. coli serotype O75 isolates are strongly associated with urinary tract infection and sepsis in humans, and virulence-associated phylogenetic group B2 (Devine et al., 1989; Obata-Yasuoka et al., 2002; Platell et al., 2012). Moreover, O75 strains are usually non-lactose fermenter (Platell et al., 2012; Chang et al., 2014). Our results were consistent with the results from previous studies as the majority of our ST1193 isolates were non-lactose fermenter (96.1%) and belonged to serotype O75 (90.2%). Because O75 isolates were never found to be associated with FQ resistance in previous studies, the recent emergence of O75-B2-FQr-ST1193 E. coli isolates are probably due to clonal expansion (Platell et al., 2012).

In this study, ciprofloxacin resistance was mainly due to the accumulation of three point mutations in DNA gyrase or topoisomerase IV (gyrA S83L, D87N, and parC S80I), which are the primary bacterial target of quinolone (Hooper and Jacoby, 2015; Correia et al., 2017). The pattern of non-synonymous mutations found in our B2-FQr-ST1193 isolates was identical to those observed in Australian and Korean ST1193 isolates (Platell et al., 2012; Chang et al., 2014). As discussed in previous study (Platell et al., 2012), the homogeneity of ST1193 isolates suggests this subclone emerged and spreaded from a common ancestor. Meanwhile, previous study suggested that more distinctive QRDR mutations probably provided E. coli ST131 H30 isolates with fitness advantages over other fluoroquinolone resistant E. coli isolates (Johnson et al., 2015). In this study, all the E. coli ST1193 isolates contained the same distinctive combination of three non-synonymous mutations (gyrA S83L, D87N, and parC S80I). These three QRDR mutations found in
ST1193 isolates are the only ones that have been experimentally proved to increase fluoroquinolone MICs at the present time (Marcusson et al., 2009). Therefore, non-synonymous QRDR mutations in gyrA and parC may play a similar role in the fitness of E. coli ST1193 isolates as what they do in ST131 lineage.

The clonal dissemination of blaCTX-M-55 among E. coli isolates causing urinary tract infections maybe due to the expansion of ST1193 clone in one previous study (Xia et al., 2017). In this study, we attempted to identify the blaCTX-M genes in our third generation cephalosporin resistant ST1193 isolates. Although our results showed a high percentage (95.7%) of third generation cephalosporin resistant isolates harboring different blaCTX-M genes, no correlation between ST1193 clone and any special blaCTX-M genes was found in this study. Regional difference may be the main reason for the disparity of these two studies. Further nationwide study should be carried out to explain the difference in China.

Identical virulence gene profiles as one common phenotypic characteristic were presenting in previous studies (Platell et al., 2012; Cremet et al., 2013). In this study, we also exhibited identical virulence genes profile (fimH, fyuA, iutA, kpsMT II, kpsK1, and PAI) among most of the ST1193 isolates. Together with the results of serotyping, fluoroquinolone resistance and phylogenetic group B2, the high level of homogeneity between ST1193 strains from different geographical regions suggests a probable divergence from a common ancestor.

Meanwhile, we identified a total of 35 PFGE types using PFGE analysis in this study, and no dominant intra-hospital PFGE type
was found using a cutoff of 85% similarity. Our results suggested that *E. coli* ST1193 clinical isolates were not spread by clonal strains in our hospital.

**CONCLUSION**

ST1193 lineage account for the majority of B2-FQ-non-ST131 *E. coli* population in this study. These ST1193 isolates are highly clonal diversity as indicated by PFGE patterns. Most of the ST1193 isolates were serotype O75 and lactose non-fermenting. Strategic surveillance and control schemes are needed in the future for this newly emerging clone of *E. coli*: B2-FQ-ST1193.

**ETHICS STATEMENT**

This study was approved by The Institutional Review Board of the Fujian Medical University Union Hospital, Fuzhou, China. No consent was needed for this study.

**AUTHOR CONTRIBUTIONS**

JW, FL, YL, and QH performed experiments. JW and FL conceived the study and analyzed the results. BL supervised the study and prepared the manuscript. All authors read and approved the final manuscript.

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**SUPPLEMENTARY MATERIAL**

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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