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Predominance of pHK01-like incompatibility group FII plasmids encoding CTX-M-14 among extended-spectrum beta-lactamase–producing *Escherichia coli* in Hong Kong, 1996–2008☆,☆☆

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ABSTRACT

This study assessed the temporal changes in the molecular epidemiology of bacteremic *Escherichia coli* isolates producing CTX-M-14 in Hong Kong. Blood isolates from 1996 to 1998 (period 1, $n = 50$) and 2007 to 2008 (period 2, $n = 117$) were investigated by molecular methods. CTX-M–type ESBL was carried by 98.2% (164/167) of the isolates. In both periods, the CTX-M-9 group and CTX-M-14 allele were the predominant ESBL type. The major clones were found to change from ST68 and ST405 in period 1 to ST131, ST69, and ST12 in period 2. Among 65 CTX-M-14–producing plasmids investigated further, 54 had the FII replicon. Replicon sequence typing and plasmid polymerase chain reaction–restriction fragment length polymorphism showed that 79.6% (43/54) of the FII plasmid subset was similar to the completely sequenced plasmid, pHK01 (human urine, Hong Kong, 2004). These pHK01-like plasmids were found to have spread to the major clones (ST68, ST405, and ST131) and multiple singleton isolates of all 4 phylogenetic groups.

1. Introduction

In Asia, the burdens of cefotaximase (CTX-M)-producing Enterobacteriaceae have increased dramatically in the last decade (Hawkey, 2008; Ho et al., 2005, 2007). Studies from Hong Kong, mainland China, South Korea, and Thailand have consistently showed that CTX-M-14 is the most prevalent enzyme (Ho et al., 2007; Lo et al., 2010; Nguyen et al., 2010; Yi et al., 2010). This CTX-M allele was first described in 2001 among *Escherichia coli*, *Klebsiella pneumoniae*, and *Shigella* isolates collected from different parts of South Korea in 1995 (Pai et al., 2001). While clonal spread has been reported in Japan and Canada (Pitout et al., 2005; Suzuki et al., 2009), the spread of CTX-M-14 has been found to be mainly caused by conjugative plasmids (Cottell et al., 2011; Ho et al., 2011c; Valverde et al., 2009). In Spain, the spread of CTX-M-14 from 2000 to 2005 was largely due to pRYC105-like plasmids of the IncK incompatibility group disseminated among diverse *E. coli* lineages (Valverde et al., 2009). Recently, it has been shown that pRYC105 is similar to the pCT plasmid that has been found in bacteria from the United Kingdom, mainland China, and Australia (Cottell et al., 2011). Recently, we sequenced the IncFII epidemic plasmid, pHK01, and showed that it has disseminated widely among *E. coli* isolates collected from patients with community-acquired urinary tract infections in 2004 (Ho et al., 2007; Ho et al., 2011c).Variants closely related to pHK01 have been identified among Enterobacteriaceae isolates from mainland China and Vietnam (Ho et al., 2011c; Nguyen et al., 2010; Yi et al., 2010).

In this study, we investigated the clonal structure and relatedness of plasmids encoding CTX-M-14 for a collection of blood culture *E. coli* isolates from patients treated in a healthcare region from 1996 to 2008.

2. Methods

2.1. Bacterial strains and susceptibility testing

The *E. coli* isolates were recovered from blood cultures of patients who were treated in a healthcare region in Hong Kong (Ho et al., 2002, 2005). The healthcare region (QMH) includes a network of 5 public hospitals, including 1 acute care university teaching hospital with 1500 beds and all the clinical disciplines including renal, liver, and bone marrow transplantation service, and 4 convalescence care hospitals with 110 to 524 beds. (Ho et al., 2005). This study included isolates recovered from 2 time periods, 1996–1998 (period 1) and 2007–2008 (period 2). The proportion of extended-spectrum β-

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lactamases (ESBL)–producing *E. coli* among all blood culture *E. coli* isolates for period 1 and period 2 was 6.6% (74/1127) and 26.8% (253/945), respectively (Ho et al., 2002, 2011b). For period 1, 67.6% (50/74) of the ESBL-producing isolates remained viable and all were included. The clinical characteristics for the 50 patients have been described (Ho et al., 2002). For period 2, 46.2% (117/253) of the ESBL-producing isolates from 253 patients (1 per patient) were randomly selected for inclusion. Only 1 ESBL-producing isolate per patient was included in this study. For those patients with more than 1 isolate, only the first isolate was tested. The VITEK GNI system (bioMérieux, Vitek, Hazelwood, MO, USA) was used for bacterial identification. The disc diffusion method was used for susceptibility testing and results were interpreted according to the Clinical and Laboratory Standard Institute (CLSI) guidelines (Clinical and Laboratory Standard Institute, 2011). Production of ESBL was determined by the double-disc synergy test (Ho et al., 2000, 2007).

2.2. CTX-M gene detection and epidemiologic typing

Genes related to the CTX-M families were sought by polymerase chain reaction (PCR) and sequencing using primers previously described (Lo et al., 2010; Saladin et al., 2002). Selected isolates were studied by pulsed-field gel electrophoresis (PFGE) of *Xba*I-digested genomic DNA (Amersham Pharmacia Biotech, Little Chalfont, UK), and patterns were analysed with Gelcompar II software (Applied Maths TX, USA). A multiplex PCR was used to assign the *E. coli* isolates to one of the 4 main phylogenetic groups (A, B1, B2, and D) (Ho et al., 2007). Multilocus sequencing was performed as described (Wirth et al., 2006). PCR reactions were used for detection of the 15 major O serotypes (O1, O2, O4, O6, O7, O11, O12, O15, O16, O18, O25, O75, O86, O102, and O157) and of the O25b subtype (Clermont et al., 2007; Clermont et al., 2009; Deschamps et al., 2009).

2.3. Conjugation and replicon typing

Conjugation experiments and sizing of plasmids were carried out as described previously (Ho et al., 2011c, 2011d). The replicon (*rep*) content in the plasmids was determined by PCR and sequencing (Carattoli et al., 2005). When a transconjugant cannot be obtained, the replicon type for the plasmid encoding CTX-M-14 was determined in the parent strains. In all the isolates, the replicon location in the plasmids was confirmed by hybridization with probes specific for $bla_{\text{CTX-M-14}}$ and *rep* amplified by PCR from different samples (Ho et al., 2011c, 2011d). The FII plasmids were categorized by the FAB (FII, FIA, FIB) formula using the replicon sequence typing scheme (Villa et al., 2010).

2.4. Analysis of $bla_{CTX\text{-}M\text{-}14}$ genetic support

Based on published studies and sequences deposited in the GenBank database, 8 different types of $bla_{\text{CTX-M-14}}$ genetic support have been reported. For purposes of description, they were denoted types I to VIII. Monoplex PCRs using different primer pair combinations were used to map the genetic support to one of the recognized type (Table S1).

2.5. PCR-RFLP analysis of FII plasmids

The FII plasmids were analysed further by a PCR–restriction fragment length polymorphism (PCR-RFLP) method previously described by us (Table S2) (Ho et al., 2011c).

2.6. Statistical analysis

The chi-square test was used to compare categorical variables between the 2 time periods. A P value of <0.05 was considered to indicate statistical significance. All statistical analysis was performed by the Epi Info software (version 3.5.1, Centers for Diseases Control and Prevention Atlanta, USA). A Dice coefficient similarity threshold of 85% was used to resolve unique PFGE groups (Ho et al., 2007; Lo et al., 2010).

3. Result

3.1. Antimicrobial susceptibilities and ESBL types

The isolates often exhibit co-resistance to the non–β-lactam antibiotics. The overall resistance rates for isolates from period 1 (n = 50) and period 2 (n = 117) were as follows: cotrimoxazole, 84% and 54.7%; ciprofloxacin, 78% and 59.8%; and gentamicin, 78% and 48.7%. Most isolates were susceptible to amikacin (98% and 97.4%) and piperacillin–tazobactam (84% and 97.4%). All isolates were susceptible to imipenem at the traditional breakpoint (inhibition zone diameter ≥16 mm, equivalent to MIC ≤4 mg/L). All isolates in period 2 were susceptible to imipenem at the new CLSI breakpoint (inhibition zone diameter ≥23 mm and equivalent to MIC ≤1 mg/L). However, only 90% of the isolates from period 1 were susceptible and 10% (5/50) of the isolates were imipenem-intermediate (inhibition zone diameters, 20–22 mm, and equivalent to MIC 2 mg/L).

PCR with CTX-M–specific primers showed that CTX-M–type ESBL was carried by 98.2% (164/167) of the ESBL-producing isolates (Table 1). The CTX-M gene in 109 isolates was sequenced, and a total of 5 alleles (CTX-M-14, -9, -27, -15, and -79) were found. In both periods, the CTX-M-9 subgroup and CTX-M-14 allele were the predominant CTX-M–type ESBL.

3.2. Epidemiologic typing of strains

Table 2 summarises the findings from the epidemiologic typing. Phylogenetic PCR showed that 56.3% (94/167) of the isolates were phylogenetic group D, 34.1% (57/167) were group B2, 7.2% (12/167) were group A, and 2.4% (4/167) were group B1. The proportion of phylogenetic group D had decreased from 84% (42/50) in period 1 to 44.4% (52/117) in period 2 (P < 0.001, chi-square test). Conversely, phylogenetic B2 isolates had increased from 6% (3/50) in period 1 to 46.2% (54/117) in period 2 (P < 0.001).

Overall, 41.3% (69/167) of the isolates could be assigned to 5 clones: ST68, ST405, ST131, ST69, and ST12 (Table 2). All 5 clones were producers of the CTX-M-14 allele. The remaining 98 isolates were either singleton (n = 85) or PFGE nontypeable (n = 13). Strains belonging to the ST68 and ST405 clones had decreased from 30% (15/50) and 26% (13/50) in period 1 to 2.6% (3/117) and 0% (0/117) in

Table 1
Distribution of CTX-M subgroups and alleles among the ESBL-producing *E. coli* isolates.

	n (%)		
	Period 1	Period 2	Total
CTX-M subgroup[a]			
M9	49 (98.0)	101 (86.3)	150 (89.8)
M1		12 (10.3)	12 (7.2)
M1 + M9		2 (1.7)	2 (1.2)
Negative	1 (2.0)	2 (1.7)	3 (1.8)
Subtotal	50 (100)	117 (100)	167 (100)
CTX-M allele[b]			
CTX-M-14	47	56	103
CTX-M-9	2	1	3
CTX-M-27		1	1
CTX-M-15		1	1
CTX-M-79		1	1
Subtotal	49	60	109

[a] PCR with CTX-M subgroup-specific primers.
[b] CTX-M-14, -9, and -27 are members of the CTX-M-9 subgroup, while CTX-M-15 and -79 are members of the CTX-M-1 subgroup. All CTX-M-14 sequences were identical to the CTX-M-14a variant (AF252622).