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Characteristics of OXA-48-Producing *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella oxytoca* from a Chinese Hospital

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Abstract

Objectives: The OXA-48-like enzymes are mainly produced by various *enterobacteriaceae*. The aim of this study was to investigate the characteristics of OXA-48-producing *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella oxytoca* from China.

Methods: Carbapenemase genes (bla_{VIM}, bla_{OXA-48}, bla_{KPC}, bla_{IMP} and bla_{NDM}) were screened by PCR and subsequent amplicons sequencing. Genetic relationship was investigated by pulsed field gel electrophoresis and multilocus sequence typing. The size and incompatibility types of bla_{OXA-48}-carrying plasmids were analyzed by the S1-PFGE, Southern blot and multiple PCR.

Results: Several OXA-48-producing enterobacterial isolates were collected, including three *E. coli*, three *E. cloacae* and one *K. oxytoca*. All isolates exhibited low-levels of carbapenem resistance or even were susceptible to imipenem and meropenem. The *E. coli* isolates belonged to ST156, ST648 and ST3554, while the *E. cloacae* were ST418 and ST414, respectively. All isolates harbored the same ~60 kb IncL/M bla_{OXA-48}-carrying plasmid.

Conclusions: This is the first report of OXA-48-producing *E. cloacae* ST414 and ST418 worldwide, and OXA-48-producing *K. oxytoca* in China. Although the bla_{OXA-48} gene prevalence is at a low frequency in China, the bla_{OXA-48}-carrying plasmid has spread among different *enterobacteriaceae* species.

Keywords: OXA-48; *Escherichia coli*; *Enterobacter cloacae*; *Klebsiella oxytoca*

Introduction

Since the first report from Turkey in 2004, the OXA-48-like enzymes have been found in many countries, which exhibit some regional specificity, particularly in Mediterranean and European countries and in India [1]. The OXA-48-like enzymes are mainly produced by *Klebsiella pneumonia*, while other *Enterobacteriaceae*, such as *Escherichia coli*, *Enterobacter cloacae*, *E. aerogenes*, *Citrobacter freundi*, *K. oxytoca*, *Serratia marcescens*, *Morganella morganii*, can also be the host [2-5].

In China, we first reported a nosocomial outbreak of OXA-48-producing *K. pneumoniae* in 2016 [6]. Thereafter, bla_{OXA-48}-carrying *E. coli* and *E. cloacae* have also been identified [7]. Meanwhile, OXA-48-producing *K. pneumoniae* and *E. coli* have been confirmed to be imported from Europe to China [8]. However, the epidemiological relationship and molecular characteristics of OXA-48-producing *Enterobacteriaceae* other than *K. pneumoniae* remain unknown. In this study, we collected several bla_{OXA-48}-positive enterobacterial isolates, including three *E. coli*, three *E. cloacae* and one *K. oxytoca*. The phenotypic and genotypic characteristics of these isolates were analyzed.

Materials and Methods

All clinical isolates were collected from Chinese PLA general hospital and identified by MALDI-TOF MS (BioMérieux). This study did not require formal ethical approval, because we only analyzed the characteristics of clinical isolates that were collected during routine bacteriological analyses and no human participants were involved. Antibiotic susceptibility testing was performed by VITEK 2 AST-GN09 and AST-GN13 cards (BioMérieux, Inc.). For the isolates that exhibited non-susceptibility to carbapenems, carbapenemase genes (bla_{VIM}, bla_{OXA-48}, bla_{KPC}, bla_{IMP} and bla_{NDM}) were screened by PCR and subsequent amplicons sequencing [6]. Genetic relationship was investigated by pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). The size and incompatibility types of bla_{OXA-48}-carrying plasmids were analyzed by the S1-PFGE, Southern blot and multiple PCR as previously described [6].

Results and Discussion

In this study, seven enterobacterial strains were isolated from urine and sputum samples of patients in the Respiratory Intensive Care Unit (RICU), including three *E. coli*, one *K. oxytoca* and three *E. cloacae*, of which two strains isolated from a single patient were reported before [7]. All isolates exhibited high-levels resistance to cefotaxime, piperacillin/tazobactam, amikacin, and ciprofloxacin, while exhibited heterogeneous carbapenem resistance patterns (Table 1). Most isolates showed low-levels of carbapenem resistance, or were even susceptible

to imipenem and meropenem, indicating the weak activity of OXA-48 against carbapenems.

No.	Species	Source	Isolation date	PFGE type	MLST	Minimal inhibitory concentration (mg/L)								
						CTX	CAZ	FEP	TZP	IPM	MEM	ETP	AMK	CIP
IR53017*	*E. coli*	Urine	7/1/2015	EC1	ST648	>64	16	>64	64	<1	<1	>8	>64	>4
IR53006	*E. coli*	Sputum	6/28/2016	EC1	ST3554	>64	4	>64	>128	8	1	4	>64	>4
IR53034	*E. coli*	Sputum	4/24/2017	EC2	ST156	>64	16	>64	>128	4	1	4	>64	>4
IR5392	*K. oxytoca*	Sputum	8/20/2015	N/A	N/A	>64	16	8	>128	8	1	4	>64	>4
IR5283*	*E. cloacae*	Sputum	8/21/2015	EL1	ST418	>64	4	16	64	8	4	4	>64	>4
IR53043	*E. cloacae*	Tissue	11/12/2017	EL2	ST418	8	<1	<1	>128	8	4	>8	<2	>4
IR5473	*E. cloacae*	Sputum	1/12/2018	EL3	ST414	>64	16	>64	>128	8	4	4	>64	1

*: Strains were isolated from a single patient that had been reported [7]. N/A: not applicable

CTX: cefotaxime; CAZ: ceftazidime; FEP: cefepime; TZP: piperacillin/tazobactam; IPM: imipenem; MEM: meropenem; ETP: ertapenem; AMK: amikacin; CIP: ciprofloxacin

Table 1: Characteristics of OXA-48 -producing enterobacterial isolates.

The *E. coli* IR53017 and IR53006 showed an identical PFGE pattern, but belonged to ST648 (allelic profile 92-4-87-96-70-58-2) and ST3554 (allelic profile 92-4-87-96-70-58-128), respectively. Their minimum inhibitory concentrations of imipenem dropped from 8 to <1 mg/L (Table 1). These suggested that with the clone's spreading, small genetic mutations appeared, and these mutations may lead to the changes in the resistance phenotype. In addition, although they have identical PFGE pattern, different genotypic and phenotypic characteristics indicate that they have significant difference in character. This also shows the defects of the PFGE method in the analysis of homology.

In total, OXA-48-producing *E. coli* ST9, ST10, ST38, ST43, ST46, ST69, ST88, ST101, ST127, ST131, ST155, ST167, ST216, ST362, T405, ST410, ST617, ST648, ST746, ST963 have been reported [2,4,9]. In China, OXA-48-producing *E. coli* ST69 and ST405 have been identified [8,10]. In this study, we found *E. coli* ST648 that appeared in Europe and North African [2,9], and *E. coli* ST156 that has never been reported before. OXA-48-producing *E. cloacae* have been identified worldwide [2,3,5]. In this study, OXA-48-producing *E. cloacae* ST414 and ST418 were identified, and to the best of our knowledge, this is the first report for these clones. The *E. cloacae* ST418 was the most common carbapenemase-producing *E. cloacae* clone in China, accounting for 20% [11]. However, the majority of ST418 produced NDM-1 [11]. This clone was genetically closer to ST127 and ST755, and may have the advantages of being the main epidemic strains. Furthermore, OXA-48-producing *K. oxytoca* have been discovered worldwide [2,3,5], but this is the first report in China.

With its global spreading, bla_{OXA-48}-like genes have been found in different species of organisms, and is mostly associated with the dissemination of a particular broad host-range conjugative ~60 kb IncL/M plasmid [12,13]. In this study, plasmid analysis revealed that all isolates harbored the same ~60 kb IncL/M bla_{OXA-48}-carrying plasmid. Although the genetic environment of bla_{OXA-48} was not analyzed in this study, it could be inferred that the IS1999 element might be located in the upstream of the bla_{OXA-48} gene and truncated by IS1R because of the same plasmid size and type as the plasmid analyzed in our previous study [6]. Noticeably, almost all of the OXA-producing strains were isolated from the same ward (RICU), and carried the same ~60kb IncL/M plasmid, indicating the great transfer ability of bla_{OXA-48} in different strains of *Enterobacteriaceae* species, as described previously [7,14,15]. It has reported that carbapenemase-producing *Enterobacteriaceae* can survive over several months [16], and infected/colonized patients may be considered as reservoirs that can act as hidden disseminators [14]. As a consequence, a dynamic surveillance is needed due to its potential transferability and prolonged persistence, although the bla_{OXA-48} gene prevalence is at a low frequency in China.

The present study has the following limitations. The main drawback of the study is the paucity of information on the clinical characteristics of the patients. Second, the susceptibility of polymyxin and tigecycline were unexplored.

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Conflicts of Interest

The authors have declared that no competing interests exist.

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