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

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Received date: Nov 05, 2018; Accepted date: Nov 15, 2018; Published date: Nov 28, 2018

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 (blaVIM, blaOXA-48, blaKPC, blaIMP and blaNDM) 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 blaOXA-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 blaOXA-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 blaOXA-48 gene prevalence is at a low frequency in China, the blaOXA-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 pneumoniae, while other Enterobacteriaceae, such as Escherichia coli, Enterobacter cloacae, E. aerogenes, Citrobacter freundii, K. oxytoca, Serratia marcescens, Morganella morgani, 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, blaOXA-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 blaOXA-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 (blaVIM, blaOXA-48, blaKPC, blaIMP and blaNDM) 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 blaOXA-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.

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

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Source</th>
<th>Isolation date</th>
<th>PFGE type</th>
<th>MLST</th>
<th>Minimal inhibitory concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CTX</td>
</tr>
<tr>
<td>IR53017*</td>
<td>E. coli</td>
<td>Urine</td>
<td>7/1/2015</td>
<td>EC1</td>
<td>ST648</td>
<td>&gt;64</td>
</tr>
<tr>
<td>IR53006</td>
<td>E. coli</td>
<td>Sputum</td>
<td>6/28/2016</td>
<td>EC1</td>
<td>ST3554</td>
<td>&gt;64</td>
</tr>
<tr>
<td>IR53034</td>
<td>E. coli</td>
<td>Sputum</td>
<td>4/24/2017</td>
<td>EC2</td>
<td>ST156</td>
<td>&gt;64</td>
</tr>
<tr>
<td>IR5392</td>
<td>K. oxytoca</td>
<td>Sputum</td>
<td>8/20/2015</td>
<td>N/A</td>
<td>N/A</td>
<td>&gt;64</td>
</tr>
<tr>
<td>IR5283*</td>
<td>E. cloacae</td>
<td>Sputum</td>
<td>8/21/2015</td>
<td>EL1</td>
<td>ST418</td>
<td>&gt;64</td>
</tr>
<tr>
<td>IR53043</td>
<td>E. cloacae</td>
<td>Tissue</td>
<td>11/12/2017</td>
<td>EL2</td>
<td>ST418</td>
<td>8</td>
</tr>
<tr>
<td>IR5473</td>
<td>E. cloacae</td>
<td>Sputum</td>
<td>1/12/2018</td>
<td>EL3</td>
<td>ST414</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

*: 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.

The 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 ST155, 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, blaOXA-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 Inc/L/M plasmid [12,13]. In this study, plasmid analysis revealed that all isolates harbored the same ~60 kb Inc/L/M blaOXA-48-carrying plasmid. Although the genetic environment of blaOXA-48 was not analyzed in this study, it could be inferred that the IS1999 element might be located in the upstream of the blaOXA-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 Inc/L/M plasmid, indicating the great transferability of blaOXA-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 blaOXA-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.

### Funding

This study was supported by the Special Key Project of Biosafety Technologies for the National Major Research and Development Program of China (2017YFC1200803).

### Conflicts of Interest

The authors have declared that no competing interests exist.

### References


Clin Microbiol, an open access journal
ISSN:2327-5073

Volume 7 • Issue 6 • 1000320