Original Article
An outbreak of carbapenem-resistant enterobacter cloacae in pediatric intensive care unit of a Chinese teaching hospital

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Abstract: Objective: Carbapenem-resistant Enterobacter cloacae infections are known to be particularly prevalent in vulnerable populations, especially children. However, the phenotypic and molecular characteristics of carbapenem-resistant E. cloacae infections in the pediatric wards of China have not been well described. The aim of the present study was to characterize an outbreak of carbapenem-resistant E. cloacae in a pediatric intensive care unit of a teaching hospital. Methods: A total of 10 non-duplicated carbapenem-resistant E. cloacae from pediatric patients were collected and analyzed by antimicrobial susceptibility testing. The Modified Hodge test (MHT) was used for the preliminary screening of carbapenemases. β-lactamase genes were examined by PCR for sequencing. The transfer-ability of the carbapenemase genes and the homology of the 10 strains were evaluated by a conjugation experiment and pulsed-field gel electrophoresis (PFGE), respectively. Results: The results showed that all of the 10 isolates exhibited carbapenemase activity and carried carbapenemase genes. The Klebsiella pneumoniae carbapenemase (blaKPC-2) genes were detected in 6 of the 10 isolates, the blaIMP-8 metallo-β-lactamase gene was detected in 1 isolate. Interestingly, the New Delhi metallo-β-lactamase (blaNDM) gene, which is rarely found in children, was identified in 3 isolates. Further, extended spectrum beta-lactamases (ESBLs) and cephalosporinases (AmpC enzyme) genes were detected in the majority of the carbapenem-resistant E. cloacae isolates. All carbapenemase genes were located on transferable plasmids. PFGE demonstrated that 10 carbapenemase-producing E. cloacae isolates had homology and that there were five different clone patterns. Conclusions: Our study indicated that carbapenemase-production was the main contributor to this nosocomial infection outbreak because of the E. cloacae found in the pediatric intensive care unit.

Keywords: Carbapenem-resistant, enterobacter cloacae, pediatric, KPC, IMP, NDM

Introduction

Enterobacter cloacae is an opportunistic pathogen. It is responsible for nosocomial infections that involve the respiratory system, urinary tract and central nervous system, as well as bacteremia [1-5]. E. cloacae often become resistant to extended-spectrum cephalosporins due to the production of either extended spectrum β-lactamases (ESBLs) or plasmid-mediated or mutational derepression chromosomal-encoded AmpC β-lactamase [6-8]. Carbapenems are the drug of choice in the treatment of serious infections caused by producing extended-spectrum β-lactamase (ESBL) E. cloacae. Carbapenems have been used as the first treatment choice for serious infections of those strains, but they often result in an increased production of carbapenemases. The main resistant mechanism of Enterobacteriaceae to carbapenems is the production of carbapenemases, including Ambler class A, class B and class D, which can hydrolyze almost all of the hydrolyzable β-lactams, but cannot be inhibited by β-lactamase inhibitors [9, 10].

In 2013, we observed increased number of nosocomial infections in pediatric wards caused by carbapenem-resistant Enterobacteriaceae in our hospital. The resistance rate increased from 0.25% in 2009 to 1.52% in 2013. However, reports of pediatric infections
due to carbapenem-resistant clinical isolates remain limited. In this study, we investigated the genotypic and molecular characteristics of an outbreak of carbapenem-resistant E. cloacae in series of cases from a pediatric intensive care unit in a teaching hospital in Wenzhou, China.

Methods

Bacterial isolates

Ten non-duplicated carbapenem-resistant E. cloacae isolates (EC1-EC10) were collected from patients in a pediatric ward in the Second Affiliated Hospital of Wenzhou Medical University between January 2013 and November 2013. The study has been approved and registered by Ethics Committee of Second Affiliated Hospital of Wenzhou Medical University in January 2013, the Ethics committee approved relating screening, treatment, and data collection of these patients, all the guardian of the subjects signed written informed consent form. All works were undertaken following the provisions of the Declaration of Helsinki.

All isolates were identified by the VITEK Compact-2 automatic system (BioMérieux, Lyon, France). Clinical data were collected from medical records. E. coli ATCC25922 was used for quality control in antimicrobial susceptibility tests. E. coli EC600 was used as the recipient in the conjugation transfer experiments. Salmonella enterica H9812 was used as the size marker for PFGE.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was analyzed using the VITEK Compact-2 automatic system, and the MICs of carbapenems were confirmed by the agar dilution method according to the Clinical and Laboratory Standards Institute 2012 (CLSI) [11]. The Modified Hodge test was performed to assess phenotypic carbapenemase production using 10 μg of ertapenem as recommended by the CLSI.

Resistance genotyping of bla genes

Total DNA was obtained from all isolates using a Genomic DNA Miniprep kit (Axygen, Scientific, Union, CA, USA). Genotypic analyses of genes encoding the carbapenemase genes (bla_SME, bla_GES, bla_SME/NMC, bla_KPC, bla_GES, bla_KPC, bla_NDM-1, bla_VIM, bla_SME, bla_GES, bla_SPA, bla_GES, bla_OXA-23, bla_OXA-24, bla_OXA-51, bla_OXA-58, bla_OXA-48), common ESBLs genes (bla_SHV, bla_tem, blaCTX-M, bla_cep, bla_REM) and plasmid-mediated AmpC genes (bla_MOX, bla_GST, bla_DHA, bla_CIT, bla_ACC) were performed by PCR using the previously described methodology and primers [12-16]. The positive PCR products were screened by electrophoresis on the 1.0% agarose gel and were sequenced by Sango Biotech Co. (Shanghai, China). Nucleotide sequences were analyzed and compared using BLAST.

Conjugal transfer experiment

The conjugal transfer experiment [17] was performed in mixed broth cultures using rifampin-resistant E. coli EC600 as the recipient strain. Obtained dubious transconjugants were initially confirmed by the automated microbiology systems, and then screened for the resistant genes by PCR. The antimicrobial susceptibility of transconjugants was analyzed by the VITEK Compact-2 automatic system.

PFGE

The clonal relationship between isolates was analyzed by PFGE as previously described [18]. The PFGE patterns were analyzed and interpreted according to the criteria proposed by Tenover et al. [19]. The BIO-RAD Quantity One analysis software was used to process images and draw the stammbaum, followed by clustering analysis performed using the UPGMA algorithm, which took 80% as a critical value to compare the genetic relatedness between different strains.

Results and discussion

Case presentation

These 10 isolates were cultured from 9 patients (2 E. cloacae were isolated from the sputum and hydrothorax of one patient) in the pediatric intensive care unit (PICU) (n=8), pediatric neurosurgery ward (PNS) (n=1). Of the 9 patients, 5 were male and 4 were female. The ages of the patients were as follows: 0-1 year (n=4); >1-2 years (n=2); ≥3 years (n=3). Retrospective review of these patients indicated that 6 of the 9 patients were infected in combination with other bacteria, and all patients accepted a wide variety of antibiotics (meropenem, cefo-
Table 1. The diagnosis and treatment of the 9 cases

<table>
<thead>
<tr>
<th>Strain NO</th>
<th>Case NO</th>
<th>Specimen source</th>
<th>Department source</th>
<th>Age</th>
<th>Underlying disease</th>
<th>Surgery</th>
<th>Tracheal intubation</th>
<th>Glucocorticoid</th>
<th>Antibiotic prevention</th>
<th>Antibiotic therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC1</td>
<td>1</td>
<td>sputum</td>
<td>PICU</td>
<td>3 yr</td>
<td>contusion and laceration of brain</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>LVX-SCF</td>
<td>cure</td>
</tr>
<tr>
<td>EC2</td>
<td>2</td>
<td>sputum</td>
<td>PICU</td>
<td>1 mon</td>
<td>pneumonia</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>LVX-SCF</td>
<td>cure</td>
</tr>
<tr>
<td>EC3</td>
<td>3</td>
<td>sputum</td>
<td>PICU</td>
<td>14 yr</td>
<td>pneumonia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>LVX-ETM</td>
<td>cure</td>
</tr>
<tr>
<td>EC4</td>
<td></td>
<td>hydrothorax</td>
<td>PICU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
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<td>sputum</td>
<td>PICU</td>
<td>6 yr</td>
<td>drowning</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>LVX-SCF</td>
<td>cure</td>
</tr>
<tr>
<td>EC6</td>
<td>5</td>
<td>sputum</td>
<td>PICU</td>
<td>2 yr</td>
<td>drowning</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>SCF-CTX</td>
<td>cure</td>
</tr>
<tr>
<td>EC7</td>
<td>6</td>
<td>ascites</td>
<td>PICU</td>
<td>8 day</td>
<td>enterobrosis</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>LVX-SCF</td>
<td>quit</td>
</tr>
<tr>
<td>EC8</td>
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<td>+</td>
<td>-</td>
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<td>convulsion</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>EC10</td>
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<td>-</td>
<td>+</td>
<td>LVX</td>
<td>cure</td>
</tr>
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</table>

+, yes; -, no. LVX: levofloxacin; SCF: cefoperazone-sulbactam; ETM: etimicin; CTX: cefotaxime.
Carbapenem-resistant *Enterobacter cloacae*

Table 2. Antimicrobial susceptibility of the 10 carbapenem-resistant *E. cloacae*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>EC1</th>
<th>EC2</th>
<th>EC3</th>
<th>EC4</th>
<th>EC5</th>
<th>EC6</th>
<th>EC7</th>
<th>EC8</th>
<th>EC9</th>
<th>EC10</th>
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<tr>
<td>FEP</td>
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<td>≥64R</td>
<td>16I</td>
<td>16I</td>
<td>32R</td>
<td>≤1S</td>
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<tr>
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<td>AZT</td>
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<td>≥64R</td>
<td>≥64R</td>
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<td>≤0.25S</td>
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<tr>
<td>LEV</td>
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<td>≥320R</td>
<td>≥320R</td>
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<td>SAM</td>
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<td>≥32R</td>
<td>≥32R</td>
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<td>≥32R</td>
<td>≥32R</td>
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<td>≥32R</td>
<td>≥32R</td>
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<td>TOB</td>
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<td>8I</td>
<td>8I</td>
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<td>8I</td>
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<tr>
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<td>8R</td>
<td>8R</td>
<td>4R</td>
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<tr>
<td>FD</td>
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<td>128R</td>
<td>128R</td>
<td>128R</td>
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<td>32S</td>
<td>64I</td>
<td>256R</td>
<td>128R</td>
<td>64I</td>
</tr>
<tr>
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<td>≥64R</td>
<td>≥64R</td>
<td>≥64R</td>
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<td>≤4S</td>
<td>≥64R</td>
<td>≥64R</td>
<td>≥64R</td>
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</tr>
</tbody>
</table>


Figure 1. Agarose gel electrophoresis of carbapenemase PCR products for 10 *E. cloacae* isolates investigated in this study. Lanes: 1-6, *bla*KPC-2 products; 7, *bla*IMP-8 products; 8-10, *bla*NDM-1 products; M, 100 bp DNA marker.

perazone-sulbactam, cefotaxime, imipenem, piperacillin-tazobactam or cefazolin) alone or in combination to prevent or treat infections within two weeks before the isolation of the resistant strains. Detailed information for these 9 patients is listed in Table 1.

Antimicrobial susceptibility testing

Ten carbapenem-resistant *E. cloacae* strains exhibited a high resistance to imipenem and ertapenem. Furthermore, they were all resistant to cephalosporins, monobactams, and β-lactamase inhibitor combinations. Almost all of them were resistant to cephemycins, nitrofurantoin and cotrimoxazole, and exhibited poor sensitivity to tobramycin and gentamicin. In contrast, all of the strains were highly sensitive to amikacin, ciprofloxacin and levofloxacin. The results of the antibiotic susceptibility testing of the 10 carbapenem-resistant *E. cloacae* are shown in Table 2.

**Determination of the presence of resistant genes**

All of the 10 *E. cloacae* isolates were positive by the MHT. The PCR and sequencing results showed that carbapenemase genes were detected in all of the isolates. Among the 10 isolates, The *KPC-2* gene was detected in 6 isolates, *NDM-1* gene was detected in 3 isolates, and *IMP-8* gene was detected in 1 isolate. ESBL and AmpC enzyme genes were detected in 8 and 5 isolates, respectively. Most isolates carried multiple resistance genes. The percentage of isolates that carried the carbapenemase gene as well as ESBLs or AmpC enzyme genes was 80% (Figure 1 and Table 3).
Transfer of carbapenem resistance genes

Transconjugants were successfully obtained in all of the 10 carbapenemase-producing E. cloacae isolates. Results showed decreased susceptibility to carbapenem and high resistance to β-lactams antibiotics. In contrast, they were almost completely sensitive to fluoroquinolone. All transconjugants were confirmed by PCR to harbor carbapenemase genes similar to those of the original donor isolates.

PFGE typing

PFGE demonstrated that the 10 carbapenemase-producing E. cloacae isolates had a certain homology, there were five different clone patterns. Among these, 5 isolates (EC1-EC5) that carried KPC-2 belonged to main pattern A. Two isolates (EC8-EC9) that carried NDM-1 were pattern B. Three isolates that carried KPC-2, IMP-8 and NDM-1 were pattern C (EC6), D (EC7) and E (EC10), respectively. We found that the isolates that had the same clone pattern carried the same resistant genes and had a similar resistance spectrum. The PFGE patterns of the 10 E. cloacae isolates are shown in Figure 2.

The emergence of carbapenem resistance in Enterobacteriaceae is gradually increasing and has drawn great global concern [20, 21]. It has impacted infection control approaches and treatment strategies. In the present study, we collected 10 carbapenem-resistant E. cloacae from nine PICU patients. The other one patient who received treatment in the PICU before being transferred to the pediatric neurology ward, and was suspected to be infected before the transfer. The patients in the PICU almost all experienced severe underlying diseases and complications, they received surgery, tracheal intubation and glucocorticoids (dexamethasone or methylprednisolone) treatment. All patients accepted a wide variety of antibiotics, including cephalosporins and carbapenems alone or in combination, to prevent or treat infections within two weeks before the isolation of the resistant strains, which were high risk factors for infection with resistant strains.

Patients in the ICU ward are seriously ill and often require intubation and mechanical ventilation. If sterile techniques are not effectively
performed by medical workers, nosocomial infection will likely occur in these patients. Moreover, due to the side effects of aminoglycoside and quinolones, these therapeutics is generally not used for children, which lead to a smaller range of antibiotics being used for children relative to adults. Thus, the appearance of carbapenem-resistant Enterobacteriaceae is an especially large threat to children.

Antibiotic susceptibility testing showed that although the 10 E. cloacae strains were multidrug resistant and exhibited high resistance to carbapenems, cephalosporins, monobactams, β-lactamase inhibitor combinations, cephapemycins, nitrofurantoin and cotrimoxazole, they showed a high sensitivity to amikacin, ciprofloxacin and levofloxacin. Therefore, according to the results of antibiotic susceptibility testing, most patients were administered a combination of levofloxacin and cefoperazone-sulbactam or cefotaxime for anti-infection treatment; ultimately, 7 patients were cured, but 2 patients quit treatment due to personal reasons.

In the present study, we investigated the primary resistance mechanism from the β-lactamases genes. All of the ten strains exhibited carbapenemase activity and carried carbapenemase genes. This finding demonstrates that MHT had high sensitivity and specificity for screening KPC carbapenemase, even metallo-β-lactamase IMP and NDM-1. According to the time of separation, \( \text{bla}_{\text{KPC-2}} \) genes in 6 isolates were detected first, followed by \( \text{bla}_{\text{IMP-8}} \) in 1 isolate and \( \text{bla}_{\text{NDM-1}} \) in 3 isolates. It had previously been reported that only \( \text{bla}_{\text{KPC-2}} \) was detected in the Klebsiella pneumoniae isolated from pediatric wards in our hospital [22]. This finding suggests that new resistance genes gradually appeared and diversified over time. NDM-1, a Class B carbapenemase, has spread globally and into multiple Enterobacteriaceae with striking rapidity [23, 24]. What the most alarming is that NDM-1, which is rarely found in E. cloacae, especially in children [25], was identified in 3 isolates in our study. Moreover, ESBLs and AmpC β-lactamases, which are associated with carbapenem-resistance in combination with the loss or reduction of permeability of outer membrane proteins in some isolates, were also detected in the majority of the E. cloacae isolates. The wide range of clinical and molecular characteristics in these infections highlights the challenges in identifying and addressing carbapenem resistance.

Conclusions

The conjugal transfer experiment demonstrated that all carbapenemase genes were located on transferable plasmids and can transfer to recipients. This provides an advantage in the dissemination of the carbapenemase genes and may contribute to their spread in our hospital in the immediate future. PFGE suggested that both clone dissemination and horizontal spread coexisted in this outbreak of nosocomial infections. The strains with the same PFGE pattern carried the same genotype and revealed a similar resistance spectrum. Our findings highlight the genotypic and molecular characteristics of the pediatric patients, and provide a basis for carbapenem-resistant E. cloacae in children. We must strengthen the monitoring of nosocomial infections through molecular epidemiological investigation to avoid the outbreaks of clinical infection.

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Disclosure of conflict of interest

None.

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