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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 transferability 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 (bla_{KPC-2}) genes were detected in 6 of the 10 isolates, the bla_{IMP-8} metallo-β-lactamase gene was detected in 1 isolate. Interestingly, the New Delhi metallo-β-lactamase (bla_{NDM-1}) 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_{KPC}, bla_{SME}, bla_{GES}, $bla_{IMI/NMC-A}$, bla_{IMP}, bla_{NDM-1}, bla_{VIM}, bla_{SIM}, bla_{GIM}, bla_{SPM}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, bla_{OXA-58}, bla_{OXA-48}), common ESBLs genes (bla_{SHV}, bla_{TEM}, $bla_{CTX-M,}$ bla_{VEB}, bla_{PER}) and plasmid-mediated AmpC genes (bla_{MOX}, bla_{FOX}, bla_{D-HA}, 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 bacterium, and all patients accepted a wide variety of antibiotics (meropenem, cefo-

Table 1. The diagnosis and treatment of the 9 cases

Strain NO	Case NO.	Specimen source	Department source	High risk factors of infection						Antibiotic therapy	Outcome
				Age	Underlying disease	Surgery	Tracheal intubation	Glucocorticoid	Antibiotic prevention		
EC1	1	sputum	PICU	3 yr	contusion and laceration of brain	+	+	-	+	LVX-SCF	cure
EC2	2	sputum	PICU	1 mon	pneumonia	-	-	+	+	LVX-SCF	cure
EC3	3	sputum	PICU	14 yr	pneumonia	+	+	+	+	LVX-ETM	cure
EC4		hydrothorax			pyopneumothorax						
EC5	4	sputum	PICU	6 yr	drowning	-	+	+	+	LVX-SCF	cure
EC6	5	sputum	PICU	2 yr	drowning	-	+	+	+	SCF-CTX	cure
EC7	6	ascites	PICU	8 day	enterobrosis	+	-	+	+	LVX-SCF	quit
EC8	7	sputum	PICU	2 yr	acute gastroenteritis	-	+	-	+	LVX-CTX	quit
EC9	8	sputum	PNS	10 mon	convulsion	-	+	+	+	LVX-SCF	cure
EC10	9	sputum	PICU	1 yr	pneumonia	+	+	-	+	LVX	cure

+, yes; -, no. LVX: levofloxacin; SCF: cefoperazone-sulbactam; ETM: etimicin; CTX: cefotaxime.

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

Antibiotics	MIC (μg/mL)									
	EC1	EC2	EC3	EC4	EC5	EC6	EC7	EC8	EC9	EC10
AK	≤2S	≤2S	≤2S	≤2S	≤2S	≤2S	≤2S	≤2S	≤2S	≤2S
KZ	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R
CAZ	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R	≥64R
CRO	≥64R	≥64R	≥64R	≥64R	≥64R	8R	≥64R	≥64R	≥64R	≥64R
FEP	32R	≥64R	16I	16I	32R	≤1S	≥64R	≥64R	≥64R	32R
CN	≥16R	≥16R	≥16R	≥16R	≥16R	≤1S	2S	≤1S	≤1S	≤1S
AZT	≥64R	≥64R	≥64R	≥64R	≥64R	16R	16R	≥64R	≥64R	16R
CIP	≤0.25S	≤0.25S	≤0.25S	≤0.25S	≤0.25S	≤0.25S	0.5S	≤0.25S	≤0.25S	0.5S
IPM	16R	8R	8R	16R	16R	16R	8R	32R	32R	16R
LEV	1S	1S	1S	1S	0.5S	1S	1S	1S	1S	1S
SXT	≥320R	≥320R	≥320R	≥320R	≥320R	≤20S	≤20S	≥320R	≥320R	≥320R
SAM	≥32R	≥32R	≥32R	≥32R	≥32R	≥32R	≥32R	≥32R	≥32R	≥32R
TZP	≥128R	≥128R	≥128R	≥128R	≥128R	32I	8I	≥128R	≥128R	≥128R
TOB	8I	8I	8I	8I	8I	≤1S	8I	≤1S	≤1S	8I
ETP	8R	8R	8R	8R	8R	4R	4R	16R	8R	8R
FD	128R	128R	128R	128R	128R	32S	64I	256R	128R	64I
CTE	≥64R	≥64R	≥64R	≥64R	≥64R	≤4S	≥64R	≥64R	≥64R	≥64R

AK: amikacin; KZ: cefazolin; CAZ: ceftazidime; CRO: ceftraxone; FEP: cefepime; CN: gentamicin; AZT: aztreonam; CIP: ciprofloxacin; IPM: imipenem; LEV: levofloxacin; SXT: cotrimoxazole; SAM: ampicillin-sulbactam; TZP: piperacillin-tazobactam; TOB: tobramycin; ETP: ertapenem; FD: nitrofurantoin; CTE: cefotetan.

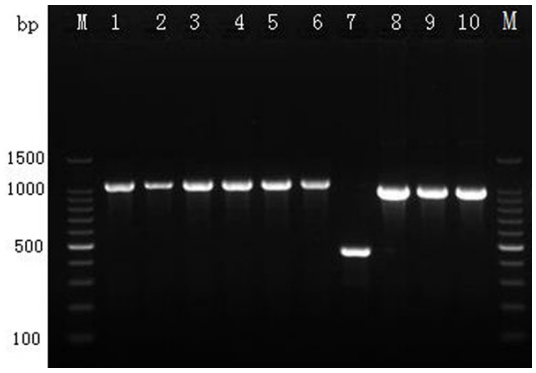

Figure 1. Agarose gel electrophoresis of carbapenemase PCR products for 10 *E. cloacae* isolates investigated in this study. Lanes: 1-6, bla_{KPC-2} products; 7, bla_{IMP-8} products; 8-10, blaNDM-1 products; M, 100 bp DNA marker.

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 cephamycins, 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**).

Table 3. MIC of carbapenems, phenotype and genotype of the 10 *E. cloacae* isolates

Isolates NO.	MICs of carbapenems (μg/mL)		MHT	Resistant genes		
	IPM	ETP		Carbapenemases	ESBLs	AmpCs
EC1	16	8	+	*KPC*-2	*TEM-1*	*DHA*-1
EC2	8	8	+	*KPC*-2	*TEM-1*	*DHA*-1
EC3	8	8	+	*KPC*-2	*TEM-1*	*DHA*-1
EC4	16	8	+	*KPC*-2	*TEM-1*	*DHA*-1
EC5	16	8	+	*KPC*-2	*TEM-1*	*DHA*-1
EC6	16	4	+	*KPC*-2	-	-
EC7	8	4	+	*IMP*-8	*TEM-1*, *CTX-M*-15	-
EC8	32	16	+	*NDM*-1	*SHV-11*	-
EC9	32	8	+	*NDM*-1	*SHV-11*	-
EC10	16	8	+	*NDM*-1	-	-

MIC: minimum inhibitory concentration; IPM: imipenem; ETP: ertapenem; MHT: Modified Hodge test; +, positive; -, not detected.

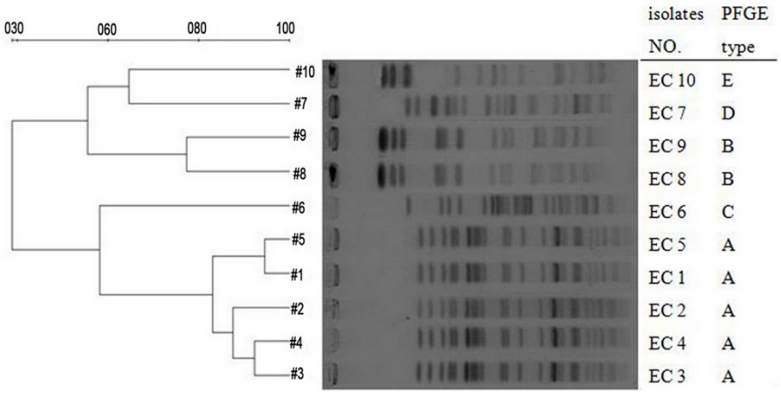

Figure 2. Pulsed-field gel electrophoresis profiles of XbaI-digested genomic DNA of the 10 E.cloacae isolates.

Transfer of carpenem 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, this 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, cephamycins, 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, bla_{KPC-2} genes in 6 isolates were detected first, followed by bla_{IMP-8} in 1 isolate and bla_{NDM-1} in 3 isolates. It had previously been reported that only bla_{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.

Acknowledegments

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Disclosure of conflict of interest

None.

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