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Original Article

First report of synergistically containing blaNDM-1 and blaVIM-19 carbapenemase producing Enterobacter cloacae ST508 in the center of China

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Abstract: Multidrug-resistant gram-negative rods are reported worldwide. Carbapenem-resistant enterobacteriaceae have been reported to an increasing extent, largely as a consequence of the acquisition of carbapenemase genes. The treatment of infections caused by Enterobacter cloacae and other enterobacteriaceae has become a troublesome clinical problem in the case of limited therapeutic options. Resistance to Carbapenem caused by Enterobacter cloacae and other enterobacteriaceae is relatively rare; therefore, this class of drugs is the last line of defense against multidrug-resistant Enterobacter cloacae for the treatment of severe infections. Unfortunately, increasing production of New Delhi metallo-β-lactamase 1 (NDM-1) has become established as a major public health threat and represents a new challenge in the treatment of infectious diseases. In this study, we describe the ability of Enterobacter cloacae to produce carbapenemase enzymes based on the results of Modified Hodge test and the biochemical Carba NP test performed according to guidelines of the Clinical and Laboratory Standards Institute (CLSI). Furthermore, we assessed the presence of genes in charge of the production of carbapenemases (blaKPC, blaVIM, blaIMP, blaOXA-48, blaNDM) among Enterobacter cloacae. The tested isolate of Enterobacter cloacae that occupied the blaNDM-1 and blaVIM-19 genes were determined. To our knowledge, this is the first detection of carbapenemase-producing Enterobacter cloacae (n=4) consisted of sequence type 508 (ST508) with NDM-1 and VIM-19, ST509 with NDM-1, and ST145 with VIM-19 in china. Our results show that on the classification ability of carbapenemase producing Enterobacter cloacae, PFGE and MLST have no much difference in two ST509. Clinical microbiology laboratories should remain vigilant in detecting Enterobacter cloacae with carbapenemases.

Keywords: Enterobacter cloacae, antimicrobial drugs, carbapenemases

Introduction

Enterobacter cloacae (E. cloacae) is an important emerging pathogen, which sometimes causes various infections including surgical site infection, urinary infection, sepsis, and many nosocomial infections such as pneumonia, bloodstream infections [1]. It is intrinsically resistant to aminopenicillins and narrow spectrum cephalosporins (such as cefazolin, and cefoxitin) due to chromosomal cephalosporinase [2]. The treatment of infections caused by E. cloacaeis a serious healthcare challenge, owing to the escalating resistance of bacteria to Carbapenem. The characteristic of multidrug resistance has been notified globally and opens the door for decline of therapeutic choice [3].

The purpose of this study was to identify the presence of bla genes responsible for carbapenemases production (blaKPC, blaVIM, blaIMP, OXA-48). Additionally, Clonal relatedness of the E. cloacae was assessed by pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) (http://pubmlst.org/perl/bigsdb/bigsdb.pl?db=pubmlst_ecloacae_seqdef).

Materials and methods

The tested E. cloacae strain was isolated in September 2014 to January 2016, were recovered from different clinical specimens processed at the microbiology laboratory of Renmin Hospital of Wuhan University, a tertiary care hospital in the center of china. Biochemical

Table 1. MIC values of antimicrobial agents tested for E. cloacae

Antimicrobial agents	1	2	3	4
Ampicillin	(R)>16	(R)>16	(R)>16	(R)>16
Piperacillin	(R)>64	(R)>64	(R)>64	(R)>64
Amoxicillin/clavulanate	(R)>16/8	(R)>16/8	(R)>16/8	(R)>16/8
Ampicillin/sulbactam	(R)>16/8	(R)>16/8	(R)>16/8	(R)>16/8
Piperacillin/tazobactam	(R)>64/4	(R)>64/4	(R)>64/4	(R)>64/4
Cefazolin	(R)>16	(R)>16	(R)>16	(R)>16
Cefotaxime	(R)>32	(R)>32	(R)>32	(R)>32
Ceftazidime	(R)>16	(R)>16	(R)>16	(R)>16
Cefepime	(R)>16	(R)>16	(R)>16	(R)>16
Aztreonam	(R)≥64	(R)>16	(S)≤2	(R)>16
Meropenem	(R)>8	(R)>8	(R)>8	(R)8
Imipenem	(R)≥16	(R)>8	(R)>8	(R)8
Amikacin	(S)≤2	(S)≤8	(S)≤8	(S)≤8
Gentamicin	(S)≤1	(S)≤2	(S)4	(R)>8
Ciprofloxacin	(S)≤0.25	(R)>2	(S)≤0.5	(R)>2
Levofloxacin	(S)≤0.25	(I)4	(S)≤1	(R)>8
Cotrimoxazole	(R)>2/38	(S)≤0.5/9.5	(R)>2/38	(S)≤0.5/9.5
Chloromycetin	(S)≤4	(S)≤4	(I)16	(S)≤4
Tetracycline	(S)≤2	(S)≤2	(S)4	(R)>8

R: resistant; S: susceptible; I: intermediate.

identification and the preliminary susceptibility test were performed using the BD Phoenix™-100 Automated Microbiology System (BD, America). Additionally, the susceptibility to antibiotics of the tested strain was performed using Kirby-Bauer disk susceptibility methods (Oxide, Britain). The results of the susceptibility tests were interpreted according to CLSI recommendations [4]. The screening detection of carbapenemases was performed according to CLSI [4]. Moreover, the biochemical Carba NP test was performed according to CLSI and the Nordmann and Poirel's protocol [5]. Further, molecular analysis was performed with the use of polymerase chain reactions (PCRs). Bacterial genome was extracted with the use of boiling lysis according to the manufacturer's instructions. PCR amplifications for genes responsible for carbapenemases production (blaKPC, blaVIM, blaIMP, OXA-48, blaNDM) were performed using appropriate primers as described previously [6-9]. The conditions were Optimized. PCR amplicons were separated electrophoretically according to a previously described protocol [9]. Moreover, sequencing of the amplicons was performed at Sangon (Shanghai, China). Multi-locus sequence typing (MLST) was performed according to the Institute Pasteur's MLST scheme. Pulsed-field gel electrophoresis (PFGE) was performed at Wuhan CDC using a CHEF Mapper apparatus (USA Richmond Bio-Rad Laboratories, Co., Ltd.) in accordance with standard operating procedures of the laboratory.

Results

The Modified Hodge Confirmatory Test for suspected carbapenemases production in enterobacteriaceae showing enhanced growth around the test or QC organism streak at the intersection of the streak and the zone of inhibition after incubating 18 h. The biochemical Carba NP test was positive within 2 hours. The obtained results indicated Carbapenem resistance mediated by carbapenemases among the tested strains of E. cloacae. The tested strain was analyzed for the presence of resistance mechanisms against β-lactam antibiotics using PCR amplifications for genes (blaKPC, blaVIM, blaIMP, OXA-48) responsible for carbapenemases production. The blaNDM and blaVIM genes were found in E. cloacae. The obtained sequence of the blaNDM gene showed identity with the sequence of the blaNDM-1 gene (Gene Bank accession no. KC460307.1). The obtained sequence of the blaVIM gene showed identity with the sequence of the carbapenemase blaVIM-19 gene (GeneBank accession no. KT820212.1). Results of minimum inhibitory concentration(MIC) values of tested antibiotics are presented in **Table 1**. The analysis of allelic profile (dnaA, fusA, gyrB, leuS, pyrG, rplB, rpoB) with use of the E. cloacae MLST sequence type database showed that the tested E. cloacaestrains respectively belonged to ST508, ST145 and ST509 type. Results of carbapenemase genes and STs of tested strains werecontained in **Table 2**. Genetic relatedness of 4 carbapenemases-positive E. cloacae isolates were acquired by pulsed-field gel electrophoresis (PFGE) with multi-locussequence typing (MLST) results in **Figure 1**.

Table 2. The results of MLST number and PCRs for Carbapenemase genes

Isolates	dnaA	fusA	gyrB	leuS	pyrG	rplB	rpoB	ST	Genes
1	63	11	58	36	77	16	19	508	NDM-1, VIM-19
2	155	109	137	107	157	8	28	509	NDM-1
3	59	40	82	9	67	6	6	145	VIM-19
4	155	109	137	107	157	8	28	509	NDM-1

DnaA, fusA, gyrB, leuS, pyrG, rplB, and rpoB are seven house keeping genes applying for E. cloacae MLST scheme.

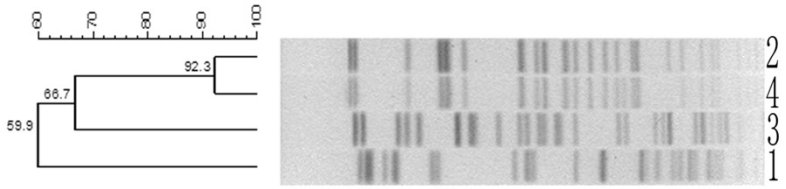

Figure 1. Dendrogram analysis showing pulsed-field gel electrophoresis (PFGE) results for 4 carbapenemases-positive E. cloacae isolates. Scale bar indicates percentage genetic relatedness.

Discussion

Carbapenems are some of the most broad-spectrum antimicrobials that we have available and are often viewed as the last-line therapeutic antimicrobial options for treatment of ESBL-producing E. cloacae. Resistance against carbapenems among E. cloacae rods is uncommon, which may be a result of AmpC β-lactamase production and loss of porins. Unfortunately, strains resistant to carbapenems due to the production of KPCs have recently been observed [10]. Producers have previously been reported in distinct geographic locations: European countries (Greece, Israel, Spain, Italy, Portugal, France, Poland, Germany, UK, and the Czech Republic), China, South America and the United States [11]. KPC production is mainly prevalent among enterobacteriaceae species. The significant majority of reports describe identification and the prevalence of blaKPC genes among nosocomial Klebsiella pneumoniae strains. Moreover, the occurrence of blaKPC genes among other enterobacteriaceae species, for example, Klebsiella pneumoniae, Escherichia coli, and Citrobacterfreundii was observed [12]. Therefore, carbapenem-resistant enterobacteriaceae (CRE) have emerged as a major concern in recent years, largely driven by the widespread dissemination of carbapenemase genes [9]. CRE almost invariably possess numerous other resistance mechanisms, drastically limiting treatment options.

A significant increase of E. cloacae isolates resistant to third generation cephalosporins has been observed in Europe [9]. In China, NDM-1 was commonly identified in Acinetobacter spp. isolated from clinical, environmental and farm animal samples but only reported sporadically in enterobacteriaceae [13-15]. Studies have shown a high percentage (65%-100%) of extended-spectrum β-lactamase (ESBL) production among E. cloacae isolates resistant to third-generation cephalosporins [16]. Clonal spread is an important factor involved in the prevalence of NDM-producing enterobacteriaceae at local and regional level. Outbreaks of NDM-producing Escherichia coli ST167 and Klebsiella pneumoniae ST11 have been reported in China and Poland, respectively [17, 18].

This study identified three clones of ST508, ST509 and ST145 among the carbapenem-resistant E. cloacae isolates in Hubei province. As we known, The most widespread ST were ST66, ST78, ST108 and ST114, each having at least 10 isolates from three to five Europe countries, diversified into multiple expanded-spectrum cephalosporins resistant phenotype, with clusters of related isolates in one or more centers [19]. Since limited numbers were obtained, the spread of the ST508, ST509 and ST145 isolates in this region still need to be further monitored. It is noteworthy that two out of the three ST isolates were identified as Multi-drug resistant (MDR) bacteria. Moreover, The E. cloacae isolates co-harbored blaNDM-1 and blaVIM-19 genes and could transfer resistance to carbapenems simultaneously by conjugation. Maybe the dissemination of E. cloacae ST508 isolates will seriously limit the future therapeutic options.

In conclusion, the study first demonstrated blaNDM-1 and blaVIM-19 genes in E. cloacae

ST508 in China, and identified two potential endemic clones of ST508 and ST509. The emergence of MDR E. cloacae ST508 and ST509 isolates is worrying. Early detection and surveillance of NDM-1 producing E. cloacae are urgently needed to prevent their further spread.

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Disclosure of conflict of interest

None.

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