EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Nationwide Surveillance of Clinical Carbapenem-resistant Enterobacteriaceae (CRE) Strains in China

Rong Zhang [a], Lizhang Liu [b], Hongwei Zhou [a], Edward Waichi Chan [c], Jiaping Li [a], Ying Fang [a], Yi Li [d], Kang Liao [e], Sheng Chen [b,c,*]

[a] Second Affiliated Hospital of Zhejiang University, Hangzhou, PR China
[b] Shenzhen Key Lab for Food Biological Safety Control, Food Safety and Technology Research Center, Hong Kong PolyU Shen Zhen Research Institute, Shenzhen, PR China
[c] State Key Lab of Chirosciences, Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong
[d] Clinical Laboratory, Medicine Department, He Nan Provincial People's Hospital, Zhengzhou, PR China
[e] First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, PR China

ARTICLE INFO

Article history:
Received 20 March 2017
Received in revised form 20 April 2017
Accepted 25 April 2017
Available online xxxx

Keywords:
Carbapenem resistance
Enterobacteriaceae
Plasmid
bla_{NDM}
bla_{KPC-2}
Molecular epidemiology

ABSTRACT

The increasing incidence of carbapenem-resistant Enterobacteriaceae (CRE) - mediated hospital infections in China prompted a need to investigate the genetic basis of emergence of such strains. A nationwide survey was conducted in China covering a total of 1105 CRE strains collected from 25 geographical locales with results showing that acquisition of two carbapenemase genes, bla_{KPC-2} and bla_{NDM}, was responsible for phenotypic resistance in 90% of the CRE strains tested (58% and 32% respectively), among which several major strain types, such as ST11 of *K. pneumoniae* and ST131/ST167 of *E. coli*, were identified, suggesting that dissemination of specific resistant clones is mainly responsible for emergence of new CRE strains. Prevalence of the *fosA3* gene which mediates fosfomycin resistance, was high, while the colistin resistance determinant *mcr-1* was rarely present in these isolates. Consistently, the majority of the bla_{NDM}-bearing plasmids recoverable from the test strains belonged to IncX3, which contained a common core structure, bla_{NDM}-*blaMBL-trpF*. Likewise, the core structure of IS*Kpn27*-bla_{KPC-2}-IS*Kpn2* was observed among plasmids harboring the bla_{KPC-2} gene, although they were genetically more divergent. In conclusion, the increasing prevalence of CRE strains in China is attributed to dissemination of conservative mobile elements carrying bla_{NDM} or bla_{KPC-2} on conjugative and non-conjugative plasmids.

1. Introduction

The scale of clinical and public health problems due to multidrug-resistant bacterial infections has further escalated in recent years following the emergence of bla_{NDM}, a plasmid-borne carbapenem resistance gene that has been widely disseminated among various species of bacterial pathogens worldwide (Kumarasamy et al., 2010; Nordmann et al., 2012). Descriptions such as "superbug", "nightmare bacteria" and "post-antibiotic era" reflected the seriousness of the antimicrobial resistance issue.

Among the major multidrug-resistant organisms that emerged within the past two decades, carbapenem-resistant *Enterobacteriaceae* (CRE), which commonly cause untreatable and hard-to-treat infections among hospitalized patients, is considered an urgent threat according to a report by the Center for Diseases Control and Prevention (CDC) in 2013 on antibiotic resistance threats in the United States. In the past two decades, utilization of carbapenems such as imipenem and meropenem in clinical treatments has become necessary due to proliferation of multidrug-resistant bacterial pathogens in clinical settings (Zilberberg and Shorr, 2013; Goel et al., 2011). Such increase in carbapenem consumption has been accompanied by the emergence of carbapenem-resistant Gram-negative pathogens (Karaiskos and Giamarellou, 2014; Livermore, 2004, 2009). According to the CDC report of 2013, >9000 healthcare-associated infections are caused by CRE each year and almost half of the hospital patients who suffer from CRE-mediated bloodstream infections died subsequently (CDC, 2013). Each year, approximately 600 deaths result from infections caused by the two most common types of CRE, namely carbapenem-resistant *Klebsiella* spp. and *E. coli* (Yong et al., 2009).

In China, the first clinical report of bla_{NDM} involved carbapenem-resistant *Acinetobacter baumannii* strains detectable in four patients who resided in different provinces, in 2011 (Chen et al., 2011). Since then it has been recoverable in most species of *Enterobacteriaceae*, including *K. pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter aerogenes* and *Citrobacter freundii*, in various cities or regions in China, such as Beijing, Changsha, Chongqing, Fuzhou, Guangzhou, Hangzhou, Hebei, Hong Kong and Zhengzhou (Berrazeg

* Corresponding author at: Shenzhen Key Lab for Food Biological Safety Control, Food Safety and Technology Research Center, Hong Kong PolyU Shen Zhen Research Institute, Shenzhen, PR China.
E-mail address: sheng.chen@polyu.edu.hk (S. Chen).

http://dx.doi.org/10.1016/j.ebiom.2017.04.032

et al., 2014; Qin et al., 2014). The first KPC producing CRE strain in China was reported in 2007, and the bla_{KPC-2} gene has since become the most widely spread carbapenemase gene in China as well as various parts of the world. In this study, we conducted a nationwide surveillance of the prevalence of CRE in China and investigated the molecular epidemiological features of these strains, and hoped to identify the key strains and mobile resistance elements responsible for causing an increase in prevalence of CRE-mediated infections in China. Findings of this work shall provide essential insight into development of effective strategies for worldwide control of CRE and reducing the rate of untreatable infections in clinical settings.

2. Materials and Methods

2.1. Carbapenem-resistant Enterobacteriaceae Isolates

Non-duplicated *Enterobacteriaceae* strains that exhibited carbapenem resistance phenotype (meropenem MIC ≥ 4 μg/ml) were collected from hospitals located in 25 Provinces and Municipalities in China, namely Anhui, Beijing, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Henan, Hubei, Hunan, Jilin, Jiangxi, Liaoning, Nanjing, Shandong, Shanxi, Shaanxi, Shanghai, Sichuan, Tianjing, Xinjiang, Zhejiang and Chengdu, during the period, June 2014 through June 2015. One representative hospital (normally the largest general hospital in the location) from each location was chosen for sample collection. All strains were subjected to species confirmation using the Vitek 2 system (bioMérieux, Marcy-l'E' toile, France), and the MALDI-TOF MS apparatus (Bruker Microflex LT, Bruker Daltonik GmbH, Bremen, Germany).

2.2. Antimicrobial Susceptibility Testing

The minimal inhibitory concentrations (MICs) of 12 antibiotics, namely amoxicillin-clavulanic acid, cefotaxime, ceftazidime, imipenem, mropenem, amikacin, ciprofloxacin, colistin, fosfomycin and tigecycline, were determined using the agar dilution method, and the results were analyzed according to the CLSI criteria of 2016 (Huang et al., 2016; CLSI, 2016). The 2017 EUCAST breakpoints were used (available at http://www.eucast.org/clinical_breakpoints/) for tigecycline.

2.3. Screening of Carbapenemase and Other Antimicrobial Resistance Genes

PCR and nucleotide sequencing were performed to screen for the presence of the carbapenemase-encoding genes bla_{VIM}, bla_{IMP}, bla_{KPC}, bla_{OXA-48} and bla_{NDM} as described previously (Dallenne et al., 2010). Screening of *fosA3* and *mcr-1* was performed as previously described (Li et al., 2016, 2017; Liu et al., 2017; Lin and Chen, 2015). An imipenem-EDTA double-disc synergy test and the modified Hodge test were used to assess the ability of the test strains to produce carbapenemases; analysis was performed according to CLSI guidelines (Huang et al., 2016; CLSI, 2016).

2.4. PFGE and ST Typing

Multi locus sequence typing (MLST) for these CRE isolates was performed according to the previously reported protocol (Liu et al., 2014). Clonal relationships of major ST stain types of *K. pneumonia* and *E. coli* were investigated by PFGE of *Xba*I-digested genomic DNA using a Rotaphor System 6.0 instrument (Whatman Biometra, Goettingen, Germany), with a running time of 24 h and pulse times of 3–40 s. *Salmonella* strain H9812 was used as the control strain. Dendrograms depicting the genetic relatedness of the test strains were generated from the homology matrix to describe the relationships of the PFGE profiles of the test strains.

2.5. Conjugation, S1-PFGE and Southern Hybridization

Conjugation experiments were carried out using the mixed broth method as previously described (Borgia et al., 2012). PFGE, S1-PFGE and Southern Hybridization were performed as previously described (Wang et al., 2015).

2.6. Plasmid Sequencing

Plasmids carrying the bla_{KPC-2} and bla_{NDM} genes were extracted from transformants using the Plasmid Midi kits (Qiagen, Germany). The plasmids were subjected to sequencing using Illumina NextSeq 500 platforms. After obtaining the raw reads, SPAdes was utilized to perform the hybrid-assembly and obtain complete plasmid sequences. Illumina short-reads were then utilized to polish the finished plasmids. The RAST annotation pipeline was chosen to perform rapid annotation of the plasmids (Overbeek et al., 2014). Comparison of the plasmids against the highly homologous plasmids in the NCBI database was performed by BRIG (Alikhan et al., 2011).

2.7. Plasmid Mapping

PCR mapping of the conservative regions of IncX3 plasmid and regions carrying bla_{NDM}-bearing mobile elements was performed on IncX3 plasmids as previously described (Huang et al., 2016). The genetic environment of bla_{KPC-2} on conjugative plasmids was analyzed by primer walking as previously described (Pfeifer et al., 2011).

3. Results

3.1. CRE Strains and Their Susceptibility to Various Antimicrobials

A total of 1105 non-duplicate CRE strains collected from hospitals in 25 Provinces and Municipalities in China were studied to obtain molecular epidemiological features of such organisms. *K. pneumoniae* was the most prevalent species (703 strains), followed by *E. coli* (164), *E. cloacae* (132), *E. aerogenes* (Alikhan et al., 2011), *Klebsiella oxytoca* (Alikhan et al., 2011), *Serratia marcescens* (Borgia et al., 2012), *C. freundii* (Borgia et al., 2012) and 16 strains of other *Enterobacteriaceae* species (Table 1). All carbapenem-resistant *K. pneumoniae*, *E. coli* and *E. cloacae* isolates were found to be resistant to almost all β-lactam antibiotics tested, with only a small proportion of the strains being susceptible to carbapenems and cephalosporins. The rate of susceptibility to amikacin, ciprofloxacin, fosfomycin and tigecycline were respectively 47.7%, 27.7%, 31.3% and 7.8% among the *K. pneumoniae* strains, 68.8%, 41.4%, 88.9% and 54.4% among the *E. coli* strains, and 62.5%, 25.0%, 35.3% and 6.8% among the *E. cloaceae* strains. Overall, resistance to colistin was extremely rare among CRE strains in China, with respectively 1.1%, 2.3% and 6.2% of the *K. pneumoniae*, *E. coli* and *E. cloaceae* strains displaying colistin MIC ≧ 4 μg/ml (Table 2).

3.2. Carbapenemase-encoding Elements Harbored by Clinical CRE Strains

The CRE strains were further tested for their ability to produce carbapenemase and carriage of carbapenemase genes. A total of 887 out of the 1105 CRE were found to produce carbapenemases. All these carbapenemase-producing CRE were found to carry different carbapenemase genes. The degree of correlation between carbapenem resistance phenotype and carriage of carbapenemase genes was over 90% for *K. pneumoniae* and *E. coli*, whereas only 80% of carbapenem-resistant *E. cloacae* strains were found to harbor carbapenemase genes. Among the CRE strains tested, the KPC-2-type carbapenemase gene (bla_{KPC-2}) was the most dominant type and detected in 627 (57%) strains, whereas the bla_{NDM} gene was detected in 343 (31%) strains; 21 strains were found to harbor both genes (1.9%). The bla_{IMP-4} gene was detected in 35 (3%) strains, one of which was found to harbor the

Please cite this article as: Zhang, R., et al., Nationwide Surveillance of Clinical Carbapenem-resistant Enterobacteriaceae (CRE) Strains in China, EBioMedicine (2017), http://dx.doi.org/10.1016/j.ebiom.2017.04.032