Journal of Infection and Public Health xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Infection and Public Health

journal homepage: http://www.elsevier.com/locate/jiph

Emergence and mechanism of carbapenem-resistant *Escherichia coli* in Henan, China, 2014

Wen-juan Liang[a,b], Hui-ying Liu[a], Guang-Cai Duan[a,b,*], Yong-xin Zhao[c], Shuai-yin Chen[a], Hai-Yan Yang[a], Yuan-Lin Xi[a]

[a] College of Public Health, Zhengzhou University, Zhengzhou, Henan 450001, China
[b] Henan Innovation Center of Molecular Diagnosis and Laboratory Medicine, Xinxiang Medical University, Xinxiang, Henan 453003, China
[c] Laboratory Department, the Third Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan 453003, China

ARTICLE INFO

Article history:
Received 26 April 2017
Received in revised form 12 July 2017
Accepted 9 September 2017

Keywords:
bla_{NDM}
Carbapenem-resistant
Escherichia coli

ABSTRACT

The emergence and dissemination of carbapenem-resistant *Escherichia coli* (*E. coli*) strains is a main risk for global public health, but little is known of carbapenemase producing *E. coli* in Henan, China. The study was undertaken to investigate the prevalence and mechanism of carbapenem-resistant *E. coli* strains in a hospital in Xinxiang, Henan, China, 2014. A total of 5 carbapenemase-producing *E. coli* strains were screened from 1014 isolates. We found that they were all resistant to meropenem and imipenem. Amikacin showed the best sensitivity, with gentamicin coming up next. The positive rate *of* bla_{NDM} was 80% (4/5). The sequencing results showed that two isolates belonged to bla_{NDM-1} whereas other 2 isolates carried the bla_{NDM-5}. Other carbapenemase genes including bla_{IMP}, bla_{VIM}, bla_{KPC} and bla_{OXA-48} were not detected. The $bla_{CTX-M-15}$, bla_{TEM-1}, *sul2, aad,* and *aac(6")–Ib–cr* were also detected. MLST analysis showed that NDM-producing *E. coli* were sporadic. Conjugation test indicated bla_{NDM} could be transferred. In conclusion, the bla_{NDM} was the principal resistance mechanism of carbapenem-resistant *E. coli* in the hospital, Henan, China.

Introduction

With the widespread use of antibiotics, the carbapenem-resistant strains have become a serious public health issue in the worldwide and are usually resistant to almost all antibiotics [1,2]. Over the past 10 years, carbapenemase-producing strains increasingly have been reported in Enterobacteriaceae strains [3–5]. The acquisition of carbapenemase genes is one of the most common mechanisms for the gram-negative bacteria resistant to the carbapenem antibiotics. Three classes of carbapenemases have been identified: class A carbapenemase (KPC), class B metallo-enzymes, and class D enzymes (OXA-48 type) [6]. The KPC and OXA-48 are mainly found in *Klebsiella pneumoniae* (*K. pneumoniae*) and many different Enterobacteriaceae strains. There are three main types in Class B metallo-β-lactamases: the Verona integron–encoded metallo-β-lactamase (VIM), IMP types, and New Delhi metallo-β-lactamase (NDM) [7]. The plasmid-mediated carbapenem-resistance is widely perceived as attributing to the dissemination of the carbapenem-resistant genes and the emergence of carbapenem-resistant strains. The carbapenem-resistant genes could co-exist with β-lactamase and other resistant genes on plasmid, which brought a new challenge to the treatment of infections caused by carbapenem-resistant strains [2].

Escherichia coli (*E. coli*), is a bacterial species with high diversity ranged from intestinal commensal strains to intestinal pathogenic, and then extra-intestinal pathogenic strains causing urinary tract infection, sepsis and meningitis [8]. *E. coli* is a leading cause of the community and nosocomial-acquired infection. Recently, it is reported that carbapenem-resistant *E. coli* has been a serious problem in the worldwide, which attracts significant interest and attention [9,10]. In China, carbapenemase have been identified in Hong Kong, Shanghai and Beijing [11–13]. However, few studies were reported on prevalence and mechanism of carbapenem-resistant *E. coli* strains in Henan which is the one of the most populous province in China. Therefore, it is necessary to explore the prevalence and mechanism of carbapenem-resistant *E. coli* strains

* Corresponding author at: College of Public Health, Zhengzhou University, Zhengzhou, Henan 450001, China./Henan Innovation Center of Molecular Diagnosis and Laboratory Medicine, Xinxiang Medical University, Xinxiang, Henan 453003, China.
E-mail addresses: wenwen3_1@126.com (W.-j. Liang), 1508853042@qq.com (H.-y. Liu), wenjuanliang@gs.zzu.edu.cn (G.-C. Duan), zyx080808@163.com (Y.-x. Zhao), sychen@zzu.edu.cn (S.-y. Chen), yhy@zzu.edu.cn (H.-Y. Yang), xyl@zzu.edu.cn (Y.-L. Xi).

http://dx.doi.org/10.1016/j.jiph.2017.09.020

Table 1
The primers used to amplify the resistance genes.

	Gene	Primer	Sequence (5′—3′)	Annealing temperature	Length of amplification fragment/bp
Carbapenem	bla_{NDM}	F	ATTGCCCAATATTATGCACCC	56	748
		R	GGAATGGCTCATCACGATCAT		
	bla_{IMP}	F	TGAGCAAGTTATCTGTATTC	52	740
		R	TTAGTTGCTTGGTTTTGATG		
	bla_{VIM}	F	TTGGTCTACATGACCGCGTCT	55	747
		R	TTTGACAACGTTCGCTGTGT		
	bla_{KPC}	F	ATGTCACTGTATCGCCGTCTA	55	821
		R	TCGCTGTGCTTGTCATCCT		
	bla_{OXA-48}	F	TTGGTGGCATCGATTATCGG	55	744
		R	GAGCACTTCTTTTGTGATGGC		
β-lactamase	bla_{TEM-1}	F	TGCGGTATTATCCCGTGTTG	55	297
		R	TCGTCGTTTGGTATGGCTTC		
	bla_{OXA-1}	F	ACACAATACATATCAACTTCGC	50	814
		R	AGTGTGTTTAGAATGGTGATC		
	$bla_{CTX-M-15}$	F	CGATGTGCAGTACCAGTAA	56	586
		R	TTAGTGACCAGAATCAGCGG		
Aminoglycoside	*aadA1*	F	GCAGCGCAATGACATTCTTG	60	282
		R	ATCCTCGGCGCGATTTTG		
	aac(6′)-Ib-cr	F	TTGCGATGCTCTATGAGTGGCTA	57	482
		R	CTCGAATGCCTGGCGTGTTT		
Quinolone	*qnrA*	F	ATTTCTCACGCCAGGATTTG	56	627
		R	GATCGGCAAAGGTTAGGTCA		
	qnrB	F	ACGATGCCTGGTAGTTGTCC	56	562
		R	GATCGTGAAAGCCAGAAAGG		
	qnrS	F	ACGACATTCGTCAACTGCAA	58	550
		R	TAAATTGGCACCCTGTAGGC		
	qepA	F	AACTGCTTGAGCCCGTAGAT	55	198
		R	GTCTACGCCATGGACCTCAC		
Sulphonamide	*sul1*	F	TTCGGCATTCTGAATCTCAC	52	822
		R	ATGATCTAACCCTCGGTCTC		
	sul2	F	CCTGTTTCGTCCGACACAGA	55	435
		R	GAAGCGCAGCCGCAATTCAT		
Tetracycline	*tetA*	F	GGTTCACTCGAACGACGTCA	56	577
		R	CTGTCCGACAAGTTGCATGA		
	tetB	F	CCTCAGCTTCTCAACGCGTG	56	634
		R	GCACCTTGCTCATGACTCTT		

Carbapenemase gene: bla_{NDM}, bla_{VIM} and bla_{KPC}, bla_{IMP} *and* bla_{OXA-48}; β-lactamase genes: $bla_{CTX-M-1}$, bla_{OXA-1} and bla_{TEM-1}.

in this area, and make appropriate recommendations for the strategically controlling of *E. coli* infection.

Materials and methods

Bacterial isolates and DNA extraction

A total of 1014 *E. coli* strains were isolated from feces, urine specimens, sputum, and excretion of patients collected in Xinxiang city (Henan, China) from January to December, in 2014. All of the isolates were identified according by API20E. The bacterial isolates were stored at −80 °C in 20% glycerol.

Total DNA was extracted from the strains using a bacterial DNA extraction kit (Shanghai Life Feng Biotechnology Co. Ltd. China). The DNA was stored at −20 °C until needed for experiments.

Antimicrobial susceptibility testing and screening for metallo-β-lactamases

Antimicrobial susceptibility tests were performed by the disc diffusion method for imipenem (IPM, 10 μg), meropenem (MEM, 10 μg), ampicillin (AMP, 10 μg), ampicillin/sulbactam (SAM, 20 μg), amoxicillin-potassium (AMC, 20 μg), cefotaxime (CTX, 30 μg), cefepime (FEP, 30 μg), gentamicin (GEN, 10 μg), amikacin (AK, 30 μg), ciprofloxacin(CIP, 5 μg), levofloxacin(LVX,5 μg), sulfamethoxazole (SXT, 30 μg), and tetracycline (TET, 30 μg). The disc diffusion method was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), 2016. The ATCC25922 was used for quality control. The production of carbapenemase was evaluated in the isolates by modified Hodge test and MEM-EDTA double-disk synergy testing [14].

PCR amplification of resistance genes

A total of 18 resistance genes including five carbapenem-resistant genes were selected: bla_{NDM}, bla_{VIM} and bla_{KPC} (this study), bla_{IMP}, *and* bla_{OXA-48} [7], and other resistant genes such as $bla_{CTX-M-15}$ [14], bla_{OXA-1} [15], bla_{TEM-1} and *aad* [16], *sul1* and *sul2* [17], *aac(6')-Ib-cr*, *qepA*, *qnrA*, *qnrB* and *qnrS* [18], *tetA and tetB* [19]. The amplification reactions were carried out in a 25 μL mixture containing DNA template (2 μL), 1 × PCR buffer, 5 pmol of primer, 100 mM dNTP, and 0.5 U of Taq DNA Polymerase (Takara, Japan). The primers and conditions used for the PCR amplification were described in Table 1. The PCR products were resolved on 2% agarose gels stained with ethidium bromide and visualized using a short wavelength ultraviolet light source. The PCR products of bla_{NDM} were sequenced both forward and reverse directions by the Sangon Biotech at Shanghai. The BLAST was applied to confirming the subtype of the bla_{NDM}.

Molecular typing

The carbapenemase-producing *E. coli* genotypes were characterized by multilocus sequence typing (MLST). Seven housekeeping genes (adk, fumC, gyrB, icd, mdh, purA and recA) were amplified according to the protocol described on the MLST webs and

Please cite this article in press as: Liang W-j, et al. Emergence and mechanism of carbapenem-resistant *Escherichia coli* in Henan, China, 2014. J Infect Public Health (2017), http://dx.doi.org/10.1016/j.jiph.2017.09.020