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Original Research Article

Community prevalence of carbapenemase-producing Gram-negative bacteria

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Abstract

Purpose: *To raise awareness of carbapenemase-producing organisms, identify "at-risk" patients when admitted in a medical healthcare facility, and to outline effective infection prevention and control measures in order to halt the entry and spread of these organisms.*
Methods: *A total of 1043 un-duplicated urine specimens of healthy volunteers who had no travel history or history of hospitalization were screened. The carbapenemase genotype of each imipenem-resistant strain was determined. Molecular typing and homology analysis of the main carbapenemase-producing strains were used to reveal the mode of transmission of resistance genes. Through transfer joint experiments, the potential risk of spread of carbapenemase genes was assessed.*
Results: *A total of 19 carbapenemase-producing strains from 1,043 non-duplicated healthy volunteers (1.82 %) were identified. The main carbapenemase-producing organism was E. coli (42.1 %, 8/19). The main carbapenemase genotype of E. coli was blaKPC-2 (7 strains). Results from multi-locus sequence typing (MLST) indicated that 7 E. coli isolates belonged to ST-10, ST-101, ST-131, ST-405, ST-410 and ST-1193 and ST-2562. Homologous cluster analysis revealed that the sequence types among the 7 E. coli were high in diversity. The blaKPC-2 gene was successfully transferred from these isolates to 10.22-14 via conjugation. All recipient cells showed marked decreases in carbapenem sensitivity to imipenem ($p < 0.05$)). The degrees of conjugation were $2.10±0.12 \times 10^{-4}$, $1.96±0.14 \times 10^{-4}$, $2.72±0.18 \times 10^{-4}$, $3.15±0.20 \times 10^{-4}$, $2.92±0.23 \times 10^{-4}$, $3.50±0.20 \times 10^{-4}$ and $4.12±0.24 \times 10^{-4}$ in recipient cells of TC7.23-51, TC8.9-42, TC8.15-11, TC8.23-59-3, TC8.23-83, TC9.08-47 and TC10.13-15, respectively.*
Conclusion: *The findings demonstrate the pattern and features of carbapenemase-insensitive E. coli. The blaKPC-2 was the main community-prevalent gene of carbapenem-resistant E. coli. In view of increasing incidence of resistance to multi-drug therapy, surveillance of insensitivity to antibiotics is vital, especially urinary system infection due to carbapenem-insensitive E. coli.*

Keywords: *Carbapenem resistance, Incidence, Communal spread, Monitoring, Antimicrobial resistance, Gene*

INTRODUCTION

Over the last decade, there has been a rapid increase in carbapenem resistance. The most frequently described carbapenemases are *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), imipenemase metallo-β-lactamase (IMP), Verona integron-borne metallo-β-lactamase (VIM), and oxacillinase β-lactamase 48 (OXA-48) [1]. The aforementioned enzymes render bacteria resistant to carbapenem group of antimicrobial drugs used to combat serious infections in critically-sick patients [2]. Several Gram-negative bacteria produce carbapenemase [3].

Urinary tract infections are second only to respiratory tract infections in human infectious diseases. *Escherichia coli* and *Klebsiella pneumonia* are common pathogens involved in urinary tract infections [4]. In recent years, several studies have reported the isolation of carbapenem-resistant bacteria from the urinary system [5-7]. However, these reports are derived from a combination of reactive screening, outbreak control, in-patient surveillance, and diagnostic samples [5-7]. Thus, the true community prevalence is unknown. Therefore, it is important to prevent the spread of carbapenem resistance genes in carriers and avert further outbreaks, based on local prevalence. Due to paucity of local communal reports on incidence of carbapenemase-producing organisms, this study was carried out amongst the community population in The Second Affiliated Hospital of Nantong University.

METHODS

Subject recruitment and sampling

A total of 1,034 adult volunteers (616 males and 418 females) were recruited from healthy people attending physical examination at The Second Affiliated Hospital of Nantong University. All volunteers had no history of hospitalization, and had not traveled in the previous 6 months. Their ages ranged from 24 to 56 years. Urine microbiota samples of the volunteers were collected from April 16 to October 31, 2019. Subjects who were treated with antibiotics or other drugs within the previous 30 days before the hospital visit were excluded. This research was approved by the ethics committee of the Second Affiliated Hospital of Nantong University (approval no. 20200016), and followed international guidelines for human studies [8]. All the subjects provided written informed consent before participation.

The method used for active screening was as previously described by Gottschick [9], with slight modification. In the active screening process, 10 ml of urine sample was aseptically collected. Non-duplicated samples were centrifuged at 4000 rpm for 15 min within 2 h, and approximately 9.5 ml of supernatant was discarded. The remaining 0.5 ml of liquid and sediment were shaken and mixed, and incubated on Colombian blood agar with 4 μg/ml imipenem (Sigma-Aldrich Corp., St. Louis, MO, USA.) for 18 – 24 h at 35 ℃ in a 5 % CO_2 incubator. All isolates were purified using Columbia blood agar (Autobio, Zhengzhou, China). The bacterial species were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Autof ms1000, Autobio, Zhengzhou, China).

A total of 19 strains of Gram-negative bacteria were collected, comprising *Escherichia coli* (*E. coli*), *Enterobacter cloacae*, *Serratia marcescens*, and *Klebsiella oxytoca,* after excluding 54 strains of *Stenotrophomonas maltophilia* which are naturally resistant to imipenem. Carbapenem resistance phenotype (MIC value of imipenem ≥ 4μg/ml) was determined in the isolates collected. *Escherichia coli* ATCC 25922 served as quality control. Drug susceptibilities were judged according to the M100-S27 standard established by Clinical and Laboratory Standards Institute (CLSI, 2017) [10].

Determination of carbapenemase genes

The carbapenemase produced by each of these isolates was determined with PCR. All isolates were subjected to a simple 10-min boiling extraction. The carbapenem-resistant genes were screened using PCR in all strains [11-14]. The primer sequences used are shown on Table 1. Moreover, DNA sequencing was performed on both strands of the PCR amplification products. The results were compared and aligned with reference sequences using the online BLAST database.

Multi-locus sequence typing

The genotypes of the KPC-2 carbapenemase-synthesizing *E. coli* were determined using MLST, with 7 housekeeping genes (Table 2) subjected to amplification as outlined on MLST website, followed by sequencing using Sangon Biotech, and the allelic numbers were obtained. Thereafter, MEGA 5.0 software was used to construct a phylogenetic tree reflecting the homology of the isolates.

Conjugation experiments

The transferability of carbapenemase genes was determined with broth-mating method, using KPC-elaborating strains of *E. coli* (7.23-51, 8.9-42, 8.15-11, 8.23-59-3, 8.23-83, 9.08-47 and 10.13-15) sensitive to amikacin as donors.

Urethral pathogenic *E. coli* (UPEC) 10.22-14, which was sensitive to imipenem and resistant to amikacin, was used as recipient. The assay was

Table 1: Primers used in PCR

Gene	Primer sequence (5'-3')	Amplification product (bp)
blaKPC	F: TCGCCGTCTAGTTCTGCTGTCTT R: GGGATGGCGGAGTTCAG	507
blaNDM	F: CGAGCATTACCAAAGGGTGA R: TAGTGCTCAGTGTCGGCATC□□	1300
blaIMP	F: ATGAGCAAGTTATCTGTATTCTTTAT R: TTAGTTGCTTAGTTTTGATGGTTT	741
blaVIM	F: GGTCGCATATCGCAACGCAGT R: CGGCGACTGAGCGATTTTTG	636
blaIMI	F: CCATTCACCCATCACAAC R: CTACCGCATAATCATTTGC	440
blaSME	F: AGATAGTAAATTTTATAG R: CTCTAACGCTAATAG	527
blaMUS-1	F: GGTCATCACTACCCACTCCAC R: AAGCTATCACGTTACCATCGGC	323
blaOXA-1	F: GCAAATATTATCTACAGCAGCGC R: GAGGATCTTGAAAGTTGAATCTGG	183
blaOXA-23	F: GATGTGTCATAGTATTCGTCG R: TCACAACAACTAAAAGCACTG	1058
blaOXA-48	F: TTGGTGGCATCGATTATCGG R: GAGCACTTCTTTTGTGATGGC	744
blaOXA-51	F: ATGAACATTAAAGCACTC R: CTALTAAAATACCTAATrGTTC	750
blaB1	F: TTGTGGTTATAGACTGTCCGTGGG R: TATTCAAGACCTCCGGCACGAT	136

Table 2: Primer sequences of *E. coli* housekeeping genes

Gene	Primer sequence (5'-3')	Amplification product (bp)
adk	F: ATTCTGCTTGGCGCTCCGGG R: CCGTCAACTTTCGCGTATTT	583
fumC	F: TCACAGGTCGCCAGCGCTTC R: GTACGCAGCGAAAAAGATTC	806
gyrB	F: TCGGCGACACGGATGACGGC R: ATCAGGCCTTCACGCGCATC	911
icd	F: ATGGAAAGTAAAGTAGTTGTTCCGGCACA R: GGACGCAGCAGGATCTGTT	878
mdh	F: ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG R: TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT	932
recA	F: CGCATTCGCTTTACCCTGACC R: TCGTCGAAATCTACGGACCGGA	780
purA	F: CGCGCTGATGAAAGAGATGA R: CATACGGTAAGCCACGCAGA	816

carried out at 37 ℃ for 12 h, at a donor: recipient cell ratio of 1:1. The screening of transconjugant cells (Tc) was done from Luria-Bertani agar plates containing imipenem (4μg/ml) and amikacin (128 μg/ml). The donor and recipient cells were inoculated on their respective screening plates, for control and calculation of degree of conjugation. The experiments were repeated three times, and the results are expressed as mean. The degree of conjugation (C) was calculated as shown in Eq 1.

(C) = Tc/Dc ………… (1)

where Tc and Dc are transconjugant and donor cells, respectively.

RESULTS

Carbapenem resistance gene profile

Carbapenem resistance genes were detected in a total of 19 strains from healthy volunteers, using PCR. The 19 strains were from non-duplicate volunteers, and the prevalence was about 1.84 % (19/1034). The main isolates in 19 carbapenem-resistant gram-negative strains were *E. coli* (42.1 %, 8/19) (Table 3). The prevalence of carbapenem-resistant *E. coli* was about 0.77 % (8/1034). The sequences of PCR amplification products were analyzed using the online BLAST database. The results showed that the carbapenemase genes carried by these strains were *blaKPC-2*, *blaIMP-4, blaOXA-23, blaIMP-1, blaNDM-1, blaMUS-1*, *blaB1.* These results are presented on Table 3.

Molecular subtyping analysis with MLST and strain homology analysis

Results from MLST indicated that 7 *E. coli* isolates from volunteers belonged to ST-10, ST-101, ST-131, ST-405, ST-410 and ST-1193 and ST-2562 (Table 4). Homologous cluster analysis revealed that the sequence types among the *E. coli* isolates had high diversity (Figure 1). This indicated that the transmission characteristics of the *blaKPC-2* gene in this study were not mainly vertical transfer.

Risk assessment of spread of *E. coli blaKPC-2*

The 10.22-14 strain was a clinically isolated UPEC used as the recipient cell which was sensitive to imipenem but resistant to amikacin. The *blaKPC*-2 genes were successfully transferred from these isolates to 10.22-14 via conjugation, and the nucleic acid fragments of the corresponding size of *blaKPC*-2 gene were obtained through PCR amplification in the transconjugant cells. The MIC of the recipient cells was determined. All recipient cells showed marked reductions in carbapenem sensitivity to imipenem, when compared to 10.22-14. Results from conjugation assay showed that *blaKPC*-2 was transferrable to UPEC, thereby promoting carbapenemase resistance of the latter. These results are shown on Table 5 and in Figure 2.

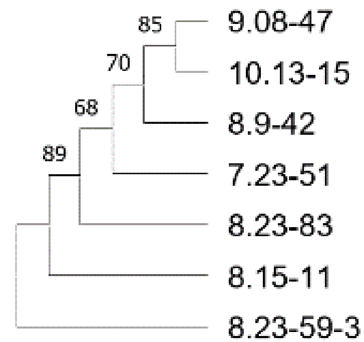

Figure 1: Phylogenetic trees of *E. coli* isolates based on 7 housekeeping gene sequences

DISCUSSION

A report by WHO states that carbapenemase-producing bacteria constitute a serious health challenge worldwide [15]. Due to lack of effective treatment strategies and high incidence of fatalities linked to carbapenem-resistant *Enterobacteriaceae* (CRE), preventive measures are of prime importance.

Active screening for identification of asymptomatic colonies, and preemptive seclusion of contacts reduce the pressure of colonization and transmissions between

Table 3: Carbapenem resistance gene profiles

Species	Number of strains	Resistance genotype
Escherichia coli	8 (42.1%, 8/19)	*blaKPC-2* (7 strains)*, blaIMP-4* (1 strains)
Acinetobacter baumannii	1 (5.26%, 1/19)	*blaOXA-23*
Klebsiella oxytoca	1 (5.26%, 1/19)	*blaIMP-1*
Klebsiella Pneumoniae	1 (5.26%, 1/19)	*blaKPC-2*
Enterobacter cloacae	1 (5.26%, 1/19)	*blaNDM-1*
Pfiodeus mirabidis	1 (5.26%, 1/19)	*blaKPC-2*
Serratia marcescens	1 (5.26%, 1/19)	*blaOXA-23*
Pseudomonas putida	1 (5.26%, 1/19)	*blaIMP-4*
Morganella morganii	1 (5.26%, 1/19)	*blaNDM-1*
Raoultella ornithinolytica	1 (5.26%, 1/19)	*blaKPC-2*
Myroides odoratus	1 (5.26%, 1/19)	*blaMUS-1*
Elizabethkingia meningoseptica	1 (5.26%, 1/19)	*blaB1*

Table 4: Genotypes of *blaKPC-2*-producing *E. coli* strains

Strain	Volunteer gender	Volunteer age (years)	Sequence type
7.23-51	Female	54	ST-1193
8.9-42	Male	39	ST-10
8.15-11	Male	27	ST-2562
8.23-59-3	Female	42	ST-131
8.23-83	Male	56	ST-405
9.08-47	Female	24	ST-410
10.13-15	Male	45	ST-101

Table 5: Relevant indicators of transconjugant cells

Strain	Imipenem (μg/ml)	*blaKPC*-2	Conjugation rate
10.22-14	0.125	-	-
TC7.23-51	16	+	$2.10 \pm 0.12 \times 10^{-4}$
TC8.9-42	16	+	$1.96 \pm 0.14 \times 10^{-4}$
TC8.15-11	16	+	$2.72 \pm 0.18 \times 10^{-4}$
TC8.23-59-3	16	+	$3.15 \pm 0.20 \times 10^{-4}$
TC8.23-83	16	+	$2.92 \pm 0.23 \times 10^{-4}$
TC9.08-47	16	+	$3.50 \pm 0.20 \times 10^{-4}$
TC10.13-15	32	+	$4.12 \pm 0.24 \times 10^{-4}$

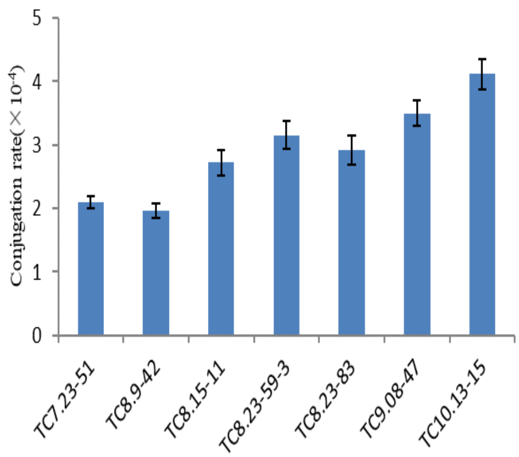

Figure 2: Conjugation rate of *blaKPC-2* gene in 7 strains of *Escherichia coli*

asymptomatic carriers and high-risk groups.

The strategies employed to screen for CRE depend on community epidemiology. In this epidemiological investigation, 19 carbapenem-resistant gram-negative strains were isolated from urinary system of 1,034 healthy community volunteers. The 19 strains were from non-duplicate volunteers, with about 1.84 % detection (19/1034). About 80 – 90 % of urinary tract infections are caused by urethral pathogenic *E. coli* [16]. *Escherichia coli* was also the main isolate in 19 carbapenem-resistant gram-negative strains (42.1 %, 8/19). Data from 1,425 hospitals in the National Bacterial Resistance Surveillance Network in China 2019 showed that the resistance of *Escherichia coli* to carbapenem antibiotic (imipenem) was about 1.5 % [17]. *Escherichia coli* was detected in all 1,034 volunteers; the detection of carbapenem-resistant *E. coli* was about 0.77 % (8/1034). Their carbapenemase genotypes were *blaKPC-2* (7 strains) and *blaIMP-4* (1 strain). Thus, the main epidemic carbapenemase genotype of *E. coli* detected from urinary system in this region was *blaKPC-2*.

However, Asia-Pacific countries are often linked to IMP from *Enterobacter spp.*, *Klebsiella spp.*, *Citrobacter spp.*, and *E. coli* [18-20]. This is inconsistent with the results of the present study, probably due to the fact that this investigation targeted only Gram-negative bacteria in the urinary system of healthy groups. Nevertheless, consistent with the present results, it has been reported that the KPC group of serine carbapenemases are the most common globally [21-24]. Thus, it was considered necessary to understand how KPC spreads in this region.

Results from MLST indicated that 7 *E. coli* strains belonged to different types. Homologous cluster analysis revealed high diversity in sequence among 7 *E. coli* isolates. The results indicated that the transmission characteristics of the *blaKPC-2* gene in this region were not mainly vertical transfer. Horizontal transfer conjugation test showed that the *blaKPC-2* genes carried by the donor bacteria were successfully transferred to the recipient cell. All recipient cells exhibited significantly reduced carbapenem susceptibility to imipenem. Therefore, the transmission of *blaKPC-2* gene in this region involves mainly horizontal transfer. Active screening for patients using urine samples should be a valuable clinical diagnostic and treatment option. Thus, it is very important to establish screening/detection protocol for carbapenemase-producing organisms in medical diagnostic laboratories.

CONCLUSION

The results obtained in this study underscore the epidemiological features and high insensitivity of carbapenemase-insensitive *E. coli*. The *blaKPC-2* was the main community prevalent gene in carbapenem-resistant *E. coli*, and it could be spread through plasmid-catalyzed horizontal gene transfer. Screening for antimicrobial resistance remains a principal strategy for tackling increases in prevalence of multi-drug resistance, especially *CRE* linked to urinary system infections, which is of serious medical concern.

DECLARATIONS

Acknowledgement

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Conflicts of interest

No conflict of interest is associated with this study.

Contribution of authors

This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents. The study was conceived and designed by Xinling Li and Xinjian Cao; Yihua Zhu and Fuying Chu collected and analyzed the data, while Yihua Zhu wrote the manuscript. All authors read and approved the manuscript prior to publication.

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