Emergence of Klebsiella pneumoniae carbapenemase-producing Proteus mirabilis in Hangzhou, China

ARTICLE *in* CHINESE MEDICAL JOURNAL · SEPTEMBER 2010

Impact Factor: 1.05 · DOI: 10.3760/cma.j.issn.0366-6999.2010.18.014 · Source: PubMed

5 AUTHORS, INCLUDING:

Zi-Ke Sheng
Fudan University
16 PUBLICATIONS **88** CITATIONS
SEE PROFILE

Zike Sheng
Fudan University
40 PUBLICATIONS **474** CITATIONS
SEE PROFILE

Original article

Emergence of *Klebsiella pneumoniae* carbapenemase-producing *Proteus mirabilis* in Hangzhou, China

SHENG Zi-ke, LI Jun-jie, SHENG Guo-ping, SHENG Ji-fang and LI Lan-juan

Keywords: *enterobacteriaceae; proteus mirabilis; carbapenem; Klebsiella pneumoniae carbapenemase*

Background Carbapenems are used to treat severe infections caused by multi-drug-resistant organisms, however, the emergence of carbapenem-resistant bacterial isolates is becoming an increasing therapeutic challenge. Since the first *Klebsiella (K.) pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* was reported in 2001, KPC-producing isolates have been found increasingly, specially in *Enterobacteriaceae*. The aim of this study was to characterize the mechanisms of a carbapenem-resistant *Proteus (P.) mirabilis*.

Methods A carbapenem-resistant *P. mirabilis* isolate was recovered from pleural drainage fluid of a patient admitted to surgical intensive care unit. Antimicrobial susceptibility testing of the isolate was performed by disk diffusion according to Clinical and Laboratory Standards Institute guidelines, and subsequent minimal inhibitory concentrations were determined with the E-test. Amplification of the bla_{KPC} gene generated a positive band and the PCR products were sequenced subsequently. The plasmid of the isolate was extracted and was successfully transformed into *Escherichia (E.) coli* DH5α.

Results The *P. mirabilis* isolate was resistant to all detected antimicrobial agents except tigecycline. KPC-2 was confirmed by DNA sequence analysis. The transformant *E. coli* was resistant to carbapenems. Further study demonstrated that upstream and downstream regions of bla_{KPC-2} were identical to that observed in *K. pneumoniae* submitted to GenBank from China in 2007.

Conclusion Carbapenem resistance in the *P. mirabilis* isolate in this study is mainly due to production of KPC-2.

Chin Med J 2010;123(18):2568-2570

Carbapenems are used to treat severe infections caused by multi-drug-resistant organisms, especially by extended-spectrum β-lactamase-producing pathogens. However, the emergence of carbapenemase is becoming an increasing therapeutic challenge because this enzyme hydrolyzes not only carpaenems but also penicillins, cephalosporins, and monobactams.[1] The carpaenem- resistant *Klebsiella (K.) pneumoniae* was first reported to produce *K. pneumoniae* carbapenemase (KPC)-1 in US in 2001. Since then, KPC-type β-lactamases have been found in *Klebsiella oxytoca,*[2] *Escherichia coil (E. coli),*[3] *Citrobater spp.*,[4] *Pseudomonas aeruginosa*,[5] *Salmonella spp.*,[6] *Serratia marcescens*,[4] as well as *Proteus mirabilis (P. mirabilis)*.[7]

P. mirabilis are clinical isolates which barely have β-lactamase activity under usual growth conditions. However, carbapenem resistance in *Proteus spp.* mediated by OXA-23 (a class D β-lactamase) and VIM-1 (a class B metallo-β-lactamase) has been identified.[7] Changes in penicillin-binding proteins or outer membrane proteins could result in carbapenem resistance in *Proteus pp*.[8,9] Carbapenem resistance in *P. mirabilis* caused by a class A β-lactamase, KPC-2, firstly reported by Tibbetts et al[7] in 2008. In this study, we characterized a clinical carbapenem-resistant isolate of *P. mirabilis* carrying bla_{KPC-2} gene in Hangzhou, China.

METHODS

Bacterial strains

In December 2009, a carbapenem-resistant *P. mirabilis* strain BP1 was isolated from pleural drainage fluid of a patient with esophageal carcinoma, in surgical intensive care unite (SICU) in the First Affiliated Hospital of Zhejiang University (Hangzhou, China). Prior to the isolation, the patient was repeatedly treated with antimicrobial agents including penicillins, cephalosporins, carbapenems, and aminoglycosides for 2 months. The isolate was identified with the Vitek-2 system (bioMe'rieux, France).

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *P. mirabilis* BP1 was assayed using disk diffusion according to guidelines of Clinical and Laboratory Standards Institute.[10] *E. coli* ATCC 25922 and *E. coli* DH5α were used as quality control in the assay.

DOI: 10.3760/cma.j.issn.0366-6999.2010.18.014

Department of Infectious Disease, State Key Laboratory for Diagnosis and Treatment of Infectious Disease, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310003, China (Sheng ZK, Li JJ, Sheng GP, Sheng JF and Li LJ)

Correspondence to: Dr. SHENG Ji-fang, Department of Infectious Disease, State Key Laboratory for Diagnosis and Treatment of Infectious Disease, the First Affiliated Hospital, School of Medicine, Hangzhou, Zhejiang 310003, China (Tel: 86-571-87236759. Fax: 86-571-87236755. Email: shengji.fang@163.com)

This study was supported by the National Basic Research Programs of China (No. 2007CB513004), and the Major National S&T Projects for Infectious Diseases (2008ZX10002-007).

PCR amplification for *bla* genes

The primers used to amplify bla_{KPC}, bla_{TEM}, bla_{SHV}, bla_{IMP-1}, bla_{IMP-2}, bla_{VIM-1}, bla_{VIM-2}, bla_{SPM-1} were described previously.[11,12] Prepared bacterial DNA of the *P. mirabilis* BP1 was used as the template for PCR amplifications.

Conjugal transfer experiment and analysis of plasmid

The conjugation experiment was carried out in mixed broth cultures and rifampicin-resistant *E. coli* 600 was used as the recipient strain. Plasmids of *P.mirabilis* BP1 were prepared with a QIAprep Spin Miniprep Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The plasmids were transformed into competent *E. coli* DH5α. The transformants, *E. coli* BP001T, were selected on a Mueller-Hinton Agar (MHA) plate containing 1 μg/ml of meropenem. *E. coli* BP1 T contained a plasmid with the size greater than 50 kb (data not shown). Amplification of bla_{KPC} gene of the *E.coli* BP1T by PCR with the same primer described above was positive. Additional antimicrobial susceptibilities of the *P.mirabilis* BP1 and *E.coli* BP1T were assayed by E-test (AB Biodisk, Solna, Sweden).

RESULTS

P. mirabilis BP1 was resistant to all the tested agents. The presence of bla_{KPC} was confirmed while the other *bla* genes mentioned above were not detected. And the DNA sequencing of the PCR products identified the gene as *KPC-2*.

The conjugation experiments failed. DNA sequencing analysis of *KPC-2* and its adjacent regions was performed using traditional Sanger method. An approximately 3-kb fragment including bla_{KPC} gene was analyzed by sequencing. Subsequent BLAST search showed that the *KPC-2* gene in this study had 100% identity at the nucleic acid level with the *KPC-2* gene sequence submitted to GenBank from Hangzhou, China (GenBank accession number DQ897687). Furthermore, the regions flanking bla_{KPC-2} in *P. mirabilis* BP1 were identical to that observed in *K. pneumoniae* by Wei et al in China.[13] Minimal inhibitory concentrations of *E. coli* BP1T were lower than those for *P.mirabilis* BP1 (Table). This may contribute to the mutation of porins which results in the decreased membrane permeability.

In addition, because a prospective study on the relationship between gut flora and nosocomial infection pathogens was performing in our laboratory, the carpaenem-resistant isolates of *P. mirabilis* were also found in fecal specimens of the patient in the week the *P. mirabilis* BP1 was isolated from pleural drainage fluid.

DISCUSSION

In this study, we described a clinical KPC-2-positive *P. mirabilis* isolate in China. Reports on carpaenem-resistant microbes carrying bla_{KPC} gene have been increasing,[14,15] since the initial discovery of KPC-producing *K. pneumoniae* in China.[13] This may contribute to the wide use of carpaenems and cephalosporins. In addition, what alarming is that several infections caused by KPC-producing *K. pneumoniae*, *E.coli*, *Enterobacter cloacae*, and *P. aeruginosa* have occurred during the past 6 months in the hospital (data unpublished). Because there is potential prevalence for those KPC-positive bacteria in the clinic, further study on the epidemiological relationship between these organisms is needed.

It has already been known that higher mortality rates associated with infection were caused by bla_{KPC}-carrying bacteria.[16] Therefore, it is essential to establish strict resistance surveillance and drug administration for avoiding the epidemic KPC-producing organisms in this country.

Table. Minimal inhibitory concentrations (μg/ml) of *P.mirabilis* BP1, *E. coli* BP1T, and *E. coli* DH5α

Antimicrobial agents	*P. mirabilis BP1* (parent)	*E. coli BP1T* (transformant)	*E. coli DH5α* (host strain)
Imipenem	≥32	12	0.125
Ertapenem	≥32	4	0.016
Meropenem	8	6	0.016
Aztreonam	≥256	≥256	0.023
Ampicillin	≥256	≥256	2.000
Cefotaxime	≥32	≥32	0.125
Cefepime	24	16	0.023
Gentamicin	4	0.380	0.380
Amikacin	2	0.750	0.750
Ciprofloxacin	12	0.023	0.023
Colistin E	≥256	0.750	0.750
Tigecycline	1.500	0.250	0.190

Acknowledgements: We thank SHEN Ping and LING Zong-xing for constructional suggestions, and Charlie Xiang for comments on this manuscript.

REFERENCES

1. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob Agents Chemother 2001; 45: 1151-1161.
2. Yigit H, Queenan AM, Rasheed JK, Biddle JW, Domenech-Sanchez A, Alberti S, et al. Carbapenem-resistant strain of Klebsiella oxytoca harboring carbapenem-hydrolyzing β-lactamase KPC-2. Antimicrob Agents Chemother 2003; 47: 3881-3889.
3. Navon-Venezia S, Chmelnitsky I, Leavitt A, Schwaber MJ, Schwartz D, Carmeli Y. Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant *Escherichia coli* clones in Israel. Antimicrob Agents Chemother 2006; 50: 3098-3101.
4. Deshpande LM, Rhomberg PR, Sader HS, Jones RN. Emergence of serine carbapenemases (KPC and SME) among clinical strains of *Enterobacteriaceae* isolated in the United States medical centers: report from the MYSTIC program (1999–2005). Diagn Microbiol Infect Dis 2006; 56: 367-372.

5. Villegas MV, Lolans K, Correa A, Kattan JN, Lopez JA, Quinn JP, et al. First identification of *Pseudomonas aeruginosa* isolates producing a KPC type carbapenem-hydrolyzing β-lactamase. Antimicrob Agents Chemother 2007; 51: 1553-1555.
6. Miriagou V, Tzouvelekis LS, Rossiter S, Tzelepi E, Angulo FJ, Whichard JM. Imipenem resistance in a Salmonella clinical strain due to plasmid-mediated class A carbapenemase KPC-2. Antimicrob Agents Chemother 2003; 47: 1297-1300.
7. Tibbetts R, Frye JG, Marschall J, Warren D, Dunne W. Detection of KPC-2 in a clinical isolate of *Proteus mirabilis* and first reported description of carbapenemase resistance caused by a KPC β-lactamase in P. mirabilis. J Clin Microbiol 2008; 46: 3080-3083.
8. Neuwirth C, Siebor E, Duez JM, Pechinot A, Kazmierczak A. Imipenem resistance in clinical isolates of *Proteus mirabilis* associated with alterations in penicillin-binding proteins. J Antimicrob Chemother 1995; 36: 335-342.
9. Villar HE, Danel F, Livermore DM. Permeability to carbapenems of Proteus mirabilis mutants selected for resistance to imipenem or other beta-lactams. J Antimicrob Chemother 1997; 40: 365-370.
10. Clinical and Laboratory Standards Institute. 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. CLSI document M7-A6. Clinical and Laboratory Standards Institute, Wayne, PA.
11. Lomaestro BM, Tobin EH, Shang W, Gootz T. The spread of *Klebsiella pneumoniae carbapenemase*-producing *K. pneumoniae* to upstate New York. Clin Infect Dis 2006; 43: e26-e28.
12. Shibata N, Doi Y, Yamane K, Yagi T, Kurokawa H, Shibayama K, et al. PCR typing of genetic determinants for metallo-β-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. J Clin Microbiol 2003; 41: 5407-5413.
13. Wei ZQ, Du XX, Yu YS, Shen P, Chen YG, Li LJ. Plasmid-Mediated KPC-2 in a Klebsiella pneumoniae isolate from China. Antimicrob Agents Chemother 2007; 51: 763-765.
14. Cai JC, Zhou HW, Zhang R, Chen GX. Emergence of Serratia marcescens, Klebsiella pneumoniae, and Escherichia coli isolates possessing the plasmid-mediated carbapenem-hydrolyzing beta-lactamase KPC-2 in intensive care units of a Chinese hospital. Antimicrob Agents Chemother 2008; 52: 2014-2018.
15. Wu Q, Liu Q, Han L, Sun J, Li Y. Plasmid-mediated carbapenem-hydrolyzing enzyme KPC-2 and ArmA 16S rRNA methylase conferring high-level aminoglycoside resistance in carbapenem-resistant Enterobacter cloacae in China. Diagn Microbiol Infect Dis 2010; 66: 326-328.
16. Bratu S, Mooty M, Nichani S, Landman D, Gullans C, Pettinato B, et al. Emergence of KPC-possessing Klebsiella pneumoniae in Brooklyn, New York: epidemiology and recommendations for detection. Antimicrob Agents Chemother 2005; 49: 3018-3020.

(Received May 18, 2010)
Edited by PAN Cheng