A *Klebsiella pneumoniae* sputum culture isolate from China carrying *bla*_{OXA-1}, *bla*_{CTX-M-55} and *aac*(6')-Ib-cr

Fluoroquinolones, antimicrobial agents used extensively in medicine and agriculture, are considered safe from naturally occurring antimicrobial-modifying enzymes (Robicsek *et al.*, 2006). Acquired resistance to fluoroquinolones in Gram-negative bacteria most often corresponds to mutations in the *gyrA* DNA sequence as well as the *parC* DNA sequence (Weigel *et al.*, 2002; Shi *et al.*, 2005). Reduced susceptibility to ciprofloxacin in clinical bacterial isolates has been conferred by a variant of the *aac*(6')-Ib gene encoding aminoglycoside acetyltransferase: *aac*-Ib-cr. This enzyme reduces the activity of ciprofloxacin by N-acetylation at the amino nitrogen on its piperazinyl substituent. The *aac*(6')-Ib-cr gene is most common in *Escherichia coli* and equally prevalent in ciprofloxacin-susceptible and -resistant strains, and not associated with *qnr* genes (Park *et al.*, 2006; Robicsek *et al.*, 2006).

In July 2006, we identified a *Klebsiella pneumoniae* CZ2987 sputum isolate producing extended-spectrum β -lactamases by using the Phoenix-100 Automated Microbiology system (Becton Dickinson). MICs of ampicillin, piperacillin, ceftazidime, ceftriaxone, cefepime, aztreonam, imipenem, ciprofloxacin, levofloxacin, gentamicin, amikacin and tobramycin were determined by agar dilution according to the guidelines of the CLSI (2005).

Whole-cell DNA from *K. pneumoniae* CZ2987, prepared by a rapid alkaline lysis procedure, was used as a template in PCR assays. Oligonucleotide primers were designed on the basis of the nucleotide sequence published in GenBank. The primers were: *bla*_{OXA-1} group, 5'-CTG TTG TTT GGG TTT CGC AAG-3' and 5'-CTT GGC TTT TAT GCT TGA TG-3'; *bla*_{CTX-M-1} group, 5'-ATG GTT AAA AAA TCA CTG CGC-3' and 5'-TCC CGA CGG CTT TCC GCC TT-3'; *aac*(6')-Ib, 5'-ATG ACT GAG CAT GAC CTT GC-3' and

5'-TTA GGC ATC ACT GCG TGT TC-3'; *gyrA*, 5'-AAA TCT GCC CGT GTC GTT GGT-3' and 5'-GCC ATA CCT ACG GCG ATA CC-3'; *parC*, 5'-AAA CCT GTT CAG CGC CGC ATT-3' and 5'-AAA GTT GTC TTG CCA TTC ACT-3'. All of the amplification reactions were performed with an initial denaturation (3 min at 93 °C) followed by 35 cycles of denaturation (1 min at 93 °C), annealing (1 min at 55 °C) and extension (1 min at 72 °C). The final extension was for 7 min at 72 °C. Twenty microlitres of each sample was subjected to electrophoresis on 2 % agarose gels. The amplicons were purified with PCR Clean Up kits (Roche Molecular Biochemicals) and sequenced on an ABI PRISM 377 analyser (Applied Biosystems).

The *K. pneumoniae* CZ2987 isolate was resistant to ampicillin (MIC >256 mg l⁻¹), piperacillin (MIC >256 mg l⁻¹), ceftazidime (MIC 128 mg l⁻¹), ceftriaxone (MIC 256 mg l⁻¹), cefepime (MIC 128 mg l⁻¹), aztreonam (MIC >256 mg l⁻¹), ciprofloxacin (MIC 64 mg l⁻¹), levofloxacin (MIC 32 mg l⁻¹), gentamicin (MIC 128 mg l⁻¹), amikacin (MIC 64 mg l⁻¹) and tobramycin (MIC 256 mg l⁻¹), and susceptible to imipenem (MIC 0.5 mg l⁻¹). After 2 % agarose gel electrophoresis, it was found that *K. pneumoniae* CZ2987 produced a band of 519 bp, which was then purified and sequenced using an ABI PRISM 377 analyser. The sequence of the *K. pneumoniae* CZ2987 *aac*(6')-Ib-cr gene had 99 % identity with that of the *aac*(6')-Ib-cr gene with accession no. EF100892. A C→A tranversion occurred at base 304; however, the variation was a silent mutation. The GenBank/EMBL/DDBJ accession number for the CZ2987 *aac*(6')-Ib-cr gene is EF443074. Furthermore, *K. pneumoniae* CZ2987 simultaneously carried *bla*_{OXA-1} and *bla*_{CTX-M-55} genes. In addition, in our study, a mutation of the quinolone resistance determining region in *gyrA* (Ser83→Leu and Asp87→Asn) and *parC* (Ser80→Ile) also occurred.

K. pneumoniae is an important pathogen that causes urinary tract infections, pneumonia and intra-abdominal infections in hospitalized immunocompromised patients with severe underlying diseases. Of the fermentative Gram-negative bacilli causing nosocomial infection, *K. pneumoniae* is second only to *E. coli*. *K. pneumoniae* is becoming multidrug-resistant and carries a variety of resistance genes. To our knowledge, this is the first report of the *aac*(6')-Ib-cr gene, which reduces the activity of both aminoglycosides and fluoroquinolones (Machado *et al.*, 2006), in PR China.

Our finding indicating the potential spread of multidrug-resistant *K. pneumoniae* is worrying. Further research is required to identify the genetic context of the *aac*(6')-Ib-cr gene and therefore understand the mechanism of spread.

Weifeng Shi,¹ Jianping Qin² and Zuhuang Mi³

¹Department of Clinical Laboratory, Third Affiliated Hospital of Suzhou University, Changzhou 213003, PR China

²Department of Clinical Laboratory, Changzhou Chinese Traditional Medicine Hospital, Changzhou 213003, PR China

³Wuxi Clone Gen-Tech Institute, Wuxi 214026, PR China

Correspondence: Weifeng Shi (shiweifeng67@163.com)

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