Imipenem resistance due to class A carbapenemase KPC-2 in a Flavobacterium odoratum isolate

Resistance to carbapenems among Enterobacteriaceae strains is mostly due to production of various carbapenemases, such as metallo-β-lactamas, expanded-spectrum oxacillinases and Ambler class A enzymes. The KPC family is an important group that contributes to carbapenem resistance in various pathogens. These enzymes, which can hydrolyse all kinds of β-lactams, have been well identified among many members of the Enterobacteriaceae (Bratu et al., 2005; Yigit et al., 2003; Hossain et al., 2004; Jiang et al., 2010), but have rarely been reported outside this family. Here, we describe for what we believe to be the first time a Flavobacterium odoratum isolate from China that can produce the carbapenem-hydrolysing enzyme KPC-2.

F. odoratum WX2856 was obtained in February 2010 from the aspirate of an intra-abdominal abscess in a 57-year-old man with acute diffuse peritonitis at Wuxi Hospital for Infectious Disease. The identification of F. odoratum WX2856 was performed using the VITEK system (bioMérieux) and confirmed using API strips (bioMérieux). The MICs of drugs were determined by using the agar dilution method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2009). The whole-cell DNA from F. odoratum WX2856 was isolated by a rapid alkaline lysis procedure and used as a template in further PCR assays. The primers for amplification of the blaKPC, blaIMP, blaVIM, blaSHV and blaOXA genes were designed as previously described (Smith Moland et al., 2003; Senda et al., 1996; Tsakis et al., 2000; Queenan et al., 2000; Donald et al., 2000). The 25 μl reaction mixture for the PCR assays contained the following: 10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 μM deoxynucleotide triphosphate and 25 pmol of each primer. The PCR conditions were as follows: 5 min at 94 °C, 35 cycles of 45 s at 94 °C, 45 s at 53 °C and 45 s at 72 °C and 7 min at 72 °C. The amplified products were purified with a PCR Clean Up kit (TaKaRa) and sequenced with an ABI sequencer analyser. The sequence similarity was determined by using the BLAST program from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST).

The MIC results suggested that F. odoratum WX2856 was only susceptible to levofloxacin and was highly resistant to imipenem, cefotaxime, cefazidime, cefoxitin, aztreonam, piperacillin/tazobactam, cefoperazone/sulbactam and amikacin. However, the PCR result suggested that F. odoratum WX2856 harbours a blaKPC gene. Moreover, the BLAST analysis suggested that this gene was identical to a KPC-2 gene reported previously (GenBank accession no. AY034847). Therefore, these results suggested that the F. odoratum WX2856 isolate harboured a KPC-2 carbapenemase. Interestingly, we detected a Klebsiella pneumoniae isolate producing a KPC-2 carbapenemase in a sputum specimen from the same patient. This isolate was successfully cleared by a combination of gentamicin, levofloxacin and amikacin. A conjugation experiment and molecular hybridization of that K. pneumoniae isolate suggested that KPC-2 was encoded on a plasmid of approximately 56 kb (data not shown).

However, conjugation failed in F. odoratum WX2856. This suggests that the KPC-2 gene in the F. odoratum isolate may not be located on a plasmid as in K. pneumoniae. A previous study suggested that a gene encoding a β-lactamase, identified as a metallo-β-lactamase, was located on the chromosome in F. odoratum (Sato et al., 1985). Therefore, these results raise the interesting questions of where this KPC-2 gene is located and what is the relationship of KPC-2 between these two species of bacteria. Do the results suggest the possibility of interspecies transmission of the KPC-2 gene? Therefore, the localization of the KPC-2 gene in F. odoratum and the relationship of KPC-2 genes between K. pneumoniae and F. odoratum will be confirmed by further research.

Since carbapenemases can hydrolyse expanded-spectrum cephalosporins and carbapenems, KPC-2 is very formidable for clinical therapy of serious infections caused by nosocomial pathogens. The detection of carbapenemases by routine microbiological tests in the clinical laboratory remains difficult. Therefore, the detection and prevention of carbapenemase infection need to be well emphasized. To our knowledge, this is the first report about F. odoratum encoding KPC-2 worldwide.

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