Identification of a blaVIM-4 gene in the internationally successful Klebsiella pneumoniae ST11 clone and in a Klebsiella oxytoca strain in Hungary

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Keywords: antimicrobial resistance epidemiology, β-lactamases, resistance genetics

Sir,
The VIM-type metallo-β-lactamases (MBLs) have been detected worldwide in Pseudomonas aeruginosa and Acinetobacter spp., and, more recently, in many species of Enterobacteriaceae. After the first detection of VIM-4 in P. aeruginosa in Greece in 2001,1 these strains were isolated in many countries, including Hungary, where VIM-4-producing P. aeruginosa and Aeromonas hydrophila were reported.2,3

In Hungarian clinical microbiological laboratories, the screening for presumptive carbapenemase producers is performed according to Hungarian guidelines, which are based on the CLSI recommendations.4 The putative production of MBLs is tested by the modified Hodge test, and disc tests containing imipenem and/or ceftazidime either alone or combined with EDTA. In 2009, 5% of Klebsiella spp. (1.4% of Enterobacteriaceae) isolates from the BP1 centre and 4.76% of Klebsiella spp. (2.4% of Enterobacteriaceae) isolates from the BP2 centre were ertapenem-resistant non-carbapenemase producers. No imipenem or meropenem non-susceptible strains were isolated during this year. Based on screening and confirmatory tests, one carbapenem-susceptible Klebsiella pneumoniae strain (KP3686) isolated from bronchoalveolar lavage (BP1 centre, February 2009) proved to be MBL producers. The aim of our study was to characterize the first Hungarian MBL-producing K. pneumoniae KP3686 and K. oxytoca KOS294/9 isolates.

MICS of antimicrobial agents were determined by Etest (bio-Mérieux, Marcy l’Étoile, France) (Table 1). In the PCR assays, primers targeting the 5′ conserved sequence (CS) and the 3′ CS of class 1 integrons,2,3 and primers specific to blaTEM, blaSHV, blaVIM, blaCTX-M,5 and blavIM,2 were used. The nucleotide sequences were determined using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

In KP3686, the chromosomally encoded non-extended-spectrum β-lactamase (non-ESBL)-type blaSHV-11, plasmid-carried blaCTX-M-15 and blatem-1, and the class 1 integron-located blavIM-4 genes were detected (Table 1). In KOS294/9, only the blavIM-4-carrying class 1 integron was found. Both integrons carried two resistance gene cassettes, namely an aac(6′)-Ib (so-called aac44) gene in the first position, followed by a blavIM-4 gene cassette. The results of integron sequencing showed the same VIM-4-containing class 1 integron in both isolates. Furthermore, the integron was found to be identical to that previously characterized from P. aeruginosa strains that originated from southern Hungary and from the first MBL-producing A. hydrophila strain.1,2 Their nucleotide sequences were assigned to GenBank under the accession numbers GU181265 for KP3686 and GU181269 for KOS294/9.

References

J Antimicrob Chemother 2010
doi:10.1093/jac/dkq133
Advance publication 21 April 2010

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In order to clarify the location of the class 1 integron, DNA hybridization, conjugation assays and transformation were performed, as described previously. 5 The DNA hybridization showed the presence of the VIM gene on plasmids of \( \text{C}24 \) 90 kb in both strains. Although the conjugation assays failed, the plasmids harbouring \( \beta \)-lactamase genes were successfully transformed to \( \text{Escherichia coli} \) DH5a. PCR experiments on the transformants and sequencing of the respective products confirmed the existence of \( \text{blaCTX-M-15 ESBL} \) and \( \text{blaTEM-1} \) on the plasmid pKP3686/2, and the presence of \( \text{blaVIM-4} \) on the plasmids pKP3686/1 and pKO5294/9 (Table 1). For fingerprinting analysis, plasmid DNA from transformants was digested with PstI (Biolabs, Beverly, MA, USA) and EcoRI (Promega, Madison, WI, USA). The similarity of pKP3686/1 and pKO5294/9 was confirmed by restriction analysis as well.

PFGE of the XbaI-digested (Biolabs) genomic DNA and multilocus sequence typing (MLST) of the \( \text{K. pneumoniae} \) strain were performed, and the results analysed as described previously. 5,6 Results of both PFGE and MLST proved that KP3686 belonged to the previously described sequence type (ST) 11 clone, detected throughout Hungary as a CTX-M-15-producing epidemic clone (EC III), 5 and subsequently reported in France, the Netherlands, Spain, and Korea. \( \text{K. pneumoniae} \) strain KP3686, isolated in Hungary, fell into the previously described sequence type (ST) 11 clone, detected throughout Hungary as a CTX-M-15-producing epidemic clone (EC III). In conclusion, this study highlights the role of horizontal transfer in the dissemination of this integron.

In conclusion, the emergence of VIM-producing isolates in the successful multiresistant \( \text{K. pneumoniae} \) ST11 clone represents a disquieting finding. To the best of our knowledge, this is also the first report in \( \text{K. oxytoca} \). To understand the dynamics of VIM-producing strains, epidemiology is very important. The homogeneity of the class 1 gene cassettes among \( \text{Klebsiella} \) spp. described in this paper and those previously described in \( \text{P. aeruginosa} \) and \( \text{A. hydrophila} \) may suggest a common origin of the VIM-4 resistance cassette. 2,3 Even though the \( \text{blaVIM} \)-containing integrons are mainly harboured by transferable plasmids in most enteric bacteria, the spread of plasmids with identical patterns among isolates of different species, as we observed, is not common. These findings highlight a role of horizontal transfer in the dissemination of this integron or plasmid and its repeated acquisition by various clinical strains.

### Acknowledgements

We gratefully acknowledge the assistance provided by Natasa Pesti.

### Funding

This work was supported in part by the Hungarian National Scientific Research Fund, grant no. OTKA F048410, and by János Bolyai, Hungarian State Fellowship.

### Transparency declarations

None to declare.
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J Antimicrob Chemother 2010
doi:10.1093/jac/dkq117
Advance publication 9 April 2010

Endoscopy-associated transmission of carbapenem-resistant
Klebsiella pneumoniae producing KPC-2 β-lactamase

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Keywords: K. pneumoniae, carbapenemases, cross-infection, contamination, infection control

Sir,

Carbapenem resistance in Enterobacteriaceae due to the production of KPC carbapenemase is becoming a significant clinical problem.1,2 Klebsiella pneumoniae producing KPC carbapenemase (KPC-Kp) have been reported from many countries worldwide, including the USA, Colombia, Israel and Greece, and have been associated with increased hospital costs, increased length of stay and higher patient mortality.2–4 Worryingly, the epidemiology in the USA appears to be changing, since outbreaks have now been described in long-term acute care hospitals.5

The positive risk–benefit relationship of endoscopy interventions has been clearly established.6 Although the risk of nosocomial infections from endoscopes appropriately reprocessed is very low, inadequate reprocessing has been reported to be the source of outbreaks.6 We report here a nosocomial outbreak of KPC-Kp in France, with regional inter-hospital dissemination mediated by a contaminated duodenoscope.

An 85-year-old patient with bladder cancer was admitted to the medical intensive care unit (Hôpital de Bicêtre, France) for severe gastrointestinal bleeding. Upon arrival, screening samples (rectal swabs) for multidrug-resistant (MDR) bacteria, as previously described using chromID ESBL (bioMérieux, Marcy-l’Etoile, France),2 revealed the presence of an extended-spectrum β-lactamase (ESBL)-producing Escherichia coli. The patient underwent endoscopy to stop the bleeding. Five days later, in the course of the weekly MDR screening of the unit, he was screened positive for an MDR K. pneumoniae isolate. Antibiogram determined by the disc diffusion method and MICs determined by Etest and interpreted according to the CLSI7 revealed that this MDR-Kp strain was resistant to penicillins, cephalosporins, fluoroquinolones, co-trimoxazole, rifampicin and tetracycline, but showed intermediate resistance to imipenem (MIC=8 mg/L) and susceptibility to gentamicin (MIC=2 mg/L) and colistin (MIC=4 mg/L). The presence of the blaKPC-2 gene was identified by PCR and sequencing. The patient underwent surgery for gastrectomy and 2 weeks later had bacteraemia with KPC-Kp that was treated successfully with gentamicin (5 mg/kg/day) and colistin (50 000 IU/kg/day). Screening of patients in the same surgical unit for gut carriage of MDR bacteria identified two contact patients that were KPC-Kp(+), indicating probable nosocomial transmission. Increased awareness, cohorting of these KPC-Kp(+) patients, dedicated nursing staff and reinforced hygiene precautions prevented further spread in the hospital. The period of time separating the initial screening of the patient and the diagnosis of KPC-Kp positivity may have contributed to the spread of KPC to other patients. Concomitantly, a patient from a neighbouring hospital who underwent endoscopy at the same gastroenterology ward was diagnosed to be KPC-Kp(+). The two patients had their endoscopy on separate days (2 weeks apart), but with the same endoscope. Bacterial cultures from the endoscope revealed KPC-Kp (102 cfu/100 mL of wash solution), Pseudomonas aeruginosa and other bacteria common in the digestive tract. Retrospective analysis of the patients that had gastroscopy with the same endoscope identified a Greek patient with KPC-Kp faecal carriage, who was transferred from a hospital in Chania (Crete, Greece) 2 months earlier. Following the endoscopic treatment of this patient, 17 patients, mostly from five regional hospitals (10 patients) and from Bicêtre hospital (7 patients), underwent gastroscopy with the same contaminated endoscope. Of these 17 patients, 10 could be screened; 6 were colonized with KPC-Kp and among these 2 developed KPC-Kp infections (one bacteraemia and...