Plasmid-mediated carbapenem-hydrolysing $\beta$-lactamase KPC-2 in a Klebsiella pneumoniae isolate from Switzerland

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Sir,

The emergence and dissemination of Enterobacteriaceae isolates producing carbapenemases in various geographical regions represent a significant threat to the management of nosocomial infections. Carbapenem-hydrolysing $\beta$-lactamases include metallo-$\beta$-lactamases, expanded-spectrum oxacillinases and Ambler class A enzymes. 1 Among class A enzymes, the most common are KPC-$\beta$-lactamases, which hydrolyse all $\beta$-lactams except cephamycins. The blaKPC-like genes have been reported most often from enterobacterial species recovered from many states in the USA.2 In addition, KPC-producing Klebsiella pneumoniae isolates are endemic in Greece and Israel, and have been reported from many countries worldwide, including South America, China and Western Europe. 2 The rapid dissemination of KPC enzymes among different enterobacterial species is related to the localization of blaKPC genes on transferable broad-host-range plasmids and their association with a particular transposon, 3 but is also linked with a disseminated international clone of KPC-producing K. pneumoniae sequence type (ST) 258. 4-5 We describe here the first identification of a KPC-producing K. pneumoniae isolate from Switzerland.

In mid-2010, a patient with methicillin-resistant Staphylococcus aureus pneumonia requiring mechanical ventilation was transferred from a hospital in Sicily to the Neuchâtel public hospital in Switzerland. During his 11 day stay in hospital in Sicily, he had been treated with ciprofloxacin, clarithromycin and teicoplanin. Upon arrival in Switzerland the patient was febrile and was treated empirically with ceftazidime and linezolid for 5 days. A week later, while the patient was afebrile, urine and sputum cultures grew a pan-resistant K. pneumoniae. This was considered as colonization, thus no antibiotic treatment was initiated and the patient eventually fully recovered.

The antibiogram determined by the disc diffusion method and MICs determined by Etest and the Vitek2 system (AST-EXN card) and interpreted according to the CLSI guidelines 6 revealed that this K. pneumoniae strain was resistant to all penicillins and expanded-spectrum cephalosporins, imipenem (MIC, 12 mg/L), ertapenem (>32 mg/L), meropenem (32 mg/L) and doripenem (16 mg/L). This strain was of intermediate susceptibility to tigecycline (MIC of 2 mg/L), but was susceptible to gentamicin (MIC of 3 mg/L) and the MIC of colistin was >16 mg/L. A blaKPC-2 gene was identified by PCR and sequencing as previously described.7 The isolate possessed additional $\beta$-lactamase genes, including those encoding the narrow-spectrum $\beta$-lactamases SHV-11 (naturally occurring), TEM-1 and OXA-9.

PCR mapping and sequencing of the blaKPC-flanking regions using combinations of primers showed that the blaKPC-2 gene was located inside a Tn4401 transposon identical to that found in the K. pneumoniae reference strain YC from France.8 Multilocus sequence typing (MLST), performed according to the protocol described on the K. pneumoniae MLST web site (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html), showed that this K. pneumoniae strain was of the ST258 type, known to be disseminated worldwide.4-5 The plasmid location of the blaKPC-2 gene was confirmed by electroporation of a
crude plasmid extract obtained using the Kieser method into Escherichia coli TOP10. Plasmid analysis of the K. pneumoniae isolate and its transformant confirmed that bla_{KPC-2} was located on an \( \sim75 \text{ kb} \) plasmid. No other antibiotic resistance marker was co-transferred. PCR-based replicon typing of the major plasmid incompatibility groups showed that the bla_{KPC-2} positive plasmid belonged to the IncFIAS incompatibility group. We believe this is the first report of a KPC-producing isolate of K. pneumoniae from Switzerland. It seems likely that the isolate was imported as a result of the transfer of a patient from Sicily. In Italy, several studies reported KPC-2- or KPC-3-producing K. pneumoniae isolates, being mostly of ST258 type. There is a need for urgent action to slow down and control the worldwide and epidemic spread of enterobacterial carbapenemase producers, including countries with a low level of antibiotic resistance, such as Switzerland.

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**Transparency declarations**

None to declare.

**References**


**CO\(_2\)**-dependent methicillin-resistant *Staphylococcus aureus*

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Sir,

A female patient in her 20s presented with a right thigh abscess. There was local swelling and erythema. There was no indication for surgical drainage and the patient was commenced on intravenous flucloxacillin with clinical improvement. This was changed to oral flucloxacillin and the patient self-discharged and was lost to follow-up.

A wound swab of the thigh abscess was sent to the laboratory. Gram staining revealed polymorphs and Gram-positive cocci. The specimen was incubated on horse blood agar with 5% CO\(_2\) and on MacConkey agar aerobically. Growth of Gram-positive cocci, which were slide coagulase positive (Remel), was noted within 24 h on horse blood agar, but not on MacConkey agar. Susceptibility testing was attempted using Vitek2, but growth in the control well was not achieved, and therefore the attempt was terminated. The organism did not grow under aerobic conditions on a purity plate.

Disc susceptibility was performed on Mueller–Hinton agar and the isolate was subcultured on MRSASelect (Bio-Rad, Australia). Both were incubated in 5% CO\(_2\) as well as O\(_2\). Growth was observed only in the presence of CO\(_2\). CLSI disc diffusion susceptibility testing was performed and the cefoxitin (30 \( \mu \)g) zone diameter was 12 mm. RT–PCR for femA and mecA genes was positive. The isolate was confirmed to be methicillin-resistant *Staphylococcus aureus* (MRSA). In addition, molecular testing for the Panton–Valentine leucocidin (PVL) gene by SYBR green RT–PCR assay was performed and was positive. The isolate was sent for typing at the Department of Microbiology and Infectious Diseases, Path West Laboratory Medicine, Nedlands, WA, Australia, and was sequence type 30 (ST30).

*Staphylococci* are common colonizers of humans and are frequently isolated in clinical specimens. Variant subpopulations of staphylococci have been described that appear and behave differently to the more commonly isolated *S. aureus*. As such, specific nutritional and atmospheric requirements may be needed to isolate and characterize these organisms.

CO\(_2\)-dependent *S. aureus* was first reported in 1955. CO\(_2\)-dependent *S. aureus* may appear as a population of small colony variant (SCV) *S. aureus* that is defective in electron transport and respiratory activity and can be easily

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