Detection of the plasmid-mediated KPC-2 carbapenem-hydrolysing enzyme in three unusual species of the Enterobacteriaceae family in Israel

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Keywords: bla genes, interspecies transfer, infection control

Sir,

Carbapenem-resistant Enterobacteriaceae (CRE) pose a major threat to public health worldwide. One of the most frequently encountered mechanisms is the Ambler class A Klebsiella pneumoniae carbapenemase (KPC)-type enzyme. Since the initial report of a KPC-producing K. pneumoniae in 2001,1 these organisms have spread worldwide, mainly due to the spread of a single successful clone, sequence type 258 (ST258).2 In addition, KPC enzymes have been detected in multiple genera and species of the Enterobacteriaceae family, including Salmonella spp., Klebsiella oxytoca, Enterobacter spp., Citrobacter freundii, Escherichia coli, Proteus mirabilis and Serratia marcescens.3 The blaKPC gene is typically located on the Tn4401 transposon, which may be carried on various plasmids. Transfer of mobile genetic elements may serve as a vector for inter-species transmission and thus poses a serious challenge for infection control measures.3,4

In this report, we describe three unusual Enterobacteriaceae species carrying the blaKPC-2 gene: Leclercia adecarboxylata, Kluyvera cryocrescens and Kluyvera ascorbata. The molecular and microbiological characteristics of these isolates are discussed.

All strains in this research were isolated from rectal surveillance cultures of patients from three medical centres: Laniado Medical Center (LMC), Tel Aviv Sourasky Medical Center (TASMC) and Rambam Medical Center (RMC). Table 1 summarizes the molecular and microbiological characteristics of the isolates. Bacterial identification was performed with the VITEK-2 system using GN-ID and GN09 cards (bioMérieux, Marcy l’Etoile, France) and confirmed by 16S rDNA sequencing. We identified the isolates as K. cryocrescens (two isolates), K. ascorbata (one isolate) and L. adecarboxylata (one isolate). All isolates carried the blaKPC-2 gene.5 Plasmid DNAs purified from the four isolates were transformed into E. coli DH10B. Transformants were selected on lysogeny broth (LB) agar supplemented with 100 mg/L ampicillin and confirmed by PCR. Susceptibility to ertapenem, imipenem and meropenem was tested by agar dilution and interpreted based on the 2012 CLSI criteria. All isolates were resistant to ertapenem, imipenem and meropenem. In the transformants, the MICs of ertapenem, imipenem and meropenem were 2–8, 4–8 and 2–4 mg/L, respectively.

Determination of plasmid sizes was done as previously described,6 revealing various sizes of the blaKPC-2-harbouring plasmids. The plasmid incompatibility group (Inc) was identified as IncIc. The blaKPC-2 gene was located on the Tn4401 transposon and was typed by sequencing as Tn4401c.3

The presence of the Tn4401c variant in these isolates is in contrast with isolates belonging to the Israeli epidemic clone, K. pneumoniae ST258, which harbours the Tn4401a variant on the pKpQIL plasmid, which belongs to the IncFII incompatibility group.7 It was shown previously that isolates harbouring Tn4401c had a 10-fold reduction in their blaKPC expression. This also correlated with lower MICs of carbapenems.8 The identical plasmid incompatibility group and Tn4401 type in all isolates suggests horizontal inter-species transmission of the Tn4401c element between different plasmids of the same type, as recently described by Mathers et al.9

Kluyvera spp. and L. adecarboxylata are present ubiquitously in the environment in water and soil and were also described as part of the normal gastrointestinal flora in humans.8,9 Human infections with these bacteria are rare. Several cases of L. adecarboxylata infections in humans have been reported, especially in immunocompromised patients. In such patients, L. adecarboxylata was reported to cause bacteremia, sepsis, peritonitis, cellulitis, endocarditis and cholecystitis.9 Reported human infections caused by Kluyvera spp. include bacteremia, soft tissue infections, intra-abdominal abscesses and urinary tract infections. Recently, Ribeiro et al.10 reported on the first isolate of a carbapenem-resistant Kluyvera georgiana possessing the blaKPC-2 gene. Although clinical infections were absent in all of these cases, the spread of blaKPC-carrying plasmids into unusual Enterobacteriaceae species is of concern from an infection control perspective. These bacteria, though commonly
regarded as avirulent, could serve as a reservoir for the spread of resistance genes between different species, leading to dissemination of resistant bacteria between patients and in the environment.

**Funding**

This work was supported in part by European Commission FP7: SATURN—Impact of Specific Antibiotic Therapies on the Prevalence of Human Host Resistant Bacteria research grant 241796.

**Transparency declarations**

None to declare.

**References**


**Table 1. Molecular and microbiological features of the four KPC-producing Enterobacteriaceae isolates**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Isolate no.</th>
<th>LMC</th>
<th>RMC</th>
<th>TASMC</th>
<th>LMC</th>
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<td>RCM</td>
<td>rectum</td>
<td>L. adecarboxylata</td>
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<td>6907</td>
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<td>Size of blaKPC-harboring plasmid (kb)</td>
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<td>Ertilpenem MIC (mg/L)</td>
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J Antimicrob Chemother 2013
doi:10.1093/jac/dks446
Advance Access publication 8 November 2012

**Identification of a novel insertion sequence element associated with carbapenem resistance and the development of fluoroquinolone resistance in Acinetobacter radioresistens**

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**Keywords:** GyrA, imipenem, ciprofloxacin, QRDR, oxacillinase

Sir,

Acinetobacter radioresistens, although sometimes isolated on the skin of healthy humans, rarely causes serious illness.1,2 In a recent study, A. radioresistens was isolated from blood and urine cultures, but made up <1% of the total Acinetobacter spp. isolated, while