was found as part of the 10 kb Tn3-like element Tn4401, PCR assays with specific primers for Tn4401 were performed.\textsuperscript{10} Amplicon sequencing revealed that the \textit{bla}_{KPC-2} gene was in all cases embedded in a Tn4401-like transposon. Published papers have reported that Tn4401 has been found on IncN and IncFII \textit{K. Klebsiella pneumoniae} ST258 background.\textsuperscript{6} Furthermore, as regards the coexistence of methylase \textit{armA} in KPC-producing \textit{K. pneumoniae}, already found to be associated on pETKp90 and pETKp50 plasmids and on the same pKP048 plasmid, Southern blot experiments on genomic and plasmid DNA from the \\
\textit{K. Klebsiella pneumoniae} producing \textit{armA} gene.

\textit{In conclusion, our findings suggest that KPC-2- and ArmA-}

\textit{producing \textit{K. pneumoniae} strains are emerging in an ST101 background.\textsuperscript{9}}

\textit{Furthermore, regards the coexistence of methylase \textit{armA} in KPC-producing \textit{K. pneumoniae}, already found to be associated on pETKp90 and pETKp50 plasmids and on the same pKP048 plasmid, Southern blot experiments on genomic and plasmid DNAs with the \textit{bla}_{KPC}, \textit{armA} and \textit{bla}_{KPC}-IT probes obtained by PCR fragments were performed. A hybridization signal on the same fragment of 97 kb in all strains was found, suggesting that these genes are located on the same element. Further studies are in progress in our laboratory in order to identify the element carrying the \textit{armA} gene.

\textit{In conclusion, our findings suggest that KPC-2- and ArmA-}

\textit{producing \textit{K. Klebsiella pneumoniae} strains are emerging in an ST101 background. These clones are extensively resistant, also due to lateral gene transfer, rendering all families of drugs useless and requiring only antibiotic combinations (G. Ceccarelli, M. Falcone, A. Giordano, M. L. Mezzatesta, C. Caio, S. Stefani and M. Venditti, unpublished results). Therefore, the diffusion of these epidemic clones requires the activation of infection control procedures.}

\begin{center}
\textbf{References}
\end{center}


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\textbf{Acknowledgements}
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We express our gratitude to Antony Bridgwood for language revision.

\begin{center}
\textbf{Funding}
\end{center}

This work was supported by Italian Minister of Universities funding (to S. S. and M. L. M.).

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\textbf{Transparency declarations}
\end{center}

None to declare.

\begin{center}
\textbf{J Antimicrob Chemother} 2013
doi:10.1093/jac/dkt124
Advance Access publication 25 April 2013
\end{center}

\begin{center}
\textbf{Isolation of carbapenem-resistant NDM-1-positive Providencia rettgeri in Mexico}
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Sir,

Bacteria of the genus Providencia are Gram-negative opportunistic pathogens that have been isolated from a wide variety of environments, including human stool samples. They comprise part of the natural human gut flora but may also cause infections, including travellers’ diarrhoea. They are also responsible for urinary tract and other nosocomial infections in humans. The New Delhi metallo-β-lactamase (NDM-1) is the most recently discovered transferable molecular class B metallo-β-lactamase. The gene encoding this enzyme was located on a 178 kb plasmid belonging to incompatibility group A/C in a Providencia stuartii clinical isolate. However, it has been described in different plasmid types (IncA/C, IncF, IncLM, IncN or untypeable) and is also chromosomally integrated.

This work describes four Providencia rettgeri clinical isolates obtained from patients with urinary tract infection in the intensive care unit (ICU) of the University Hospital of Monterrey, Mexico, between January and June 2012 (Table 1). The P. rettgeri isolates were identified using the API 20E galleries (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and interpreted with the Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA). In addition, the 16S rRNA gene was characterized by using primers previously described.

A possible epidemiological link between the patients was a surgical resident involved in the care of the four patients while in the ICU. None of the patients had evidence of infection prior to being transferred to the ICU; the average time to positive urine cultures for P. rettgeri was 29 days (range 12–68 days).

MICs were determined by broth microdilution following CLSI recommendations and the phenotypic screening to determine the production of a carbapenemase enzyme was carried out using a double-disc synergy test (meropenem, imipenem and EDTA). The isolates were screened using PCR for genes encoding a range of carbapenemase enzymes, including KPC, GES, IMP, SIM, GIM, SPM, VIM and NDM. Genotyping was performed using PFGE and the results were analysed following the guidelines of Tenover et al. using GelCompar II (Applied Math, Kortrijk, Belgium). The plasmid profile was analysed using ion-interchange columns (Qiagen, Valencia, CA, USA); subsequently, a Southern hybridization was carried out with a non-radioactive probe for the NDM-1 gene. Mating and transformation experiments and identification of plasmid incompatibility groups by PCR replicon typing were undertaken as described previously.

The genotyping analysis of the four P. rettgeri isolates showed one clonal group (data not shown). All isolates were susceptible to tigecycline (1 mg/L), but resistant to imipenem, meropenem, and is also chromosomally integrated.

**Keywords:** antimicrobial resistance, Enterobacteriaceae, Gram-negative

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**Table 1. Characteristics of metallo-β-lactamase NDM-1-producing Providencia isolates**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Patient</th>
<th>Date of admission (days)</th>
<th>Patient gender/age</th>
<th>Number of days after admission that P. rettgeri was isolated</th>
<th>Plasmids (kb)</th>
<th>Incompatibility group, Inc</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>06-1617</td>
<td>2b</td>
<td>January 2012 (17)</td>
<td>male/32</td>
<td>17</td>
<td>256</td>
<td>64</td>
<td>IPM MEM CIP GEN PIP CAZ CTX TGC CST</td>
</tr>
<tr>
<td>06-1619</td>
<td>3c</td>
<td>January 2012 (21)</td>
<td>female/50</td>
<td>12</td>
<td>256</td>
<td>64</td>
<td>IPM MEM CIP GEN PIP CAZ CTX TGC CST</td>
</tr>
<tr>
<td>06-1622</td>
<td>4d</td>
<td>May 2012 (31)</td>
<td>female/53</td>
<td>21</td>
<td>256</td>
<td>64</td>
<td>IPM MEM CIP GEN PIP CAZ CTX TGC CST</td>
</tr>
<tr>
<td>06-1623</td>
<td>5e</td>
<td></td>
<td>female/53</td>
<td>32</td>
<td>256</td>
<td>64</td>
<td>IPM MEM CIP GEN PIP CAZ CTX TGC CST</td>
</tr>
</tbody>
</table>

IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; GEN, gentamicin; PIP, piperacillin; CAZ, ceftazidime; CTX, cefotaxime; TGC, tigecycline; CST, colistin.

aThe plasmids appearing in bold or underlined correspond to the plasmids obtained in the mating and transformation experiments, respectively.

bThe patient died of non-infectious complications.

cThe patient died of neurological complications.

dThe patient was transferred to a community hospital 10 days after isolation with no signs of infection.

eThe patient was discharged without clinical signs of infection and remained asymptomatic 6 weeks after discharge.
Ivermectin lacks antituberculous activity

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Keywords: Mycobacterium tuberculosis, tuberculosis, susceptibility

Sir,

It was recently reported by Lim et al.\(^1\) that avermectins, including ivermectin, selamectin and moxidectin, are bactericidal against several isolates of \textit{Mycobacterium tuberculosis}, including multidrug-resistant and extensively drug-resistant clinical isolates. Ivermectin is a semi-synthetic avermectin (macrocyclic lactone) produced by the soil actinomycete \textit{Streptomyces avermitilis}.\(^2\) It has been successfully used to treat parasitic infections, including onchocerciasis, strongyloidiasis, ascariasis, cutaneous larva migrans, filariases, gnathostomiasis and trichuriasis, as well as pediculosis.\(^3,4\) Apart from the study by Lim et al.,\(^5\) only one study has shown the \textit{in vitro} antimicrobial activity of ivermectin against \textit{Chlamydia trachomatis} using a cellular model.\(^5\)

Here, we tested an additional set of 13 \textit{M. tuberculosis} complex isolates from France as no European isolate had been incorporated into the previous study.\(^5\) This collection consisted of 10 \textit{M. tuberculosis} clinical isolates and three reference strains (\textit{M. tuberculosis} H37Rv, \textit{Mycobacterium canetti} CIP 140010059 and \textit{Mycobacterium bovis} BCG Pasteur 1173P2). Ivermectin concentrations were chosen to match those used by Lim et al.\(^1\) Briefly, mycobacteria were cultured at 37°C in 7H9 Middlebrook medium supplemented with 10% (v/v) oleic acid/albumin/dextran/catalase (OADC) (Becton Dickinson, Sparks, MD, USA) and 0.5% (v/v) glycerol to mid-log phase (optical density of 0.5 at 600 nm). MICs were determined using an agar dilution method after dilution of ivermectin in Middlebrook 7H10 medium supplemented with OADC. Sterile 6-well tissue culture plates (Dominique Dutschker, 1936

References


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