was found as part of the 10 kb Tn3-like element Tn4401, PCR assays with specific primers for Tn4401 were performed.10 Amphlicon sequencing revealed that the 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 plasmids (pKpQL-IT, S9, S12, S15, pKPN101-IT); therefore, for the detection of these plasmids, we used the following primers: S9-F, 5′-GCATTGACCTTGCGACTTCTC-3′; S9-R, 5′-GTGATTTACACCACACCTCTACATA-3′; S12-F, 5′-CAGGACGGTGATCGAATCGGATG-3′; S12-R, 5′-ATTGCTGCTAGGGCTGCTATTCT-3′; S15-F, 5′-GGGGGATGGTTTTTCGCAAGCA-3′; S15-R, 5′-GCTTTACCGAGGGAGAATGGCTAG-3′; and pKpQL-IT-F, 5′-GGAATGCTTGGGATGATAGGCAGTG-3′; pKpQL-IT-R, 5′-GAGTGCAGGAGGAGCAGACGG-3′—designed on the basis of published sequences and specific for each plasmid (GenBank accession numbers FJ223607.1, FJ223605.1, FJ223601.1, HQ589350.1, EU176011.1 and GU595196.1, respectively).10

In all strains amplicon sequence analysis (1071 bp) showed that plasmid sequences matched the pKpQL-IT plasmid, circulating in Italy and already detected in a strain of K. pneumoniae ST258 background.6

Furthermore, as regards the coexistence of methylase armA in KPC-producing K. pneumoniae, already found to be associated on pETKp90 and pETKp50 plasmids and on the same pKP048 plasmid,4,5 Southern blot experiments on genomic and plasmid DNAs with the Bla
KPC, armA and pKpQL-IT probes obtained by PCR fragments were performed. A hybridization signal on the same fragment of 97 kb in all strains was found, suggesting PCR fragments were performed. A hybridization signal on the basis of published sequences and specific for each plasmid (GenBank accession numbers FJ223607.1, FJ223605.1, FJ223601.1, HQ589350.1, EU176011.1 and GU595196.1, respectively).10

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In conclusion, our findings suggest that KPC-2- and ArmA-producing K. 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antibiotic combinations (G. Ceccarelli, M. Falcone, A. Giordano, M. L. Mezzatesta, C. Caio, S. Stefani and M. Venditti, unpublished results). Furthermore, the diffusion of these epidemic clones requires the activation of infection control procedures.

References


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KPC-2, bla


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Isolation of carbapenem-resistant
NDM-1-positive Providencia rettgeri
in Mexico

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**Transparency declarations**

None to declare.
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.1 They are also responsible for urinary tract and other nosocomial infections in humans.2 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.3 However, it has been described in different plasmid types (IncA/C, IncF, IncL/M, IncN or untypeable) and is also chromosomally integrated.4

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 confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Durham, NC, USA) and confirmed by Sensititre (bioMérieux, Dur...
piperacillin, ceftazidime, cefotaxime, ciprofloxacin and colistin (Table 1). The isolates were positive for carbapenemase activity and the PCR assays and sequencing demonstrated the presence of the gene encoding NDM-1. According to the Southern hybridization (data not shown) and PCR replication typing results obtained with the transconjugants and recombinants, the NDM-1 gene was identified on a 310 kb IncK plasmid (Table 1). However, the mating and the transformation experiments showed the respective transconjugant (310 kb and 160 kb) and transformant (310 kb and 50 kb) with two different plasmids harboured in the clinical isolates (Table 1). Similar results have been recently described suggesting that a helper plasmid is necessary for the mobilization of the plasmid-borne NDM-1. In this work, the 160 kb plasmid could be playing the role of helper. This is the first known report of an NDM-1-producing P. rettgeri in Mexico. This finding points to the need to enforce the molecular epidemiological surveillance of these pathogens and enzymes in order to prevent their dissemination among hospitals as well as to other bacterial genera.

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**Transparency declarations**

None to declare.

**References**


**Ivermectin lacks antituberculous activity**

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**Keywords**: Mycobacterium tuberculosis, tuberculosis, susceptibility

Sir,

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

Here, we tested an additional set of 13 *M. tuberculosis* complex isolates from France as no European isolate had been incorporated into the previous study. This collection consisted of 10 *M. tuberculosis* clinical isolates and three reference strains (*M. tuberculosis* H37Rv, *Mycobacterium canetti* CIP 140010059 and *Mycobacterium bovis* BCG Pasteur 1173P2). Ivermectin concentrations were chosen to match those used by Lim et al. Briefly, mycobacteria were cultured at 37°C in 7H9 Middlebrook medium supplemented with 10% (v/v) oleic acid/albumin/dextrose/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 Dutscher, Poirel L, Dortet L, Bernabeu S et al. Genetic features of *bla*<sub>NDM-1</sub> positive *Enterobacteriaceae*. *Antimicrob Agents Chemother* 2011; 55: 5403–7.

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