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## Emergence of colistin resistance in *Klebsiella pneumoniae* from veterinary medicine

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Sir,

Colistin is among the very few antimicrobials that retain activity against MDR Gram-negative bacteria. It binds to the negatively charged LPS, leading to the disruption of the membrane.<sup>1</sup> Since its discovery, colistin has been used in veterinary medicine, in particular in cattle and swine, usually collectively through prophylactic or metaphylactic practices but also as individual treatment.<sup>2,3</sup> On the other hand, colistin is a last-resort antibiotic to treat multiresistant bacteria in human medicine, although colistin-resistant clinical isolates have recently been reported. This raises concerns about the possibility that colistin-resistant strains might be selected in animals and then might be the sources of human infections.

Colistin resistance in *Klebsiella pneumoniae* is related to modifications of LPS by addition of cationic charges, such as addition of 4-amino-4-deoxy-L-arabinose to lipid A, which decreases the affinity between colistin and its target.<sup>4</sup> The modification of LPS is mediated by the *pmrHFIJKLM* operon, regulated by the PhoPQ and PmrAB two-component systems.<sup>5</sup> It has been demonstrated that MgrB, a small transmembrane protein, negatively regulates the PhoPQ system by interaction with the sensor kinase PhoQ in the periplasmic domain, preventing activation of the *pmrHFIJKLM* operon.<sup>6</sup>

It was recently shown that insertional inactivation of the *mgrB* gene in *K. pneumoniae* could result in up-regulation of the PhoPQ

system, leading to overexpression of the *pmrHFIJKLM* operon, resulting in colistin resistance due to addition of positive charges to lipid A.<sup>7,8</sup> This was demonstrated in a KPC-producing isolate from Italy and then among a series of clonally unrelated isolates with worldwide origins. Interestingly, different genetic events were identified at the origin of this resistance, being either the insertion of different types of IS at different locations into the *mgrB* gene or the occurrence of a premature stop codon in the MgrB coding sequence. All these genetic events led to truncations of the *mgrB* gene and consequently impaired the production of a functional MgrB protein.

The aim of this study was to evaluate the occurrence of colistin resistance among *K. pneumoniae* isolates recovered from bovine mastitis, a disease that can be treated by intra-mammary application of colistin (mostly in combination with penicillins) in France, and to decipher the corresponding mechanism(s). Ninety-seven non-duplicate *K. pneumoniae* isolates that caused mastitis were recovered through the Resapath network ([www.resapath.anses.fr](http://www.resapath.anses.fr)) in 2013 and sent to Anses (Lyon) for further analysis.

Antimicrobial susceptibility testing was performed by broth microdilution according to the EUCAST recommendations, using cation-adjusted Mueller-Hinton broth.<sup>9</sup> MICs of colistin were determined by Etest<sup>®</sup> (bioMérieux, La Balme-les-Grottes, France) and breakpoints were those recommended by the EUCAST: >2 mg/L, resistant; and ≤2 mg/L, susceptible. Only 1 of the 97 isolates (isolate NK34373) showed resistance to colistin, with an MIC of 8 mg/L. MLST, performed as previously described<sup>10</sup> and interpreted using the public MLST web site (<http://bigsd.web.pasteur.fr/klebsiella/klebsiella.html>), identified isolate NK34373 as being ST37. This isolate was susceptible to all other antibiotics tested, including broad-spectrum cephalosporins, all aminoglycosides, quinolones, fluoroquinolones, chloramphenicol and tetracyclines.

A PCR specific for the *mgrB* gene was performed using primers mgrB-Kp-F (5'-TTAAGAAGGCCGTGCTATCC-3') and mgrB-Kp-R (5'-AAGGCGTTCATTCTACCACC-3'). It revealed a larger amplicon compared with a WT *mgrB* amplicon (data not shown) and sequencing showed that the *mgrB* gene was interrupted by a 1057 bp IS903B element (98% nucleotide identity) belonging to the IS5 family (<https://www-is.biotoul.fr>) (GenBank accession number X02527). The IS was inserted into the *mgrB* gene between nucleotides 67 and 68 and was bracketed by a 9 bp target site duplication (5'-ACTCAGATG-3'), likely being the signature of a transposition event. In parallel, sequence analysis of the *pmrCAB* operon as previously described<sup>11</sup> identified WT genes.

Complementation experiments were then performed as previously described using recombinant plasmid pTOPO-mgrB or pTOPO-pmrB, encoding WT *K. pneumoniae* MgrB and PmrB proteins, respectively.<sup>10</sup> Electro-transformation was performed by electroporation into the colistin-resistant isolate NK34373 and electrotransformants were selected onto Mueller-Hinton agar plates supplemented with 100 mg/L zeocin (resistance marker of cloning vectors). Complementation with plasmid pTOPO-mgrB fully restored susceptibility to colistin, while the MIC of colistin remained unchanged upon transformation with plasmid pTOPO-pmrB.

These results demonstrated that the loss of functional MgrB was responsible for the colistin resistance trait observed in the *K. pneumoniae* isolate. To the best of our knowledge, this is the first description of a mechanism responsible for colistin resistance in a veterinary strain. Notably, it corresponds to a mechanism that is identical to that identified among human *K. pneumoniae* isolates, i.e. the inactivation of the *mgrB* gene. As opposed to those colistin-resistant isolates that have been identified among human isolates, this isolate was susceptible to all other antibiotics. The routes of selection of colistin resistance in this isolate remain unknown. It may result from local injection of colistin in the infected udder, but colistin resistance might also have been selected in the farm environment as a consequence of oral administration of colistin to calves, then further eliminated in the faeces. Owing to the wide use of colistin in veterinary medicine, these results suggest that more extensive epidemiological surveys should now be conducted to evaluate the prevalence and molecular features of colistin-resistant isolates in animals.

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## Transparency declaration

None to declare.

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## References

- 1 Yahav D, Farbman L, Leibovici L et al. Colistin: new lessons on an old antibiotic. *Clin Microbiol Infect* 2012; **18**: 18–29.
- 2 Kempf I, Fleury MA, Drider D et al. What do we know about resistance to colistin in Enterobacteriaceae in avian and pig production in Europe? *Int J Antimicrob Agents* 2013; **42**: 379–83.
- 3 Callens B, Persoons D, Maes D et al. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. *Prev Vet Med* 2012; **106**: 53–62.
- 4 Lee H, Hsu FF, Turk J et al. The PmrA-regulated *pmrC* gene mediates phosphoethanolamine modification of lipid A and polymyxin resistance in *Salmonella enterica*. *J Bacteriol* 2004; **186**: 4124–33.
- 5 Gunn JS, Miller SI. PhoP-PhoQ activates transcription of *pmrAB*, encoding a two-component regulatory system involved in *Salmonella typhimurium* antimicrobial peptide resistance. *J Bacteriol* 1996; **178**: 6857–64.
- 6 Lippa AM, Goulian M. Feedback inhibition in the PhoQ/PhoP signaling system by a membrane peptide. *PLoS Genetics* 2009; **5**: e1000788.
- 7 Cannatelli A, D'Andrea MM, Gianì T et al. In vivo emergence of colistin resistance in *Klebsiella pneumoniae* producing KPC-type carbapenemases mediated by insertional inactivation of the PhoQ/PhoP *mgrB* regulator. *Antimicrob Agents Chemother* 2013; **57**: 5521–6.
- 8 Poiré L, Jayol A, Bontron S et al. The *mgrB* gene as a key target for acquired resistance to colistin in *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2014; doi:10.1093/jac/dku323.
- 9 Diancourt L, Passet V, Verhoef J et al. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J Clin Microbiol* 2005; **43**: 4178–82.
- 10 Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Ninth Edition: Approved Standard M07-A9*. CLSI, Wayne, PA, USA, 2012.
- 11 Jayol A, Poiré L, Brink A et al. Resistance to colistin associated with a single amino acid change in protein PmrB among *Klebsiella pneumoniae* isolates of worldwide origin. *Antimicrob Agents Chemother* 2014; **58**: 4762–6.