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ePoster Viewing
The evolution of colistin resistance

THE MGRB GENE AS A KEY TARGET FOR ACQUIRED RESISTANCE TO COLISTIN IN KLEBSIELLA PNEUMONIAE

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Objectives: Alterations in the PhoPQ two-component regulatory system may be associated with polymyxin resistance in *Klebsiella pneumoniae.* MgrB is a small transmembrane protein of 47 amino acids produced upon activation of the PhoPQ signalling system, and acting as a negative regulator on this system. A recent study showed that insertional inactivation of the *mgrB* gene was involved in the acquired resistance to colistin in *K. pneumoniae.* The aim of this study was to investigate the mechanisms responsible for colistin resistance in a series of *Klebsiella pneumoniae* isolates recovered from different countries, with a focus on the *mgrB* gene.

Methods: Thirty-five colistin-resistant *K. pneumoniae* isolates were included in our study. Susceptibility testing for colistin was evaluated by E-test. Genotyping comparison of the isolates was performed by Pulsed-Field Gel Electrophoresis (PFGE) and MultiLocus Sequence Typing (MLST). The *mgrB* gene and its corresponding promoter sequences were analyzed by PCR and sequencing. Complementation assays were performed by cloning a wild-type *mgrB* gene into a cloning vector, and then electroporating this recombinant plasmid into the colistin-resistant clinical isolates.

Results: PCR experiments revealed that seven isolates had an insertion into the *mgrB* gene, resulting in a truncated MgrB protein. Five isolates harboured an IS*5*-like (93% nucleotide identity) insertion sequence always located between nucleotides 74 and 75 of the *mgrB* gene. Interestingly, these isolates were of four different clonal lineages. The same IS was recently identified at the same location but in another orientation in a colistin-resistant *K. pneumoniae.* Another isolate also harboured an insertional inactivation at the exact same position, but the IS was different (IS*Kpn13*). Also, another isolate harboured an insertional inactivation linked to another insertion sequence (IS*Kpn14*) at another location (between nucleotides 127 and 128). Two clonally-related isolates harboured an insertion sequence (IS*10R*) in the promoter region of *mgrB* (between nucleotides -26 and -27). Finally, two clonally-unrelated isolates harboured a substitution corresponding to an anticipated stop codon into the MgrB protein (leading to 27 and 29 amino acid long proteins, respectively). Complementation assays performed on all isolates listed above always restored a full susceptibility to colistin, confirming that lack of MgrB production was responsible for colistin resistance.

Discussion: This study showed that lack of protein MgrB leads to colistin resistance. Interestingly, a hot-spot of insertional inactivation was identified in the corresponding gene. However, we showed that multiple genetic events could lead to such inactivation. This study highlights the crucial role of MgrB in maintaining the susceptibility to colistin.