eP224
ePoster Viewing
The evolution of colistin resistance

RESISTANCE TO COLISTIN ASSOCIATED TO AN IDENTICAL MUTATION IN PROTEIN PMRB AMONG CLONALLY-UNRELATED KLEBSIELLA PNEUMONIAE ISOLATES

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Objectives: Previous works have shown that mutations in the PmrAB two-component system may confer polymixin resistance. 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 corresponding *pmrAB* operon.

Methods: A total of 35 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 *pmrAB* operon was analyzed by PCR and sequencing. Expression of the different *pmr* genes was evaluated by RT-PCR. Complementation assays were performed by cloning a wild-type *pmrB* gene into a cloning vector, and then electroporating this recombinant plasmid into the colistin-resistant clinical isolates. Carbapenemase-encoding genes were searched by PCR and further sequenced.

Results: Six isolates harboured a single nucleotide substitution in the *pmrB* gene, resulting in a single amino acid change (Thr157Pro). PFGE analysis revealed that the six isolates belonged to four distinct clones, recovered in South Africa (Sequence type [ST] 14), Turkey (ST101), and Colombia (ST15 and ST258), respectively. Three out of the four different clones produced a carbapenemase, being respectively OXA-181, OXA-48, and KPC-3, whereas a single isolate from Colombia (ST15) did not produce any carbapenemase. Complementation assays restored the full susceptibility to colistin in all isolates, confirming that this substitution in PmrB was responsible for the resistance phenotype observed.

Expression assays revealed an overexpression of the *pmrC* gene (170-fold), the *pmrA* gene (70-fold), the *pmrB* gene (70-fold), and the *pmrK* gene (40-fold) in the *pmrB*-mutated isolates as compared to wild-type *K. pneumoniae* isolates, confirming that the PmrB substitution was responsible for an increased expression of those genes.

Discussion: This study identified a key amino acid located in the PrmB protein to be responsible for the overexpression of *pmrCAB* and *pmrHFIJKLM* operon, consequently leading to resistance to colistin. Very surprisingly, the same substitution was identified in four distantly-related *K. pneumoniae* isolates, recovered over three different continents. This suggests that Thr157Pro substitution in PmrB corresponds to a hot spot leading to colistin resistance.