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Paper Poster Session V

Assaying and explaining multidrug resistance in Gram-negative bacteria

MgrB inactivation can be found also in colistin susceptible KPC-producing *Klebsiella pneumoniae* of clinical origin

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Objectives: Insertional inactivation of the *mgrB* gene, encoding a negative feed-back regulator of the PhoQ/PhoP signaling system, has been reported to be responsible for colistin resistance in KPC-*Klebsiella pneumoniae*, due to the resulting upregulation of the Pmr lipopolysaccharide modification system. In this work we have investigated seven pairs of colistin-susceptible and colistin-resistant sequential isolates obtained from stool samples of seven patients with a KPC-*Klebsiella pneumoniae* infection before and after colistin treatment, respectively.

Methods: Colistin resistant *K. pneumoniae* strains isolated in surveillance specimens of 7 patients, who were on colistin treatment, as well as sensitive strains isolated from the same patients before colistin use, were included in the study. Exact MICs for colistin were determined by the E-test. Sensitive and resistant isolates were epidemiologically studied by repetitive extragenic palindromic (REP)-PCR methodology. The *mgrB* and *pmrB* genes from the respective resistant and susceptible isolates were amplified by PCR and sequenced. Expression of the *phoP*, *phoQ* and *pmrK* genes was measured by qRT-PCR.

Results: Only clonally related pairs of isolates (susceptible and resistant) were included in the study. MICs of the colistin resistant isolates ranged between 4 and 256 mg/L while susceptible isolates had MICs of 0.125 to 2mg/L. All fourteen strains carried a bla_{KPC} gene. The *pmrB* gene was identical in all isolates, while *mgrB* was interrupted by an IS5-like insertion at nucleotide 75 in a forward or reverse orientation, in three colistin resistant isolates and in their clonal related susceptible ones.

Transcriptional upregulation of the *phoP, phoQ* and *pmrK* genes was observed in three colistin-resistant isolates (MIC range 16-256mg/L) compared to their susceptible pairs (MIC 0.125mg/L). Two of those isolates harbored an interrupted *mgrB,* identical to that found on their isogenic susceptible strain. The third isolate and its isogenic susceptible one harbored an intact *mgrB* with an Asn42Lys substitution.

Conclusion: Our findings shows that upregulation of the PhoQ/PhoP system and activation of the *pmrHFIJKLM* operon, which eventually leads to resistance to polymyxins by modification of the lipopolysaccharide target is not always associated with the inactivation of the *mgrB* gene. Other molecular factors mediating colistin resistance in *K. pneumoniae* are likely to be the focus of future studies.