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CORRESPONDENCE

MgrB variants in colistin-susceptible and colistin-resistant *Klebsiella pneumoniae* ST258

KEYWORDS
Colistin resistance;
Klebsiella pneumoniae;
MgrB;
PmrB

Abstract Resistance determinants of a colistin susceptible and five colistin resistant *Klebsiella pneumoniae* ST258 from a Hungarian outbreak were investigated. A novel MgrB variant in colistin susceptible strain was found. Elevated *phoP* and *arn* gene expressions and wild-type PmrB in all colistin resistant *K. pneumoniae* were detected. All strains lacked *mcr-1*.

To the Editor,

Klebsiella pneumoniae is a frequently identified nosocomial Gram-negative pathogen and found to be resistant to multiple classes of antibiotics, including extended-spectrum cephalosporins, carbapenems, aminoglycosides, and fluoroquinolones. The emergence of colistin resistance in *K. pneumoniae* was reported in several countries and most frequently ST258 clone was identified.[1] The main mechanism of resistance to colistin in Gram-negative bacteria is explained by the modification of lipopolysaccharides, the target molecules of polymyxins. The PmrA/PmrB and PhoP/PhoQ two-component regulatory system confers resistance to polymyxin B, and insertional inactivation of *mgrB*-encoding regulator has also been associated with colistin resistance.[2] Furthermore, *ugd*, *eptB*, *pagL*, and *cdtA* genes were also detected as determinants in colistin resistance.[3] Recently, *mcr-1* a plasmid-mediated colistin resistance determinant was identified in *Escherichia coli* in animals and in humans in China.[4]

In Hungary, the first colistin-resistant *K. pneumoniae* strains were detected between 2008 and 2009 during an outbreak of a KPC-2 producing ST258 clone. Interestingly, during that outbreak no polymyxins were used in the hospital wards but at the beginning colistin-susceptible (minimum inhibitory concentration, MIC: 0.125 μg/mL) and later on colistin-resistant (MIC: 8–24 μg/mL) *K. pneumoniae* were identified. Pulsed-field gel electrophoresis found identical patterns for all strains and based on multi-locus sequence typing all belonged to ST258.[5] In our study five colistin-resistant and a susceptible KPC-2 producing *K. pneumoniae* ST258 were investigated; all tested strains were detected in the aforementioned outbreak.

Polymerase chain reaction (PCR) amplifications of *mgrB*, *pmrB*, and *mcr-1* resistant determinants were performed with specific primers and thermal profiles.[2,4] All PCR amplicons were sequenced and analyzed based on the NCBI Genbank database. The transcription levels of *phoP*, *pmrD*, and *arn* were determined, as total cellular RNA was extracted using the RNeasy Mini kit (Qiagen, Courtaboeuf, France). Reverse transcription PCR was carried out in a LightCycler (Roche Applied Science, Meylan, France). Oligonucleotide primers of this study were designed by online tools of Eurofins MWG Operon, Ebersberg, Germany: phoP, forward GCGTCACCACCTCAAAGTTC and reverse AAACCGTCTTCATCCGGCAG; pmrD, forward AGTACAGGACAACGCTTCGG and reverse GGAGTGAGTTTATCCCCTTCC; and arnT, forward ATAATCGGCGACAGGATAGC and reverse CAGTATCGGTCAGTGGCTGT. Amplifications were performed in duplicate from two different RNA preparations. The cycle threshold (CT) values of the target genes were compared with CT values of *rpoB* housekeeping gene.

In our study *mgrB* and *IS5* were detected by PCR and sequencing. A 940-bp amplicon was amplified in the colistin-susceptible strain (Fig. 1), and a novel amino acid sequence of MgrB (MKKLRWVLLIVIIAGCLLLIRTFLNVMCDQDVQFFSGIC

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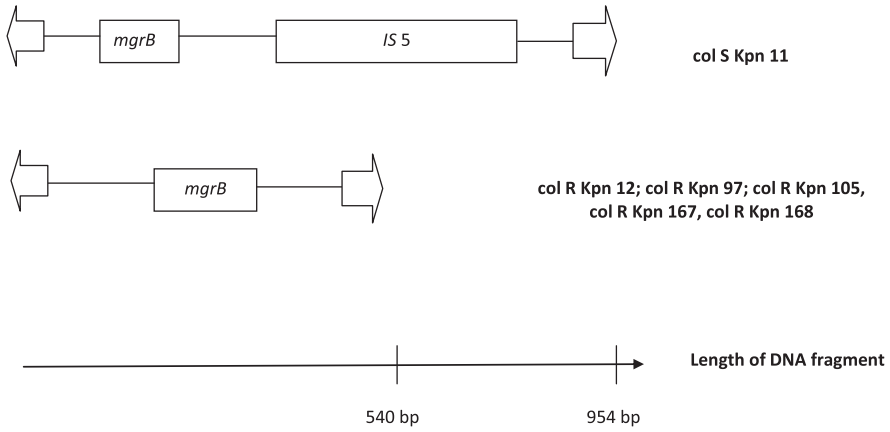

Figure 1. Nucleic acid sequence analysis of flanking regions of *mgrB* in tested strains. bp = base pair; col R Kpn = colistin resistant *Klebsiella pneumoniae*; col S Kpn = colistin susceptible *K. pneumoniae*; *IS*5 = insertion sequence 5.

TINKFIPW) was identified. By contrast, in each resistant *K. pneumoniae* the same set of primers amplified 540 bp DNA fragments, whereas these PCR amplicons were subjected to nucleic acid sequencing and a MgrB variant was uniformly present in all resistant strains (MKKLRWVLLI-VIIAGCLLLWTQMLNVMCDQDVQFFSGICTINKFIPW) and insertion sequence 5 (*IS*5) was abscent (Fig. 1). Amino acid substitution in PmrB was not found in colistin-susceptible strains and in all resistant strains PmrB remained wild-type. This is in contrast to Jayol et al,[2] who pointed out that T157P amino acid substitution is a factor of colistin resistance in *K. pneumoniae*. Overexpression of the *phoP-pmrD-arn* regulatory system was detected in the resistant *K. pneumoniae* strains. Relative gene expression rates of *phoP* and *arn* of colistin resistant strains were elevated compared with susceptible strains. A novel MgrB variant in colistin susceptible *K. pneumoniae* ST258 was detected and all tested strains in our study were negative for the *mcr-1* gene.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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