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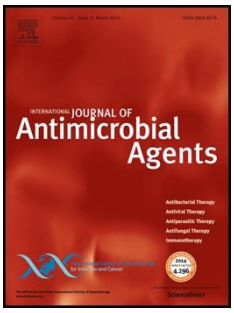

Title: Deciphering the complete deletion of the *mgrb* locus in an unusual colistin-resistant *klebsiella pneumoniae* colonizing the gut of a traveler returning from india

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Deciphering the Complete Deletion of the *MgrB* Locus in an Unusual Colistin-Resistant *Klebsiella pneumoniae* Colonizing the Gut of a Traveler Returning from India

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Sir,

most colistin-resistant (Col-R) *Klebsiella pneumoniae* strains possess alterations of the two-component systems PhoP/Q and PmrA/B. These systems respond to environmental stimuli increasing the expression of the operon *pmrHFIJKLM* whose products are responsible for lipid A modifications leading to decreased affinity for polymyxins. This process is regulated by a negative feedback of the *mgrB* gene that encodes for a small protein repressing the PhoP/Q system. Thus, inactivation of *mgrB* leads to polymyxin resistance. The most common *mgrB* alteration is the insertional inactivation, but nonsense point mutations leading to premature stop codon, as well as partial or complete deletion (Δ) of the *mgrB* locus are also described [1]. In the latter case, no PCR amplification of the locus is obtained and the Δ site remains unknown [2-4].

During an ongoing survey [5], a Col-R *K. pneumoniae* (96R-Kp) was found in the stools of a 46-year old Swiss healthy woman collected after a 35-day trip to India in August 2015. Screening for Col-R strains was specifically achieved by plating overnight enrichments of the stools (Luria-Bertani broth without and with 2 μg/mL colistin) on selective agar plates (CHROMagar Orientation plus 4 μg/mL of colistin and 8 μg/mL of vancomycin without or with 2 μg/mL of cefotaxime). Colonies were then identified using MALDI-TOF MS (Bruker), while MICs were obtained using microdilution GNX2F panels (Trek Diagnostics) [5]. Notably, the pre-trip stools did not contain any Col-R strains and the follow up screening of the stools at 3, 6, 12 months resulted negative. Moreover, 96R-Kp was the only Col-R *K. pneumoniae* strain identified after screening the pre- and post-trip stools of 47 travelers to South-Asian countries.

96R-Kp showed to be resistant to polymyxins (both colistin and polymyxin B MICs >4 μg/mL; Etest MIC for colistin of 32 μg/mL), but not to other antibiotics (e.g., cefotaxime, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole MICs of ≤1, ≤0.25, ≤1, ≤0.5

µg/mL, respectively) using the EUCAST criteria (version 7.0, 2017). The plasmid-mediated colistin resistance *mcr-1* gene was not detected by PCR amplifications [5]. PCR mapping of the *mgrB* locus was also attempted with three previously described primers (flanking the gene: 1F/R and 2F/R and internal: 3F/R;) [3], but no amplifications were obtained. As anticipated, this phenomenon was already observed, but not further explored with whole genome sequencing (WGS) [2-4]. Using primers 1F/R, only Cannatelli *et al*. could detect a Δ*mgrB* locus of 1'142 bp (from nucleotides -400 to 599 respectively to the *mgrB*) in a unique *K. pneumoniae* isolate [3].

To decipher the underlying molecular mechanism of colistin resistance, 96R-Kp underwent WGS with Illumina MiSeq and *de novo* assembly was performed with SPAdes v3.9.0 (GenBank: NIJI00000000). The strain was of ST2261 and capsular type K18 based on the *wzi* allele. Reads were mapped with the Geneious software v10.0.3 (Biomatters) against the reference genome of *K. pneumoniae* RJF999 (GenBank: CP014010) indicating that 96R-Kp lacked a large region of 5.4-kb containing 10 genes including *mgrB* (Figure 1).

A BLAST search was performed with a ~16-kb region (nucleotides from 3'334'049 to 3'350'144; Fig. 1) containing the *mgrB* locus of *K. pneumoniae* RJF999. The comparison recognized over a hundred deposited sequences sharing >99% identity with the query, suggesting a highly conserved location of *mgrB* on the chromosome of *K. pneumoniae* strains (Supplementary Fig. S1). More importantly, a BLAST search of the ~10.6-kb homologous region found in 96R-Kp did not identify other deposited *K. pneumoniae* genomes with the same 5.4-kb Δ*mgrB* locus (Supplementary Fig. S2).

To rapidly characterize Col-R strains possessing large Δ*mgrB* not amplified with primers 1F/R [3], we designed primers *mgrB*Δ-FW (5'-ACCCTGGATAGCGGAGAAGT-3') and *mgrB*Δ-RII (5'-CCGTCCCTTTACCGAAGGTC-3') and performed long PCRs implementing iProof High Fidelity Taq (Bio-Rad). For 96R-Kp, the PCR gave a product of 561 bp and its

DNA sequence (GenBank: MF287165) confirmed the 5.4-kb Δ of the *mgrB*-containing region corresponding to nucleotides 3'339'825 to 3'345'246 of the *K. pneumoniae* reference genome (Fig. 1). As a proof of concept, we also tested 9 Col-R *K. pneumoniae* strains and 2 that were fully-susceptible to polymyxins. As expected, the two groups of *K. pneumoniae* isolates yielded PCR products of ~6-kb (Supplementary Fig. S3), and DNA sequencing using primers *mgrB*Δ-FW and *mgrB*Δ-RII confirmed that the regions flanking the 5.4-kb Δ*mgrB* locus were identical to both 96R-Kp and *K. pneumoniae* RJF999.

This is the first study reporting the WGS of a not previously reported and unusual Col-R *K. pneumoniae* lacking a large region within the *mgrB* locus, thus providing detailed knowledge on the chromosomal location and genetic environment of the excised site. We are unable to define either the mechanism responsible for the deletion and the benefit for *K. pneumoniae* of having such an important deletion in a highly conserved chromosomal locus. However, the implementation of our new primers will allow determining whether more Col-R isolates contain the same or similar mechanisms of colistin resistance.

DECLARATIONS

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The study was approved by the Ethikkommission Nordwest- und Zentralschweiz (EKNZ 239/12).

Competing Interests: None

Ethical Approval: The study was approved by the Ethikkommission Nordwest- und Zentralschweiz (EKNZ 239/12)

REFERENCES

[1] Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. Clin Microbiol Rev. 2017;30:557-96.

[2] Olaitan AO, Diene SM, Kempf M, Berrazeg M, Bakour S, Gupta SK, et al. Worldwide emergence of colistin resistance in *Klebsiella pneumoniae* from healthy humans and patients in Lao PDR, Thailand, Israel, Nigeria and France owing to inactivation of the PhoP/PhoQ regulator mgrB: an epidemiological and molecular study. Int J Antimicrob Agents. 2014;44:500-7.

[3] Cannatelli A, Giani T, D'Andrea MM, Di Pilato V, Arena F, Conte V, et al. MgrB inactivation is a common mechanism of colistin resistance in KPC-producing *Klebsiella pneumoniae* of clinical origin. Antimicrob Agents Chemother. 2014;58:5696-703.

[4] Cheng YH, Lin TL, Pan YJ, Wang YP, Lin YT, Wang JT. Colistin resistance mechanisms in *Klebsiella pneumoniae* strains from Taiwan. Antimicrob Agents Chemother. 2015;59:2909-13.

[5] Bernasconi OJ, Kuenzli E, Pires J, Tinguely R, Carattoli A, Hatz C, et al. Travelers Can Import Colistin-Resistant *Enterobacteriaceae*, Including Those Possessing the Plasmid-Mediated *mcr-1* Gene. Antimicrob Agents Chemother. 2016;60:5080-4.

Figure 1. Linear comparison of 16-kb containing the *mgrB* locus of *K. pneumoniae* RJF999 and the Δ*mgrB* locus of *K. pneumoniae* 96R-Kp using the EasyFig software. Arrows indicate the open reading frames, while their direction indicate the gene orientation. Green arrows represent the gap content, the red one the *mgrB* gene. Protein names are indicated above each gene and the number of the first nucleotide is shown below. Beginning and end of the gap, as well as position and direction of the primers used, are indicated on the top and bottom of the figure. The size of the arrows and position of primers is not in scale. The grey areas indicated that sequences share >99% identity.

Supplementary Figure S1. BLAST search performed with the 16-kb region (nucleotides from 3'334'049 to 3'350'144; Fig. 1) containing the *mgrB* locus of *K. pneumoniae* RJF999. The comparison recognized over a hundred deposited sequences sharing >99% identity with the query, suggesting a highly conserved location of *mgrB* on the chromosome of *K. pneumoniae* strains.

Supplementary Figure S2. BLAST search performed with the ~10.6-kb sequence found in 96R-Kp. Other deposited *K. pneumoniae* genomes with the same ~5.4-kb Δ*mgrB* were not found.

Supplementary Figure S3. Agarose gel showing amplicons of the partial *mgrB* environment for colistin-resistant (Col-R) or colistin-susceptible (Col-S) *K. pneumoniae* strains. DNA was amplified using iProof High Fidelity Taq (Bio-Rad) and primers *mgrB*Δ-FW/-RVII. A 2-log DNA ladder was used to determine the fragment sizes. Neg, negative control.