

# Accepted Manuscript

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

Author: Odette J. Bernasconi, Valentina Donà, João Pires, Esther Kuenzli, Christoph Hatz, Francesco Luzzaro, Vincent Perreten, Andrea Endimiani

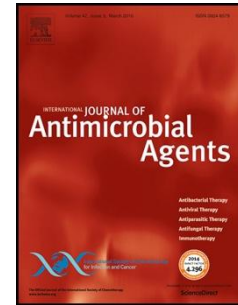
PII: S0924-8579(17)30362-X  
DOI: <https://doi.org/doi:10.1016/j.ijantimicag.2017.09.014>  
Reference: ANTAGE 5271

To appear in: *International Journal of Antimicrobial Agents*

Received date: 12-6-2017  
Accepted date: 28-9-2017

Please cite this article as: Odette J. Bernasconi, Valentina Donà, João Pires, Esther Kuenzli, Christoph Hatz, Francesco Luzzaro, Vincent Perreten, Andrea Endimiani, Deciphering the complete deletion of the *mgrb* locus in an unusual colistin-resistant *klebsiella pneumoniae* colonizing the gut of a traveler returning from india, *International Journal of Antimicrobial Agents* (2017), <https://doi.org/doi:10.1016/j.ijantimicag.2017.09.014>.

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

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5 Odette J. Bernasconi,<sup>1,2§</sup> Valentina Donà,<sup>1,§,¥</sup> João Pires,<sup>1,2</sup> Esther Kuenzli,<sup>3,4</sup> Christoph  
6 Hatz,<sup>3,4</sup> Francesco Luzzaro,<sup>5</sup> Vincent Perreten,<sup>6</sup> and Andrea Endimiani<sup>1\*</sup>

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9 <sup>1</sup> Institute for Infectious Diseases, University of Bern, Bern, Switzerland; <sup>2</sup> Graduate School of  
10 Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland; <sup>3</sup> Swiss Tropical  
11 and Public Health Institute, Basel, Switzerland; <sup>4</sup> Division of Communicable Diseases,  
12 Institute for Social and Preventive Medicine, University of Zurich, Zurich, Switzerland; <sup>5</sup>  
13 Laboratory of Microbiology, A. Manzoni Hospital, Lecco, Italy; <sup>6</sup> Institute of Veterinary  
14 Bacteriology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

15  
16 § Contributed equally

17  
18 ¥ Present address: Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern,  
19 Bern, Switzerland

20  
21  
22  
23  
24  
25 **\*Corresponding Author:**

26 Prof. Andrea Endimiani MD, PhD

27 Institute for Infectious Diseases, University of Bern

28 Friedbühlstrasse 51, CH-3001, Bern, Switzerland

29 Phone: +41-31-632 8 632; Fax: +41-31-632 8 766

30 Emails: [andrea.endimiani@ifik.unibe.ch](mailto:andrea.endimiani@ifik.unibe.ch); [aendimiani@gmail.com](mailto:aendimiani@gmail.com)

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

42 During an ongoing survey [5], a Col-R *K. pneumoniae* (96R-Kp) was found in the  
43 stools of a 46-year old Swiss healthy woman collected after a 35-day trip to India in August  
44 2015. Screening for Col-R strains was specifically achieved by plating overnight enrichments  
45 of the stools (Luria-Bertani broth without and with 2  $\mu\text{g}/\text{mL}$  colistin) on selective agar plates  
46 (CHROMagar Orientation plus 4  $\mu\text{g}/\text{mL}$  of colistin and 8  $\mu\text{g}/\text{mL}$  of vancomycin without or  
47 with 2  $\mu\text{g}/\text{mL}$  of cefotaxime). Colonies were then identified using MALDI-TOF MS (Bruker),  
48 while MICs were obtained using microdilution GNX2F panels (Trek Diagnostics) [5].  
49 Notably, the pre-trip stools did not contain any Col-R strains and the follow up screening of  
50 the stools at 3, 6, 12 months resulted negative. Moreover, 96R-Kp was the only Col-R *K.*  
51 *pneumoniae* strain identified after screening the pre- and post-trip stools of 47 travelers to  
52 South-Asian countries.

53 96R-Kp showed to be resistant to polymyxins (both colistin and polymyxin B MICs  $>4$   
54  $\mu\text{g}/\text{mL}$ ; Etest MIC for colistin of 32  $\mu\text{g}/\text{mL}$ ), but not to other antibiotics (e.g., cefotaxime,  
55 ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole MICs of  $\leq 1$ ,  $\leq 0.25$ ,  $\leq 1$ ,  $\leq 0.5$

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

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

70 A BLAST search was performed with a ~16-kb region (nucleotides from 3'334'049 to  
71 3'350'144; Fig. 1) containing the *mgrB* locus of *K. pneumoniae* RJF999. The comparison  
72 recognized over a hundred deposited sequences sharing >99% identity with the query,  
73 suggesting a highly conserved location of *mgrB* on the chromosome of *K. pneumoniae* strains  
74 (Supplementary Fig. S1). More importantly, a BLAST search of the ~10.6-kb homologous  
75 region found in 96R-Kp did not identify other deposited *K. pneumoniae* genomes with the  
76 same 5.4-kb  $\Delta mgrB$  locus (Supplementary Fig. S2).

77 To rapidly characterize Col-R strains possessing large  $\Delta mgrB$  not amplified with primers  
78 1F/R [3], we designed primers *mgrB* $\Delta$ -FW (5'-ACCCTGGATAGCGGAGAAGT-3') and  
79 *mgrB* $\Delta$ -RII (5'-CCGTCCCTTTACCGAAGGTC-3') and performed long PCRs implementing  
80 iProof High Fidelity Taq (Bio-Rad). For 96R-Kp, the PCR gave a product of 561 bp and its

81 DNA sequence (GenBank: MF287165) confirmed the 5.4-kb  $\Delta$  of the *mgrB*-containing region  
82 corresponding to nucleotides 3'339'825 to 3'345'246 of the *K. pneumoniae* reference genome  
83 (Fig. 1). As a proof of concept, we also tested 9 Col-R *K. pneumoniae* strains and 2 that were  
84 fully-susceptible to polymyxins. As expected, the two groups of *K. pneumoniae* isolates  
85 yielded PCR products of ~6-kb (Supplementary Fig. S3), and DNA sequencing using primers  
86 *mgrB* $\Delta$ -FW and *mgrB* $\Delta$ -R11 confirmed that the regions flanking the 5.4-kb  $\Delta$ *mgrB* locus were  
87 identical to both 96R-Kp and *K. pneumoniae* RJF999.

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

95

## 96 **DECLARATIONS**

97 **Funding:** This work was supported by Swiss National Science Foundation (SNF grant No.  
98 153377 to AE). Odette J. Bernasconi is a PhD student (2015-2018) supported by Hans Sigrist  
99 Foundation (Bern, Switzerland). JP was a PhD student (2014–2017) supported by the SNF.

100 The study was approved by the Ethikkommission Nordwest- und Zentralschweiz (EKNZ  
101 239/12).

102 **Competing Interests:** None

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

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124

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

133  
134 **Supplementary Figure S1.** BLAST search performed with the 16-kb region (nucleotides  
135 from 3'334'049 to 3'350'144; Fig. 1) containing the *mgrB* locus of *K. pneumoniae* RJF999.  
136 The comparison recognized over a hundred deposited sequences sharing >99% identity with  
137 the query, suggesting a highly conserved location of *mgrB* on the chromosome of *K.*  
138 *pneumoniae* strains.

139  
140 **Supplementary Figure S2.** BLAST search performed with the ~10.6-kb sequence found in  
141 96R-Kp. Other deposited *K. pneumoniae* genomes with the same ~5.4-kb  $\Delta mgrB$  were not  
142 found.

143  
144 **Supplementary Figure S3.** Agarose gel showing amplicons of the partial *mgrB* environment  
145 for colistin-resistant (Col-R) or colistin-susceptible (Col-S) *K. pneumoniae* strains. DNA was  
146 amplified using iProof High Fidelity Taq (Bio-Rad) and primers *mgrB* $\Delta$ -FW/-RVII. A 2-log  
147 DNA ladder was used to determine the fragment sizes. Neg, negative control.