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Hypervirulent *Klebsiella pneumoniae* ST23 producing OXA-48 in Switzerland

Editor: Stefania Stefani

Sir,

The population of *Klebsiella pneumoniae* (Kp) comprises genetically diverse strains that may be gathered in two distinct groups: classical Kp associated with nosocomial infections and the less common hypervirulent Kp (hvKp) associated with more severe and aggressive infections, often recovered from healthy and community-residing individuals [1]. First reported from East Asia, the hvKp strains were generally quite susceptible to antibiotics. However, hvKp, like classical Kp, may also be reservoirs of several antibiotic resistance determinants, including the problematic carbapenemases (mostly KPC and OXA-48 types) [1,2]. Such association of high-level virulence and high-level resistance explains the great threat made by the circulation of those strains in the community. The latter belong to a relatively small number of clonal backgrounds and therefore sequence types (STs), as shown through multilocus sequence typing (MLST), with sequence type (ST) ST23 being the most prevalent clone worldwide, particularly in China [1,2]. A warning of the possible emergence of hvKp ST23 producing carbapenemases in the EU.EEA has very recently been published [3].

Here, we report the case of a 25-year-old Russian patient who was initially hospitalised in Russia for multiple orthopaedic trauma. He was then hospitalised at the University hospital of Lausanne in 2018 for treatment of several bone injuries and bone infection. Upon admission, but during the entire year of his hospitalisation, a total of 6 Kp isolates (namely Kp1 to Kp6) were identified while screening for carbapenemase-producing isolates in his rectal flora by using selective SuperCarba medium (CHROMagar mSuperCABA, CHROMagar, Paris France).

During his long hospitalisation, he received several courses of antibiotics related to fevers and urinary tract infections, but Kp was never identified as a source of infection. Antibiotic susceptibility patterns of the Kp isolates were determined by disk diffusion and interpreted according to the 2021 EUCAST guidelines (https://eucast.org). All those isolates displayed very similar multidrug-resistance patterns to all ß-lactams, except to meropenem, imipenem and cefoxitin; they all remained susceptible to the ceftazidime/avibactam combination. Isolate Kp1 was resistant to colistin, whereas the other five were not, a phenomenon frequently observed among clonally-related strains, probably due to the fitness disadvantage related to the colistin-resistance phenotype. Conversely, isolate Kp6 was resistant to quinolones, although the previous isolates were not.

Carbapenemase activity was biochemically detected in all isolates by using the NitroSpeed Carba NP test [4]. For further understanding of the genetic basis underlying antibiotic resistance and virulence, the genomes of the six isolates were sequenced using the Illumina MiSeq platform. Sequence reads were analysed using BioNumerics™ (version 8.0, created by bioMérieux, available at http://www.applied-maths.com) with the default setting, except the de novo assembly, which was performed using the Unicycler pipeline. Multilocus sequence typing (MLST) analysis identified those Kp isolates as belonging to ST23. The six genomes were compared with 19 available ST23 Kp genomes in Genbank. Genome comparison was performed using core genome MLST (cgMLST) according to the scheme developed by Bionumerics. Analysis showed that the six genomes were genetically highly related (0 to 2 loci differences), and that the closest genome was that of strain MAR 14-456 identified in Moscow in 2020 [3] (Figure). All these isolates belonged to the main K1 ST23 clade described in the ECDC warning [3].

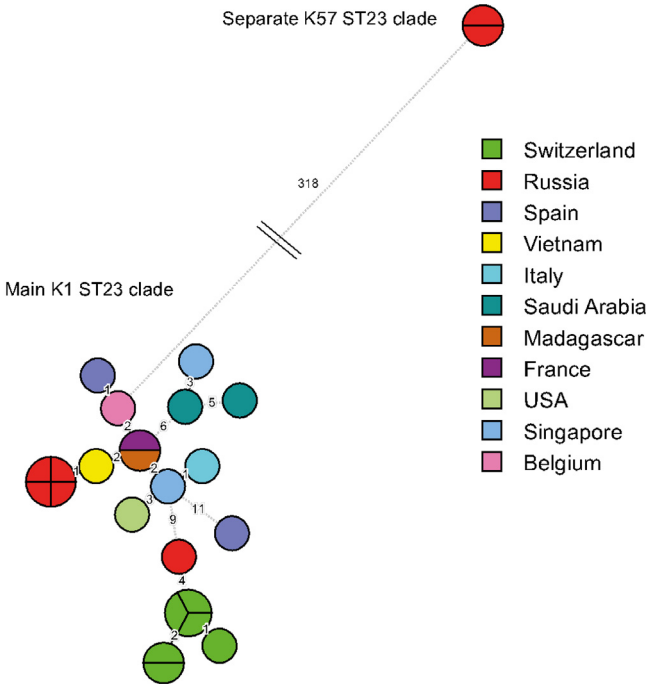


Figure. Minimum spanning tree of ST23 Kp isolates obtained from cgMLST data. Each circle represents one or several isolates. The number between circles is the number of loci differences.

Many acquired antimicrobial resistance genes were identified, including genes encoding resistance to β-lactams (bla_{OXA-48}, $bla_{CTX-M-15}$, bla_{TEM-1D}), quinolones (*qnrB*), aminoglycosides (*strA*, *strB*), sulfonamides (*sul2*), and trimethoprim (*dfrA14*) (Table). Interestingly, some of those resistance genes were absent in some isolates, likely corresponding to plasmid loss in the latter; as an

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Table
Genetic features associated with the different *Klebsiella pneumoniae* isolates.

Strain	ST type	ß-lactamase content	Other resistance determinants	Colistin resistance determinants	Virulence determinants[a]
Kp1	ST23	OXA-48, CTX-M-15, TEM-1, SHV-11	dfrA14, sul2, QnrB1 strA, strB	MgrB stop codon	*rmpA2, ybt1, iuc1, clb2*
Kp2	ST23	OXA-48, CTX-M-15, TEM-1, SHV-11	dfrA14, sul2, QnrB1 strA, strB	MgrB stop codon	*rmpA2, ybt1, iuc1, clb2*
Kp3	ST23	OXA-48, CTX-M-15, TEM-1, SHV-11	dfrA14, sul2, QnrB1 strA, strB	MgrB wild-type	*rmpA2, ybt1, iuc1, clb2*
Kp4	ST23	OXA-48, SHV-11		MgrB wild-type	*ybt1, clb2*
Kp5	ST23	OXA-48, CTX-M-15, TEM-1, SHV-11	dfrA14, sul2, QnrB1 strA, strB	MgrB wild-type	*ybt1, clb2*
Kp6	ST23	OXA-48, CTX-M-15, TEM-1, SHV-11	dfrA14, sul2, QnrB1 strA, strB	MgrB wild-type	*ybt1, clb2*

[a] *rmpA2* encodes hypermucoid phenotype, *ybt1* encodes yersiniabactin, *iuc1* encodes an aerobactin, and *clb2* encodes a colibactin

example, isolate Kp4 lacked the $bla_{\text{CTX-M-15}}$ gene, in line with the susceptibility to ceftazidime observed for only that isolate.

Noteworthy, the $bla_{\text{OXA-48}}$ gene was reported as the most common carbapenemase gene found among hvKp strains in Europe [3]. Resistance to colistin observed in isolates Kp1 and Kp2 could be explained by amino acid deletion in the MgrB protein coding for a regulatory protein involved in the lipopolysaccharide biosynthesis, while resistance to nalidixic acid in the latest strain was associated to an amino-acid substitution in gyrase A.

Analysis of the occurrence of virulence genes showed that isolates Kp1, Kp2 and Kp3 possessed the *rmpA* genes encoding regulatory proteins enhancing the mucoid phenotype, whereas Kp4 to Kp6 did not (Table) [5]. All isolates possessed the yersiniabactin and colibactin encoding genes, responsible for the hypermucoid phenotype, while the aerobactin encoding gene was only identified in isolates Kp1, Kp2 and Kp3.

With respect to the $bla_{\text{OXA-48}}$ carbapenemase gene, it was identified onto a ca. 62-kb IncL plasmid in all isolates, that plasmid being spread worldwide and known to be the main source of the $bla_{\text{OXA-48}}$ gene.

Analysis of the core genome of the six genomes showed that all isolates were clonally related. Noteworthy, the three latter recovered isolates (Kp4 to Kp6) have lost three virulence markers compared to the three former ones (Table).

This report constitutes the first identification of hvKp in Switzerland. Such identification highlights the silent spread of those strains combining resistance and virulence traits. Although worldwide reference centres dealing with antibiotic resistance mainly focus on detection of emerging antibiotic resistance, it may also be worthwhile considering associated virulence traits in the global analysis, with the objective of identifying and therefore trying to prevent the spread of those multidrug-resistant and hypervirulent strains.

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Competing Interests

None.

Ethical Approval

Not required.

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Dominique S. Blanc
Service of Hospital Preventive Medicine, Lausanne University hospital and University Hospital, Lausanne, Switzerland
Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), Universities of Fribourg and Lausanne, Switzerland

Laurent Poirel
Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), Universities of Fribourg and Lausanne, Switzerland
Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland
INSERM European Unit (IAME), University of Fribourg, Fribourg, Switzerland

Mathias Van Singer
Service of Hospital Preventive Medicine, Lausanne University hospital and University Hospital, Lausanne, Switzerland

Gilbert Greub
Institute for Microbiology, Lausanne University hospital and University of Lausanne, Lausanne, Switzerland

Patrice Nordmann*
Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), Universities of Fribourg and Lausanne, Switzerland
Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland
INSERM European Unit (IAME), University of Fribourg, Fribourg, Switzerland
Institute for Microbiology, Lausanne University hospital and University of Lausanne, Lausanne, Switzerland

*Corresponding author: Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Chemin du Musée 18, CH-1700 Fribourg, Switzerland.
E-mail address: patrice.nordmann@unifr.ch (P. Nordmann)