Supplementary Material

Bacteriologic Studies

Bacterial isolates were obtained from routine microbiological cultures of clinical samples (urine, feces, blood and proximal ureteral stent tip).

Surveillance swabs (rectal, pharyngeal and cutaneous) were screened for CP *Enterobacteriaceae* by direct inoculation in selective chromogenic agar, namely ChromID ESBL and ChromID Carba plates (bioMérieux Marcy l'Etoile, France).

Carbapenemase activity was detected by using the Rapid Carba NP test (1).

MICs were performed by broth microdilution method in Mueller-Hinton broth (Bio-Rad, Marnes-La-Coquette, France), with the exception of the combinations CZA and ceftolozane-tazobactam that were determined by Etest (AB bioMérieux; Solna, Sweden).

Results were interpreted according to EUCAST breakpoints (2).

CZA resistance was defined as > 8 µg/mL for Enterobacteriales. MICs of β-lactams were determined alone or in combination with a fixed concentration of clavulanic acid (2 µg/ml), tazobactam (4 µg/ml) or avibactam (4 µg/ml).

Identification of isolates at the species level was obtained by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (bioMérieux).

Identification of carbapenemase genes was performed by PCR using previously reported primers (3).

Carbapenemase detection directly from rectal swabs was performed with the Xpert CarbaR test (GeneExpert, Cepheid, USA).

Isolation of non-antibiotic resistant bacterial flora from feces, rectal swabs and urine was done on Mac Conkey and CNA agar, supplemented with 5% Sheep Blood (bioMérieux).

Multilocus Sequence Typing consisted in PCR amplification followed by sequencing of 7 *Kp* housekeeping genes (4).

Clonal relationship of the isolates was evaluated by pulsed-field gel electrophoresis (PFGE). Briefly, total DNA from the *K. pneumoniae* isolates was digested by using the XbaI enzyme (New England Biolabs®, Ipswich, USA). The generated fragments were separated by PFGE using a CHEF-DR III System (Bio-Rad, Cressier, Switzerland) creating a unique PFGE profile for each clonal strain (data not shown).

Phage Studies

The bacteriophage that revealed lytic activity against the MDR *K. pneumoniae* isolates recovered from the patient's clinical samples was selected from the Eliava Institute's phage collection, as already described (5). Lytic activity and stability of the bacteriophage was increased by phage adaption procedures on 5 isolates recovered overtime from the patient's urine, ureteral stent and rectal swab.

The efficiency of plating (EOP) of the phage was investigated on 18 susceptible strains (see Supplementary Table 1). EOP was calculated as the ratio of the titer on the test strain to the titer on the phage host strain. The range of EOP mainly varied between 1.0 - $2x10^{-3}$ with the exception of only 3 isolates, for which the EOP was low ($5x10^{-4}$, $5x10^{-5}$ and $5x10^{-7}$) (see Supplementary Table 1). The higher the EOP, the more significant is the phage's lytic activity against its bacterial host and hence its therapeutic potential. This highly active virulent phage preparation was used for therapeutic application, with a titer estimated at $1x10^{6}$ pfu/ml.

The host range of the selected bacteriophage was studied in Tbilisi and Fribourg, using the soft-agar overlay method, as described (6). The phage preparation was tested against

130 *Klebsiella* spp. (20 *Klebsiella oxytoca* and 110 *K. pneumoniae*) clinical isolates from Tbilisi recovered in the year 2018 from distinct patients and with variable antibiotic resistance profiles. Susceptibility to the phage was evidenced for 62 isolates, namely, 11 *K. oxytoca* (55%) and 51 *K. pneumonia*e (46%).

Then, a series of 94 KPC-producing *K. pneumoniae* isolates from worldwide was challenged against the phage in Fribourg. The virus showed a lytic effect towards 32 out of those isolates (34%), thus showing a significant, but restricted, lytic activity against geographically diverse *K. pneumoniae* strains. Next, a series of 40 clonally-unrelated, non KPC-producing *K. pneumoniae* isolates recovered from worldwide was tested, including 20 carbapenemase producers (10 producing OXA-48 and 10 producing NDM-1), 18 extended-spectrum ß-lactamase producers (8 producing either TEM or SHV derivatives and 10 producing CTX-M derivatives), and two wild-type reference strains (ATCC700603 and ATCC10031). Out of those additional 40 *K. pneumoniae* isolates tested, a significant lytic activity was observed for nineteen of them (47,5 %), including 6 OXA-48 producers, 5 NDM producers, 3 SHV-type ESBL producers, 2 CTX-M-15 producers, and the two wild-type reference strains. Finally, by testing 80 isolates belonging to other species (*Pseudomonas aeruginosa*, *Enterobacter* spp., *Escherichia coli*, and *Acinetobacter baumannii*), no lytic effect could be observed, further highlighting the species-specific lytic action of this phage.

Noteworthy, the phage action was additionally tested against the two wild-type ST1109 and ST247 *K. pneumoniae* isolates recovered from the patient. Interestingly, the firstly identified isolate (ST1109) showed a resistance phenotype, although the lately recovered one (ST247) was susceptible to the lytic action of the phage. However, since we did not investigate for how long the phage persisted in the patient following treatment interruption, we cannot ascribe this phenomenon to the selective pressure of the phage or to chance alone.

Transmission Electron Microscopy (Jeol, Akishima-Shi, Tokyo, Japan) studies were performed on the phage preparation and revealed a homogeneous population of phage particles belonging to the *Myoviridae* morphological group. The virion revealed an icosahedral head structure of 122.7 nm x 8.6 nm; a collar of 3.6 nm x 0.45 nm; a contractile tail of 109.1 nm x 2.7 nm; and a baseplate of 3.6 nm x 0.9 nm (see Supplementary Figure 1).

Next generation sequencing was used to determine the phage's genomic sequence. Briefly, a volume of 5 ml of the phage preparation used for BT and containing 10^6 plaque-forming units (pfu) of phage per ml was used for DNA extraction. Phage DNA was extracted with the Phage DNA isolation kit (NORGEN Biotek Corp., Thorold, ON, Canada). Genomic libraries were assessed using the NexteraXT library preparation kit (Illumina Inc., San Diego, CA, USA) and sequencing was performed using the Illumina MiniSeq system with 300-bp paired-end reads and a coverage of 50X. Generated FastQ data were compiled and analyzed using the CLC genomic workbench 7.5.1 (CLC bio, Aarthis, Denmark). Reads were de novo assembled with automatic bubble and word size and contigs were generated using the mapping mode "map reads back to contigs" with a minimum contig length of 1,000 nucleotides. The resulting contigs were uploaded into the RAST server using the RASTtk annotation scheme (7). The sequence of phage vB_KpnM_GF has been deposited in the Genbank databases under accession number MK421971.

The length of the phage's genome was 161,728-bp. Sequence analysis allowed to confirm the electron microscopy findings and its affiliation to the *Myoviridae* family, *Caudovirales* order, subfamily *Tevenvirinae*. That phage, named vB_KpnM_GF according to the phage nomenclature (8), shared the highest identity (69% at the nucleotide level) with a 171-kb-long phage recovered from a KPC-2-producing ST101 *K. pneumoniae* strain. Analysis of the phage's whole genome showed that it possessed a series of genes encoding common

phage-related features, including a typical phage polymerase, a DNA topoisomerase subunit, tail and head structure proteins. Importantly, *in-silico* analysis did not reveal putative virulence or antibiotic resistance or integrase sequences in the genome of the phage.

Bacteriophage Therapy

Briefly, 10 ml of the phage preparation containing 10^6 plaque-forming units (pfu) of phage per ml was taken orally in an empty stomach every 12 hours, for 3 weeks. Bacteriophage administration was preceded by alkalinisation of the gastric contents with the use of carbonated mineral water. During the first two weeks of treatment, 1 million phages were also given daily intra-rectally, *via* suppository. BT was well tolerated, without adverse events. The patient was visited weekly during BT and the clinical examination was normal. Furthermore, no alterations were noted in the complete blood count, the serum C-reactive protein levels, and the concentrations of liver enzymes and serum electrolytes. Blood urea nitrogen and serum creatinine remained unchanged from baseline. In addition, the fecal calprotectin levels remained within the normal range throughout the period of BT. Notably, the patient did not experience modifications of her stool habits (i.e. between 2 to 3 stool movements per day, with feces of variable consistency from unformed to fully formed) or abdominal discomfort.

Patient follow-up

In the 11 months following BT interruption, the patient was visited every 7-14 days. On each visit a rectal swab, urine and stool cultures and blood tests were obtained. The rectal swabs and the stools underwent growth on both selective and non-selective media (see

above). In addition, the rectal swabs underwent molecular screening for the presence of carbapenemase genes with the Xpert Carba Assay (see above for details).

The ureteral stent was replaced every 4 - 6 weeks and its proximal tip was subject to culture, except in 1 circumstance (November 2018).

Whenever the patient experienced signs and symptoms of UTI (i.e. in the months of February, March, October (in 2 circumstances) and December 2018), she was hospitalized. Blood cultures were obtained before the initiation of antimicrobial therapy and were always negative except for the last episode in December 2018 when blood, as well as the urine, cultures grew *E. faecalis* and *P. aeruginosa*. Antimicrobial treatment consisted in cycles of 4-5 days duration in case of UTIs and of 14 days when sepsis was documented and consisted in: ertapenem plus trimethoprim-sulfamethoxazole (2 days), meropenem (1 day) and finally ceftazidime (2 days) after the urine culture turned positive for *Enterobacter aerogenes* and *Citrobacter braaki* (February, 2018); ampicillin, ceftriaxone and trimethoprim-sulfamethoxazole when *Enterobacter ludwigii* was recovered from the urine culture (March, 2018); ceftazidime, when *Citrobacter freundii* was isolated from the urine (October 2018, first episode); ceftazidime plus linezolid, when *Enterococcus faecalis* was recovered from the urine (October 2018, second episode); and, finally, ceftazidime when *E. faecalis* and *P. aeruginosa* were isolated from both blood and urine cultures (December 2018).

Supplementary References

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Supplementary Table and Figure Legends

Supplementary Table 1

Efficiency of Plating of phage vB_KpnM_GF

Supplementary Figure 1

Transmission electron microscopy image of the *Klebsiella pneumoniae* phage vB_KpnM_GF particles (Magnification: X 220 000)

Supplementary Table 1

Klebsiella spp.	**vB_KpnM_GF**	**EOP**
	Titer	
*K.p.90a**	$2x10^9$	1
*K.p.91a**	$2x10^9$	1
*K.p.92a**	$2x10^9$	1
*K.p.93a**	$2x10^9$	1
*K.p.94a**	$2x10^9$	1
K.p.128	$1x10^3$	$5x10^{-7}$
K.o.105a	$2x10^9$	1
K.p.111a	$1x10^8$	$5x10^{-2}$
K.p.112a	$1x10^8$	$5x10^{-2}$
K.p.113a	$1x10^8$	$5x10^{-2}$
K.p.118a	$3x10^8$	$1.5x10^{-2}$
K.p.130a	$3x10^8$	$1.5x10^{-2}$
K.o.151a	$8x10^8$	$4x10^{-1}$
K.p.153a	$9x10^8$	$4,5x10^{-1}$
K.p.154a	$1x10^9$	$5x10^{-1}$
K.p.157a	$1x10^9$	$5x10^{-1}$
K.p.158a	$5x10^7$	$2.5x10^{-3}$
K.p.161a	$7x10^7$	$3.5x10^{-3}$
K.p.191a	$5x10^6$	$2.5x10^{-4}$
K.p.204a	$2x10^7$	$1x10^{-2}$

**K.p.90a-(rectal swab); K.p.91a (ureteral stent); K.p.92a (urine#1); K.p.93a(urine#2); K.p.94a(urine#3)* are the strains isolated from patient

Supplementary Figure 1