ACTIVITY OF BACTERIOPHAGE AGAINST MULTIDRUG RESISTANT *KLEBSIELLA PNEUMONIAE*

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Introduction

The prevalence of extended-spectrum beta-lactamase (ESBL) producing and carbapenem resistant (CR) *Klebsiella pneumoniae* (*KP*) has significantly risen worldwide. Infections due to these bacteria are of increasing clinical concern and associated with high mortality across different infection types (Castanheira et al., 2019, Martin et al., 2018). Even with newer treatment options, such as ceftazidime-avibactam, meropenem-vaborbactam, plazomicin, and cefiderocol, there remains an unmet need for safe and effective therapeutic options to treat infections caused by ESBL and CR *KPs*. Bacteriophage (phage) therapy offers a promising alternative to antibiotics as phage are usually indifferent to the antibiotic resistant state of the bacterial strain they target. Phages thus offer a novel approach with an unprecedented and orthogonal mechanism of action for treatment of acute infections caused by resistant bacteria that are insufficiently addressed by available antibiotics. Phage-based therapies confer a high strain-level specificity and have a strong intrinsic safety profile. Here we describe the identification of novel phages that can effectively target antibiotic resistant *KP* isolates.

Methodology

- *KP* clinical strains were isolated from human stool specimens preserved in glycerol. Selective culturing was carried on MacConkey and CHROMagar Orientation plates, followed by testing of individual colonies for motility, indole and urease production using UMI agar tubes. Isolates were then sequenced by NGS and preserved as glycerol stocks.
- *KP* isolates sequences were analyzed in-silico using the Kleborate tool (https://doi.org/10.1099/mgen.0.000102) to determine the multi-locus sequence type (MLST), capsule type, and antibiotic resistant genes.
- Natural phages were isolated from plaques that developed on susceptible bacterial targets. These were sequenced and characterized. Characterization included taxonomic assignment, examination of the phage sequences for undesirable genes (toxins, antibiotic resistance genes, lysogeny potential) as defined by the regulatory authorities, and testing the host range on all target bacteria.

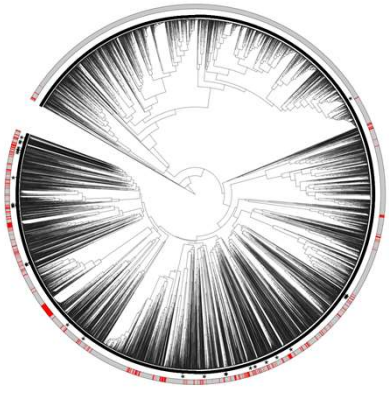
Results

1. ESBL producing and CR *KP* isolates are highly variable

Antibiotic-resistant *KP* strains encoding beta lactamase genes or a carbapenemase (ESBL or CR *KP* strains, n=33) were isolated from healthy individuals (n=4), and patients with inflammatory bowel disease (IBD, n=26) or primary sclerosing cholangitis (PSC, n=3).

To evaluate the similarity of CR and ESBL *KP* isolates, a phylogenetic tree based on the sequences of these and other *KP* strains was generated. This revealed that the ESBL and CR isolates are highly variable and dispersed on the entire *KP* phylogenetic tree.

Phylogenetic tree of *KP* strains



KP Public Sequence
BiomX IBD/PSC clinical *KP* isolate
* BiomX CR or ESBL clinical *KP* isolate

A total of 33 ESBL and CR isolates (n=33) belonging to 19 MLST types were found. 18 (54%) MLST types were isolated from more than one participant.

2. Characterization of ESBL producing and CR *KP* isolates

The sequences of the antibiotic resistance genes of 33 ESBL or CR *KPs* revealed two bla CTX-M15 encoding isolates, one double bla KPC-2 CTX-M-15 and bla SHV encoding isolate, one carbapenamase KPC2 strain and 29 bla SHV encoding isolates.

Characterization of 33 *ESBL / CR resistant KPs*

Isolate ID	MLST	Antibiotic Resistance genes
Isolate 1	ST485	SHV-27
Isolate 2	ST3345	SHV-27
Isolate 3	ST36	SHV-13
Isolate 4	ST4097-1LV	SHV-27
Isolate 5	ST13	SHV-101
Isolate 6	ST13	SHV-101
Isolate 7	ST4097	SHV-27
Isolate 8	ST1841	SHV-27
Isolate 9	ST13	SHV-101
Isolate 10	ST1966	SHV-27
Isolate 11	ST13	SHV-101
Isolate 12	ST2026-1LV	SHV-27
Isolate 13	ST661	SHV-27
Isolate 14	ST661	SHV-27
Isolate 15	ST2597	SHV-38
Isolate 16	ST611	SHV-27
Isolate 17	ST1274	SHV-41
Isolate 18	ST485	SHV-27
Isolate 19	ST678-1LV	SHV-41
Isolate 20	ST661	SHV-27
Isolate 21	ST987	SHV-27
Isolate 22	ST278	SHV-27
Isolate 23	ST29	CTX-M-15
Isolate 24	ST827	SHV-38
Isolate 25	ST1593	CTX-M-15
Isolate 26	ST280	CTX-M-15;SHV-27
Isolate 27	ST678	SHV-41
Isolate 28	ST661-1LV	SHV-27
Isolate 29	ST678-1LV	SHV-41
Isolate 30	ST399	SHV-27
Isolate 31	ST13	SHV-101
Isolate 32	ST13	SHV-101
Isolate BAA-1705	ST258	KPC

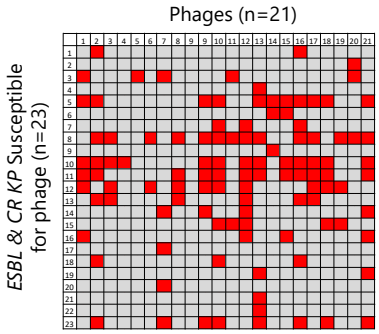
3. Broad host range phages for *KP* can infect ESBL producing and CR *KP* isolates

Screening of a large library of environmental samples resulted in the isolation of 21 phages recognizing drug resistant KP isolates. Sequence analysis of the phages revealed that they belong to different genera in the Caudovirales order, contain linear dsDNA genomes between ~40-180 kbp in length and are devoid of undesirable genes. Production titers were acceptable for manufacturing (> 10^9 PFU/mL).

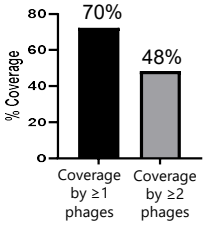
Solid infection was carried out to determine the coverage of the phages on ESBL and CR *KP* clinical isolates.

Phage susceptibility of ESBL producing/CR *KP* isolates

Host range analysis of the 21 phages on the 33 ESBL or CR *KP* isolates demonstrated that 70% coverage (n=23) of these isolates has been achieved (red squares).



The 21 phages cover 70% of the ESBL and/or CR *KP* panel. 48% of ESBL and/or CR *KPs* were targeted by more than one phage. Several wide host range phages individually targeted 27% of ESBL and CR *KPs*.



Discussion and Conclusions

Collectively, these results demonstrate the feasibility of identifying phage with potent *in vitro* activity against antibiotic resistant *KP* isolates. *In vivo* studies, testing phage efficacy in animal models of infections, are currently on going to support phage therapy as a novel therapeutic approach for treatment of ESBL and/or CR *KP* infections.

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References

Castanheira et al., *OFID*, 2019
Martin et al., *OFID* 2018