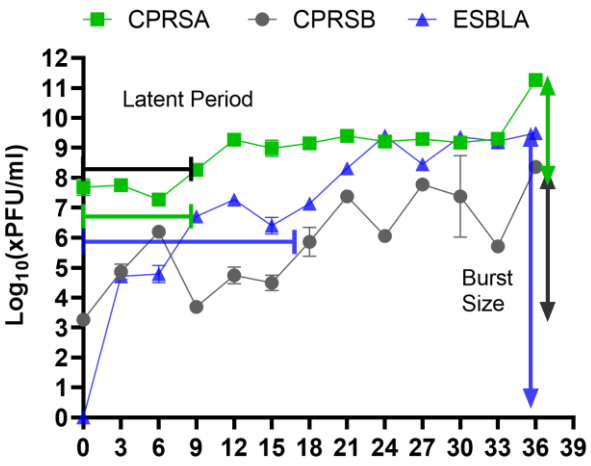
Note: This is Online Supplementary Document 1 of Michodigni NF, Nyachieo A, Akhwale JK, Magoma G, Ouédraogo A-S, Kimang'a AN. Formulation of phage cocktails and evaluation of their interaction with antibiotics in inhibiting carbapenemase-producing *Klebsiella pneumoniae* in vitro in Kenya. Afr J Lab Med. 2022;11(1), a1803. https://doi.org/10.4102/ajlm.v11i1.1803

Supplementary Methods 1: VITEK protocol

Single colony of overnight bacterial culture of KP20 was inoculated in approximately 25 ml TSB and the optical density was measured at 600 nm using in order to obtain an absorbance of 0.5–0.63 McFarland units using the VITEK DensiCHEK™ colorimeter (BioMerieux, Inc., Hazelwood, MO). After obtaining this absorbance, 10-fold serial dilution was conducted on the bacterial culture at an exponential growth phage using 0.45% saline as diluent. Then, approximately 5 ml of the first diluted bacterial suspension was used to load the VITEK 2 Systems Version 9.2 (bioMérieux) using GN Bar Code: 2411227403347768 for identification of Gram-negative rods and Gram-positive and the card was read by kinetic fluorescence measurement, and the results reported within 4.03-9.87 h. For the antibiotic susceptibility testing (AST), only bacteria correctly identified with the VITEK 2 Systems were included in the experiments. Briefly, 5 ml of each bacterial suspension was prepared as described above and was loaded into the VITEK 2 AST-XN05 Bar Code: 1481424403205844 card (BioMerieux, Inc., Hazelwood, MO). A total of 18 antimicrobials were screened for Gram-negative rods: Ampicillin/Sulbactam, Ticarcillin/Clavulanic acid, Piperacillin, Cefuroxime, Cefuroxime Axetil, Cefixime, Ceftriaxone, Cefepime, Aztreonam, Levofloxacin, Moxifloxacin, Chloramphenicol, Trimethopin, Meropenem, Minocycline, Tretracycline and Tigecycline. They represented five (5) antibiotic families including beta-lactams, quinolones, tetracyclines, phenicols and trimethoprim/Sulfonamides. The card was also analyzed by kinetic fluorescence measurement and the results was completed within 7.45-9.68 hours. The minimum concentration of imipenem was investigated using conventional method. *E. coli* (ATCC) 25922 *and Klebsiella pneumoniae* NCTC 13438 were used as reference strains for quality controls.

Supplementary Methods 2: Bacterial isolates for host range analysis

The bacteria tested during the host range analysis included bacterial reference strains such as methicillin-resistant Staphylococcus aureus National collection of Type Cultures (NCTC) 12493, *S. aureus* NCTC 1312, *S. aureus* NCTC 6571, *Esherischia coli* NCTC 13353, *E. Coli* NCTC 12202, *Klebsiella pneumoniae* NCTC 13438, *E. Coli* American Type Culture Collection (ATCC) 25922 and *Pseudomonas aeruginosa* ATCC 27850. The Multidrug resistant (MDR) bacterial strains included *Klebsiella pneumoniae* (KP) strains namely K2, K13, K15, K16, and K20 (KP20), K2 and K15, and one *Klebsiella oxytoca* strain K3, and one *E. Coli* strain K14. The bacterial isolates K13, K16 and K20 were reported as carbapenemase producers whereas K2 and K15 were extended-spectrum *beta*-lactamase producers. Observation of inhibition of bacterial growth by presence of clearing zone was considered as susceptibility of the bacteria to the phages.



Supplementary Figure 1: One-step growth curves of the precipitated phages isolated from Ruai and Rongai in February 2021. *Klebsiella* phage CPRSA had the highest burst size (610) and a latent period of 9 minutes.

Supplementary Table 1: The concentrations of the individual bacteriophages and phage cocktails used in formulating the phage cocktails.

Phage ID	Titer (PFU/ml)[a]	Concentration (MOI)[b]
CPRSA[c]	1.21 x 10^10	[1; 0.1; 0.001]
CPRSB[d]	4.4 x 10^9	[1; 0.1; 0.001]
2φ MA1[e]	2.87 x 10^9	1
2φ MA2[f]	2.87 x 10^9	0.1
2φ MA3[g]	2.87 x 10^9	0.001
3φ MB1[h]	2.6 x 10^9	1
3φ MB2[i]	2.6 x 10^9	0.1
3φ MB3[j]	2.6 x 10^9	0.001

[a]Plaque Forming Unit per milliliter, [b]Multiplicity Of Infection, [c & d]Individual *Klebsiella* phages, [e]Combination of two bacteriophages belonging to the family of *Myoviridae* at MOI 1. [f]Combination of two (2) bacteriophages belonging to the family of *Myoviridae* at MOI 0.1. [g]Combination of two (2) bacteriophages belonging to the family of *Myoviridae* at MOI 0.001. [h]Combination of 2φ MA and one belonging to the family of *Podoviridae* at MOI 1 [i]Combination of 2φ MA and one belonging to the family of *Podoviridae* at MOI 0.1 [j]Combination of 2φ MA and one belonging to the family of *Podoviridae* at MOI 0.001

Supplementary Table 2: The concentrations of the antibiotics and phage cocktails.

PHAGE/Antibiotic	Concentration	Phage Titer (PFU/ml)[a]
IMP1[b]	0.3 g/ml	-
TG1[c]	0.5856g/ml	-
IMP2[d]	0.3 g/ml	-
TG2[e]	0.5856/ml	-
2φ MA2[f]	0.1	6.3×10^9
2φ MA3[g]	0.001	6.3×10^9
3φ MB2[h]	0.1	2.67×10^9
3φ MB3[i]	0.001	2.67×10^9

[a]Plaque Forming Units per millilitre, [b]Single volume of imipenem, [c]Single volume of tigecycline, [d]Double volume of imipenem, [e]Double volume of tigecycline, [f]Combination of two bacteriophages belonging to the family of *Myoviridae* at MOI 0.1, [g]Combination of two bacteriophages belonging to the family of *Myoviridae* at MOI 0.001, [h]Combination of 2φ MA and one belonging to the family of *Podoviridae* at MOI 0.1, [i]Combination of 2φ MA and one belonging to the family of *Podoviridae* at MOI 0.001.