

TARGETING *E COLI* CELLS

TECHNICAL FIELD

The technology described herein relates to methods and compositions for targeting *E coli* cells, such as for treating or preventing an infection by *E coli* cells in human or animal subjects. The method, in an embodiment, comprises administering to the subject a particle cocktail comprising a plurality of different transduction particles to *E coli* cells. The method, in an embodiment, comprises administering to the subject a plurality of transduction particles that encode a nuclease for targeting the genomes of B2 phylogroup *E coli* cells.

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BACKGROUND

E coli infection has been identified as harmful or life-threatening in various settings, such as UTI infections, transplant patients, cancer patients and other patients that are immunocompromised or on immunosuppressant.

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Nuclease targeting of *E coli*, such as by means of CRISPR/Cas systems, has been proposed with delivery using transduction particles that can target the nuclease to *E coli* cells for chromosomal or episomal cutting, thereby killing cells or reducing their growth or proliferation. Suitable transduction particles are phage or engineered particles (such as non-self-replicative transduction particles) comprising capsids that contain nucleic acid encoding at least crRNAs or gRNAs (or additionally a Cas nuclease) for targeting. Advantageously, selective targeting can be achieved which is not possible using conventional antibiotics, such as broad-spectrum antibiotics. Such selective targeting can avoid killing of beneficial species and strains in treated patients. Indeed, disturbances of the microbiome with broad-spectrum antibiotics is a risk-factor in the prophylactic management of cancer patients at risk of febrile neutropenia.

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Bacteriophage (phage) therapy has been used prior to the broad availability of antibiotics, but has now re-gained interest due to the rise in bacterial antimicrobial resistance (AMR) combined with several successful individual case reports.

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Bacteriophages (phages) are a phylum of viruses that infect bacteria, and are distinct from the animal and plant viruses. Phages can have either a “lytic” life cycle, a “lysogenic” life cycle that can potentially become lytic, or a “non-lytic” life cycle. Phages replicating through the lytic cycle cause lysis of the host bacterial cell as a normal part of their life cycles. Phages replicating through the lysogenic cycles are called temperate phages, and can either replicate by means of the lytic life cycle and cause lysis of the host bacterium, or they can incorporate their DNA into the host bacterial DNA and become noninfectious prophages.

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The natural capability of phages to infect and kill bacteria, together with the specificity of the phage-bacterial interactions, is the basic phenomena on which the concept of phage therapy is built.

Therefore, phages that possess lytic life cycle are suitable candidates for phage therapy.

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International Patent Application No. WO 00/69269 discloses the use of certain phage strain for treating infections caused by Vancomycin-sensitive as well as resistant strains of *Enterococcus faecium*, and International Patent Application No. WO 01/93904 discloses the use of bacteriophage, alone or in combination with other anti-microbial means, for preventing or treating gastrointestinal diseases associated with the species of the genus *Clostridium*.

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US Patent Application No. 2002/0001590 discloses the use of phage therapy against multi-drug resistant bacteria, specifically methicillin-resistant *Staphylococcus aureus*, and International Patent Application No. WO 02/07742 discloses the development of bacteriophage having multiple host range.

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The use of phage therapy for the treatment of specific bacterial-infectious disease is disclosed, for example, in US Patent Application Nos. 2002/0044922; 2002/0058027 and International Patent Application No. WO 01/93904.

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US20160333348 describes the use of CRISPR/Cas systems delivered to host bacterial cells using phage as vectors.

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Amongst the several phylogroups of *E coli*, it has been observed that antibiotic-resistant (eg, fluoroquinolone (FQ)-resistant) and multi-drug resistant (MDR) strains are frequently found in the B2 phylogroup. B2 strains ST131 and ST1193 have been found that are associated with antibiotic resistance. ST131 is a globally dominant multidrug resistant clone associated with high rates in rUTI. ST131 is a major contributor to hospital- and community-acquired UTI, as well as *E coli* bloodstream infections and infections in companion animals and poultry. Originally identified in 2008, ST131 is associated with the worldwide spread of the CTX-M-15 extended spectrum β -lactamase (ESBL) resistance gene. ST131 is now strongly associated with multidrug resistance (MDR), including resistance to fluoroquinolones. Recent reports have also identified strains that are resistant to last-line carbapenems. Sequence type 1193 has recently emerged as a new, virulent and resistant lineage among fluoroquinolone resistant *E coli*.

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Classic antibiotics, such as FQ and broad-spectrum antibiotics are not, thus, sufficiently effective for combatting such infections. There is, therefore, a need to find alternative means to address these infections.

SUMMARY OF THE INVENTION

- 5 The invention provides means for treating or preventing B2 phylogroup *E coli* infections in humans and animals by combining the use of selective killing with nucleases targeted using specific types of transduction particles. The particles of the invention target by adhesion to LPS, LamB or Tsx, which has surprisingly been found highly advantageous for killing and inhibiting growth of B2 *E coli* cells of many different strains (including the potentially lethal ST131 and ST1193 strains). As exemplified
10 herein, surprisingly more than 10 different ST131 strains were killed (plaques formed) and more than 10 different ST1193 strains were killed (plaques formed).

- The invention finds utility, for example, to treat or prevent potentially life-threatening B2 phylogroup *E coli* infections in patients, such as in immunosuppressed, cancer, transplant and UTI patients, who are
15 susceptible to infection by B2 strains (and often by multiple different B2 strains). As demonstrated in the Examples, the invention is useful for preventing *E coli* B2 phylogroup bacteraemia (infection of the blood stream by *E coli*) in a subject.

To this end, the invention provides:-

20 In a First Configuration

In a First Aspect:-

A composition comprising a plurality of transduction particles for use in a method of treating or preventing an infection by *E coli* cells in a human or animal subject, wherein the method comprises administering the particles to the subject, wherein

- 25 (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
(b) the *E coli* cells are cells of *E coli* phylogroup B2; and
30 (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

In a Second Aspect:-

- A composition comprising a plurality of transduction particles for use in a method of treating or
35 preventing *E coli* bacteraemia in a human or animal subject, wherein the method comprises administering the particles to the subject, wherein

(a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of cells of

said *E coli*, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;

(b) the *E coli* cells are cells of *E coli* phylogroup B2; and

- 5 (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

In a Second Configuration

In a First Aspect:-

- 10 A method for treating or preventing an infection by *E coli* cells in a human or animal subject, the method comprising administering to the subject a plurality of transduction particles, wherein
- (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the
- 15 cells or reducing growth or proliferation of the cells in the subject;
- (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
- (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

- 20 In a Second Aspect:-

- A method for treating or preventing an infection by *E coli* cells in a human or animal subject, the method comprising administering to the subject a plurality of transduction particles, wherein
- (a) each particle comprises a nucleic acid encoding a crRNA or guide RNA that is operable with a Cas nuclease for chromosomal targeting in the cells, wherein the said administered particles contact the
- 25 cells and introduce therein the nucleic acid, wherein the crRNA or guide RNA is expressed and guides the Cas nuclease wherein the nuclease cuts the chromosomes of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
- (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
- (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety
- 30 selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

In a Third Aspect:-

- A method for treating or preventing *E coli* bacteraemia in a human or animal subject, the method comprising administering to the subject a plurality of transduction particles, wherein
- 35 (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of cells of said *E coli*, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing

the cells or reducing growth or proliferation of the cells in the subject;

(b) the *E coli* cells are cells of *E coli* phylogroup B2; and

(c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

5 In a Fourth Aspect:-

A method for treating or preventing *E coli* bacteraemia in a human or animal subject, the method comprising administering to the subject a plurality of transduction particles, wherein

(a) each particle comprises a nucleic acid encoding a crRNA or guide RNA that is operable with a Cas nuclease for chromosomal targeting in cells of said *E coli*, wherein the said administered particles

10 contact the cells and introduce therein the nucleic acid, wherein the crRNA or guide RNA is expressed and guides the Cas nuclease wherein the nuclease cuts the chromosomes of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;

(b) the *E coli* cells are cells of *E coli* phylogroup B2; and

(c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety
15 selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

In a Third Configuration

In a First Aspect:-

Use of a composition comprising a plurality of transduction particles in a method for treating or
20 preventing an infection by phylogroup B2 *E coli* cells in a human or animal subject, the method comprising administering to the subject the composition, wherein

(a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the
25 cells or reducing growth or proliferation of the cells in the subject; and

(b) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

In a Second Aspect:-

30 Use of a composition comprising a plurality of transduction particles in a method for treating or preventing an infection by phylogroup B2 *E coli* cells in a human or animal subject, the method comprising administering to the subject the composition, wherein

(a) each particle comprises a nucleic acid encoding a crRNA or guide RNA that is operable with a Cas nuclease for chromosomal targeting in the cells, wherein the said administered particles contact the
35 cells and introduce therein the nucleic acid, wherein the crRNA or guide RNA is expressed and guides the Cas nuclease wherein the nuclease cuts the chromosomes of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;

- (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
- (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

5 In a Third Aspect:-

Use of a composition comprising a plurality of transduction particles in a method for treating or preventing phylogroup B2 *E coli* bacteraemia in a human or animal subject, the method comprising administering to the subject the composition, wherein

- (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of cells of said *E coli*, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject; and
- (b) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

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In a Fourth Aspect:-

Use of a composition comprising a plurality of transduction particles in a method for treating or preventing phylogroup B2 *E coli* bacteraemia in a human or animal subject, the method comprising administering to the subject the composition, wherein

- (a) each particle comprises a nucleic acid encoding a crRNA or guide RNA that is operable with a Cas nuclease for chromosomal targeting in cells of said *E coli*, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the crRNA or guide RNA is expressed and guides the Cas nuclease wherein the nuclease cuts the chromosomes of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
- (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
- (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

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In a Fourth Configuration

30 In a First Aspect:-

A composition comprising a plurality of transduction particles for use in a method of treating or preventing an infection by *E coli* cells (optionally B2 phylogroup *E coli* cells) in a human or animal subject, wherein the method comprises administering the particles to the subject, wherein (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells,

- wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject; and

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(b) each particle comprised by the composition is a T-even phage capsid; optionally a capsid of a T2 phage, T2-like phage, RB69 phage or a RB69-like phage.

A composition comprising a plurality of different types of transduction particles, wherein each of said particles comprises a nucleic acid and said particles are capable of contacting *E coli* cells and introducing therein the nucleic acid, wherein

(a) said nucleic acid of each particle comprises a nucleotide sequence encoding a product of interest (POI), wherein the nucleic acid is capable of expressing the POI in an *E coli* cell;

(b) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx (optionally selected from LPS and Tsx) displayed on the surface of *E coli* cells; and

(c) said plurality of different types of transduction particles comprises (i) a first type of particles that comprise a LPS adhesion moiety; and (ii) a second type of particles that comprise a Tsx adhesion moiety.

In a Second Aspect:-

A method of treating or preventing sepsis, septicaemia or diarrhoea in a human or animal subject, the method comprising administering to the subject the composition of the invention, wherein the *E coli* cells comprise a strain of *E coli* that causes sepsis, septicaemia or diarrhoea in humans or animals.

In a Third Aspect:-

A method of treating or preventing infection of by *E coli* cells in a human or animal subject, wherein the method comprises administering the composition of the invention to the subject, wherein the infection is treated or prevented.

In a Fourth Aspect:-

A method of detecting the presence of *E coli* (optionally B2 phylogroup *E coli* cells) in a sample, the method comprising

(a) contacting the sample with a composition according to this Configuration; and

(b) detecting that *E coli* cells have been killed or the growth or proliferation thereof has been reduced.

In a Fifth Aspect:-

A method of detecting the presence of *E coli* (optionally B2 phylogroup *E coli* cells) in a sample, the method comprising

(a) contacting the sample with a composition according to this Configuration; wherein particles of the composition comprise a nucleic acid comprising or encoding a detectable label, wherein the said particles contact the cells and introduce therein the nucleic acid, wherein optionally the label is

expressed in the cells; and

(b) detecting that *E coli* cells comprising the label.

In a Sixth Aspect:-

- 5 A method for modifying the genomes of *E coli* cells, the method comprising contacting the cells with a composition of this Configuration, wherein nucleic acid encoding the POI is introduced into the cells thereby modifying the genomes of the cells.

10 BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Killing and growth inhibition activity of a composition of the invention when tested against *E coli* of several different phylogroups and different strains within each group (*E coli* taken from clinical samples); the composition was surprisingly highly effective at killing across many phylogroups (and particularly in the B2 phylogroup, where multiple strains – including those known to be potentially life-threatening and related with antibiotic resistance – were killed or whose growth was effectively inhibited). The 6 negatives comprised non *E coli* and non FQ resistant samples.

Figure 2: Phylo-grouping of strains used in the study and showing particularly advantageous and extensive targeting of many clinical B2 phylogroup strains.

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Figure 3: A) CRISPR-Cas driven elimination of an abbreviated panel of 82 *E. coli* clinical isolates by conjugation of CGV-EcCAS and empty vector. The conjugation efficiency was determined by spotting a dilution series of the conjugation reaction on LB agar supplemented with antibiotics and calculated as CFU/ml. The limit of detection (LOD) of this assay was 200 CFU/mL. The experiment was carried out in triplicates and is indicated by dots. CGV-EcCAS conjugation experiments indicated by arrow; empty vector conjugations indicated above the dotted line labelled LOD. B) Fraction of CRISPR-armed phageTM (CAP) and WT phage during co-culture with a host strain susceptible to both phages. CAP $\alpha 15.2$ increases its relative abundance compared to the WT phage from 7% to 86% over two consecutive passages. C) CAP $\alpha 20.4$ outcompeted WT $\alpha 20$ by increasing its relative abundance during co-culture with the common target *E. coli* strain b230 from 10% to 68% over four consecutive passages. B-C) The ratio of CAP and WT phage during co-culture with a host strain (*E. coli* b230) susceptible to $\alpha 15.2$, $\alpha 15$, $\alpha 20.4$ and $\alpha 20$. CAP $\alpha 15.2$ increase its relative abundance compared to the WT phage from 7% to 86% (G) while CAP $\alpha 20.4$ outcompeted WT $\alpha 20$ from 10% to 68% (H).

35 **Figure 4:** Specificity evaluation of SNIPR001 and individual CAPs against a panel of clinically relevant bacteria and *E. coli* strain b2480 (positive control) showing no off-target effects. Positive values represent bacterial growth (absence of observed killing), while negative values indicate

bacterial killing following phage treatment within the assessed time-period. All values indicated as means of four biological replicates with standard deviations, measuring growth over a 4-hour period, measured in CFU/mL.

- 5 **Figure 5:** A) An unrooted phylogenetic tree of the JMI strains displaying a clinical panel of 382 *E. coli* strains encompassing nine phylogroups and 118 MLSTs. Plaquing data reflects a single plaquing replicate. One strain, *E. coli* b4038, with a long branch has been truncated to 37% of the original length. Phylogenetic distance scale indicated below the phylogenetic tree, as computed by MASH. B) A spot assay was used to analyze the efficacy of SNIPR001 against the clinical panel of 382 *E. coli* strains (from JMI, North Liberty, IA, USA) isolated from bloodstream infections and the internal 429 *E. coli* strain panel. The spot assay was conducted as two independent experiments, with results presented as mean $\pm$ SD values. The standard deviation shown is based on the discrepancy of results between the two runs, calculated as the average absolute deviation of the mean prevalence of a given spotting type, normalized to the panel size. C) Coverage of SNIPR001 does not depend on antibiotic resistant phenotypes; consequently, SNIPR001 targets >90% of *E. coli* strains that are carbapenem-resistant, extended-spectrum β -lactamase (ESBL)-producing or multi-drug resistant (MDR), and 89% of fluoroquinolone resistant *E. coli* strains. Numbers indicated in each green or grey bar indicates number of bacteria susceptible or resistant to SNIPR001, respectively, for each resistance category. D) A midpoint rooted phylogenetic tree of the 72 fluoroquinolone resistant *E. coli* strains isolated from fecal samples of hematological cancer patients. 67 of the 72 strains are susceptible to at least one of the four CAPs in SNIPR001. E) Redundancy distribution showing 82% of the fluoroquinolone resistant *E. coli* strains (n = 72) from panel D are targeted by at least 2 different CAPs.

- Figure 6:** A) CAP recovery in minipigs feces after a single p.o. dose of 2×10^{12} PFU of SNIPR001 (n = 8, shown in green) or vehicle (n = 6, shown in grey) over one week with daily sampling. Trend lines indicate average recovered phage in PFU/g feces, dots indicate individual measurement points. LOD of 33 PFU/g feces indicated by the dotted line. B) CAP recovery in minipig feces after a single p.o. dose of 2×10^{12} PFU of a single CAP (n = 8 minipigs received either $\alpha 15.2$, $\alpha 20.4$ or $\alpha 51.5$, n = 7 minipigs received $\alpha 48.4$) over one week with daily sampling. Trend lines indicate average recovery, while points indicate individual measurements. Recovery was measured in PFU/g feces. LOD of 33 PFU/g feces is indicated by the dotted line. C) CAP recovery in mouse feces 8h, 24h, and 48h after the start of treatment with three times daily administration of varying doses of SNIPR001 (n = 10 for low, medium, and high, indicated in green), vehicle (n = 10, indicated in grey), or gentamicin (n = 4, indicated in grey). Recovery is measured in PFU/g feces, LOD of 371 PFU/g feces is indicated by the dotted line. D) *E. coli* b17 recovery in mouse feces indicates increased SNIPR001 effect with increased dose. Statistical analyses were performed using two-sided Kruskal-Wallis tests for

comparison of the SNIPR001-treated groups, two-sided Mann-Whitney U test for comparison of treated groups with vehicle. $P < 0.05 = *$, $0.01 = **$, $0.001 = ***$, FDR corrected using Holm's method separately for each day. Recovery is measured in CFU/g feces, with a limit of detection of 371 CFU/g feces. Animals that have begun SNIPR001 treatment are indicated in green, others in grey. E) *E. coli* b17 recovery in mouse feces 8h and 24h after the start of treatment with three times daily administration of CAPs $\alpha 15.2$, $\alpha 20.4$, $\alpha 48.4$ or $\alpha 51.5$ and in combination as SNIPR001 confirming synergy of the CAPs.

DETAILED DESCRIPTION

The invention finds application to combat harmful or life-threatening B2 *E. coli* infections in various settings, such as UTI infections, transplant patients, cancer patients and other patients that are immunocompromised or on immunosuppressant.

Cancer treatment continues to advance and survival rates for people with hematological malignancies are increasing. However, this population is immunocompromised and chemotherapy regimens cause bone-marrow suppression and gastrointestinal mucositis with associated increased intestinal permeability. Translocation of gut bacteria, including *E. coli*, from the gastrointestinal tract is a frequent cause of bloodstream infections (BSIs). The mortality-related to BSIs can be up to 50%; thus, antimicrobial prophylaxis is applied in people at risk of febrile neutropenia. There are no approved therapies for the prevention of BSIs in patients with hematological cancers, yet fluoroquinolones are used off-label in the United States. This antibiotic prophylaxis practice is at odds with the emerging paradigm that maintaining a normal microbiome is important for upholding immunological tonus potentially benefiting the outcome of oncology treatments. Indeed, disturbances of the microbiome with broad-spectrum antibiotics is a risk-factor in the prophylactic management of patients at risk of febrile neutropenia. Beyond the side effects of fluoroquinolones, including safety warnings and precautions, bacterial resistance is rising and approaching 60% in USA.

In immunocompromised patients with hematological malignancies at risk of developing neutropenia, *E. coli* is responsible for 25.1-30% of all bacteraemia cases with a 35.8% 90-day mortality rate. Moreover, up to 65% of *E. coli* isolated as the causative pathogen from BSIs in patients with hematological cancers undergoing hematopoietic stem cell transplantation (HSCT) were resistant to fluoroquinolones. Accordingly, novel narrow-spectrum treatment and prophylactic options are needed to prevent infections in these vulnerable patients. The invention addresses this need.

To this end, the invention provides compositions, methods and uses according to the above Configurations. There is, thus, provided the following description with numbered Embodiments.

1. A composition comprising a plurality of transduction particles for use in a method of treating or preventing an infection by *E coli* cells in a human or animal subject, wherein the method comprises administering the particles to the subject, wherein
 - 5 (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
 - (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
 - 10 (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of phylogroup B2 *E coli* cells.

Optionally,

- (i) each particle comprises a phage capsid containing the nucleic acid;
- 15 (ii) the particles of the composition target a plurality of different *E coli* genes, optionally selected from essential and virulence genes; and
- (iii) the composition comprises
 - A: particles which comprise a LPS adhesion moiety and a LamB adhesion moiety;
 - B: particles which comprise a LPS adhesion moiety and a Tsx adhesion moiety; or
 - 20 C: : particles which comprise a Tsx adhesion moiety but is devoid of LamB and LPS adhesion moieties.

2. A method for treating or preventing an infection by *E coli* cells in a human or animal subject, the method comprising administering to the subject a plurality of transduction particles, wherein the method comprises administering the particles to the subject, wherein
 - 25 (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
 - 30 (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
 - (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

Optionally,

- 35 (i) each particle comprises a phage capsid containing the nucleic acid;
- (ii) the particles of the composition target a plurality of different *E coli* genes, optionally selected from essential and virulence genes; and

(iii) the composition comprises

A: particles which comprise a LPS adhesion moiety and a LamB adhesion moiety;

B: particles which comprise a LPS adhesion moiety and a Tsx adhesion moiety; or

C: : particles which comprise a Tsx adhesion moiety but is devoid of LamB and LPS adhesion

5 moieties.

Optionally, the genomic DNA is chromosomal DNA of the cells. Additionally or alternatively, the genomic DNA is plasmid DNA of the cells.

10 Optionally, each particle comprises a nucleic acid encoding a nuclease for chromosomal targeting, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts the chromosomes of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject.

15 The human may be a male or female. The human may be an adult or child. The human may be 18 years of age or older, eg, 40, 50, 60, 70 80 or older. The human may be younger than 18, eg, a teenager, eg, a baby, eg, up to 5 years of age, eg, up to 2 years of age. The animal may be a livestock or companion animal eg, a dog or cat). The animal may be a bird (eg, a poultry bird, eg, a chicken, turkey or duck, preferably a chicken), cow, sheep, goat or pig (eg, a neonatal swine or a
20 swine under 6 months of age).

The infection may be a bloodstream infection. The infection may be a nosocomial infection.

In an example, each adhesion moiety is a tail fibre protein. In an example, each particle comprises a
25 phage tail fibre that comprises or is fused to a said adhesion moiety. In an example, each adhesion moiety is an antibody fragment, eg, an antibody single variable domain. In an example, each adhesion moiety is a nanobody. In an example, each adhesion moiety comprises an antibody binding site that is capable of binding to the cognate moiety. For example, each adhesion moiety comprises an antibody single variable domain (ie, a dAb), such as a nanobody. In an example, each particle
30 comprises one or more phage tail fibres or spikes, each fibre or spike comprising a said adhesion moiety.

For example, at least 2, 3 or 4 different types of said transduction particle are administered to the subject and each type comprises one or a plurality of types of tail fibres comprising adhesion moieties,
35 wherein the other particle types do not comprise said one or plurality of tail fibre types. In an example, each type of said plurality of tail fibre types differs from the other types by the type of adhesion moiety it comprises.

Optionally, the particles comprise adhesion moieties for binding to LPS, LamB and Tss. Optionally, the particles comprise adhesion moieties for binding to LamB and Tss. Optionally, the particles comprise adhesion moieties for binding to LPS and Tss. Optionally, the particles comprise adhesion moieties for binding to LPS and LamB.

In Gram-negative bacteria, the peptidoglycan layer is relatively thin and is located inward of the outer membrane, the major component of the cell wall. These two layers are connected by Braun's lipoproteins. The outer membrane is a sophisticated structure composed of a lipid bilayer ornamented with proteins, polysaccharides and lipids; the latter two molecules form the LPS layer. LPSs are complexes that consist of three parts: lipid A, the core polysaccharide and the O-polysaccharide. Lipid A is, in general, composed of fatty acids attached to glucosamine phosphate disaccharides. The core polysaccharide is connected to the lipid A through a ketodeoxyoctonate linker. The core polysaccharide and the O-polysaccharide (O-chain or O-antigen) contain several units of sugar residues extending outward to the outer membrane. Cells that contain all three components of the LPS are denominated as smooth (S) type and those that lack the O-polysaccharide portion are distinguished as rough (R) type.

Optionally, the LPS is smooth LPS or rough LPS.

For example, the particles comprise at least one type of particle whose adhesion moiety is capable of binding to O-antigen of LPS.

E coli is a very versatile species for which diversity has been explored from various perspectives highlighting, for example, phylogenetic groupings, pathovars as well as a wide range of O serotypes. The highly variable O-antigen, the most external part of the lipopolysaccharide component of the outer membrane of *E coli*, is linked to the innermost lipid A through the core region of LPS of which 5 different structures, denominated K-12, R1, R2, R3 and R4, have been characterized so far. Phylogroups B2 and C strains are mainly dominated by the R1 type. Strains within phylogroup B2 may carry a K-12 core, eg, belonging to the complex STc131, one of the major clone of extra-intestinal pathogenic *E coli* (ExPEC) strains.

Preferably, the LPS comprises a R1 core region. In an example, the LPS comprises a R2 core region. In an example, the LPS comprises a R3 core region. In an example, the LPS comprises a R4 core region. In an example, the LPS comprises a K-12 core region.

Optionally, the LamB comprises the amino acid of SEQ ID NO: 1 or an amino acid sequence that is at least 70, 80, 90 or 95% identical to SEQ ID NO: 1. Optionally, the Tsx comprises the amino acid of SEQ ID NO: 2 or an amino acid sequence that is at least 70, 80, 90 or 95% identical to SEQ ID NO: 2. Optionally, the LamB is encoded by the nucleotide sequence of SEQ ID NO: 3 or an amino acid sequence that is at least 70, 80, 90 or 95% identical to SEQ ID NO: 3. Optionally, the Tsx is encoded by the nucleotide sequence of SEQ ID NO: 4 or an amino acid sequence that is at least 70, 80, 90 or 95% identical to SEQ ID NO: 4.

The *Escherichia coli* tsx gene encodes an integral outer-membrane protein (Tsx) that functions as a substrate-specific channel for deoxynucleosides and the antibiotic albicidin. In an example, the Nucleoside-specific channel-forming protein Tsx of *E. coli* has a Uniprot Accession Number of P0A927 or is a homologue thereof. In an example, the maltose outer membrane porin (maltoporin) LamB of *E. coli* has a Uniprot Accession Number of P02943 or is a homologue thereof.

Homologue: A gene, nucleotide or protein sequence related to a second gene, nucleotide or protein sequence by descent from a common ancestral DNA or protein sequence. The term, homologue, may apply to the relationship between genes separated by the event of or to the relationship between genes separated by the event of genetic duplication.

In an embodiment, the *E. coli* cells comprise UPEC *E. coli*. In an embodiment, the *E. coli* cells comprise intestinal pathogenic *E. coli* (ExPEC) cells.

3. The composition or method of Embodiment 1 or 2 respectively, wherein the method is for treating or preventing infection of the subject by an *E. coli* strain selected from the group ST131, ST1193, ST648, ST315, ST405, ST361, ST88 and ST453.

In a preferred example, the strain is ST1193. In another preferred example, the strain is ST131.

4. The composition or method of any preceding Embodiment, wherein the method is for treating or preventing infection of the subject by a plurality of different phylogroup B2 strains of *E. coli*; optionally wherein the plurality comprises *E. coli* ST131 and ST1193 cells.

E. coli ST131 and/or ST1193 have been found to be virulent and associated with fluoroquinolone resistance. As shown in the Example section herein, the invention is useful for treating or preventing infection by such strains. Optionally, said plurality of strains comprises *E. coli* ST131 and/or ST1193 strains. Optionally, said plurality of strains comprises fluoroquinolone-resistant strains. For example, said plurality of strains comprises *E. coli* ST131 and/or ST1193 fluoroquinolone-resistant strains.

E. coli strains B2-ST73 (CH24-30); B2-ST73 (CH24-103); B2-ST131 (CH40-30); B2-ST141 (CH52-5); B2-ST372 (CH103-9); B2-ST404 (CH14-27); B2-ST404 (CH14-807) and B2-ST1193 (CH14-64)

have been found in UTI settings. In an embodiment (eg, wherein the subject is suffering from or at risk of UTI), the B2 *E coli* comprise one or more strains selected from B2-ST73 (CH24-30); B2-ST73 (CH24-103); B2-ST131 (CH40-30); B2-ST141 (CH52-5); B2-ST372 (CH103-9); B2-ST404 (CH14-27); B2-ST404 (CH14-807) and B2-ST1193 (CH14-64).

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5. The composition or method of any preceding Embodiment, wherein the subject is a transplant or cancer patient (optionally a haematological cancer patient), or wherein the patient is suffering from or at risk of a urinary tract infection (UTI); and optionally wherein the transplant is a solid organ or stem cell transplant (optionally a haematopoietic cell transplant) or wherein the

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transplant is a transplant of a medical device.

For example, the subject is a haematological cancer patient suffering from neutropenia. For example, the subject is a haematopoietic stem cell transplant patient.

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A suitable medical device may be, eg, heart device (eg, ventricular assist device, such as a left ventricular assist device (LVAD)), a catheter (eg, a biliary catheter) or a prosthesis (eg, a joint) prosthesis.

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In an example, the patient is suffering from a sequestered B2 phylogroup *E coli* infection. In an example, the *E coli* are sequestered B2 phylogroup *E coli*.

For example, the subject is suffering from or at risk of acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis or gastroenteritis caused by B2 phylogroup *E coli*. For example, the subject is a male human prostate surgery patient.

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6. The composition or method of any preceding Embodiment, wherein the method is carried out prior to the subject receiving a transplant.

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For example, the transplant is a solid organ or stem cell transplant (optionally a haematopoietic cell transplant).

7. The composition or method of any preceding Embodiment, wherein the B2 *E coli* cells comprise a strain of *E coli* that causes sepsis, septicaemia or diarrhoea in humans.

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Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 is a human pathogen responsible for outbreaks of bloody diarrhoea and haemolytic uremic syndrome (HUS) worldwide. Conventional antimicrobials trigger an SOS response in EHEC that promotes the release of the potent Shiga toxin

that is responsible for much of the morbidity and mortality associated with EHEC infection. Cattle are a natural reservoir of EHEC, and approximately 75% of EHEC outbreaks are linked to the consumption of contaminated bovine-derived products. EHEC causes disease in humans but is asymptomatic in adult ruminants. Characteristics of *E. coli* serotype O157:H7 (EHEC) infection includes abdominal cramps and bloody diarrhoea, as well as the life-threatening complication haemolytic uremic syndrome (HUS). Currently there is a need for a treatment for EHEC infections (Goldwater and Bettelheim, 2012). The use of conventional antibiotics exacerbates Shiga toxin-mediated cytotoxicity. In an epidemiology study conducted by the Centers for Disease Control and Prevention, patients treated with antibiotics for EHEC enteritis had a higher risk of developing HUS (Slutsker et al., 1998). Additional studies support the contraindication of antibiotics in EHEC infection; children on antibiotic therapy for hemorrhagic colitis associated with EHEC had an increased chance of developing HUS (Wong et al., 2000; Zimmerhackl, 2000; Safdar et al., 2002; Tarr et al., 2005). Conventional antibiotics promote Shiga toxin production by enhancing the replication and expression of *stx* genes that are encoded within a chromosomally integrated lambdoid prophage genome. The approach of the present invention may rely on nuclease cutting of target cell genomic DNA. *Stx* induction also promotes phage-mediated lysis of the EHEC cell envelope, allowing for the release and dissemination of Shiga toxin into the environment (Karch et al., 1999; Matsushiro et al., 1999; Wagner et al., 2002). Thus, advantageously, the invention provides alternative means for treating B2 phylogroup EHEC in human and animal subjects. In an example, the subject (eg, a human) is suffering from or at risk of haemolytic uremic syndrome (HUS), eg, the subject is suffering from an *E. coli* infection, such as an EHEC *E. coli* infection.

8. The composition or method of any preceding Embodiment for preventing haemolytic uremic syndrome (HUS), a UTI infection, sepsis, septicaemia or diarrhoea in the subject.

25 The composition or method may be for treating or preventing a blood stream infection by pathogenic B2 phylogroup *E. coli* cells in the subject.

9. The composition or method of any preceding Embodiment, wherein each particle comprises a phage capsid containing the nucleic acid; optionally wherein the capsid comprises capsid proteins of a T-even (optionally T4) or lambda phage.

As is known to the skilled addressee, transduction particles are operable to infect their cognate host cells to introduce therein nucleic acid by transduction.

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10. The composition or method of any preceding Embodiment, wherein each particle is a phage (optionally a lytic phage) or packaged phagemid.

11. The composition or method of any preceding Embodiment, wherein at least 2, 3 or 4 different types of transduction particle are administered to the subject.

For example, each particle types comprises a type of adhesion moiety or collection of adhesion moiety types that differs from the other types of particles.

10 In an example, 2 different types of transduction particle are administered to the subject. In an example, 3 different types of transduction particle are administered to the subject. In an example, 4 different types of transduction particle are administered to the subject. In an example, 5 different types of transduction particle are administered to the subject. In an example, 6 different types of transduction particle are administered to the subject.

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12. The composition or method of any preceding Embodiment, wherein a first type of transduction particle and a second type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to a first cognate moiety selected from the group LPS, LamB and Tsx displayed on B2 *E coli*, and the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to a second cognate moiety selected from said group, wherein the first and second adhesion moieties different from each other.

For example, the first and second adhesion moieties different from each other by their tail fibres, optionally wherein the first adhesion moiety is cognate to LPS and the second moiety is cognate to LamB; or optionally wherein the first adhesion moiety is cognate to LPS and the second moiety is cognate to Tsx; optionally wherein the first adhesion moiety is cognate to Tsx and the second moiety is cognate to LamB.

30 For example, each particle comprises a phage capsid containing a said nucleic acid; wherein at least 2, 3 or 4 different types of transduction particle are administered to the subject; and wherein a first type of transduction particle and a second type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to a first cognate moiety selected from the group LPS, LamB and Tsx displayed on B2 *E coli*, and the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to a second cognate moiety selected from said group, wherein the first and second adhesion moieties different from each other.

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For example, each particle comprises a phage capsid containing a said nucleic acid; wherein at least 2, 3 or 4 different types of transduction particle are administered to the subject; and wherein a first type of transduction particle and a second type of transduction particle are administered to the subject,
5 wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to LPS displayed on B2 *E coli*, and the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to LamB displayed on B2 *E coli*.

For example, each particle comprises a phage capsid containing a said nucleic acid; wherein at least 2,
10 3 or 4 different types of transduction particle are administered to the subject; and wherein a first type of transduction particle and a second type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to Tsx displayed on B2 *E coli*, and the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to LamB displayed on B2 *E coli*.

15 For example, each particle comprises a phage capsid containing a said nucleic acid; wherein at least 2, 3 or 4 different types of transduction particle are administered to the subject; and wherein a first type of transduction particle and a second type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and
20 binding to LPS displayed on B2 *E coli*, and the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to Tsx displayed on B2 *E coli*.

For example, each particle comprises a phage capsid containing a said nucleic acid; wherein at least 3 different types of transduction particle are administered to the subject; and wherein a first type of
25 transduction particle, a second type of transduction particle and a third type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to LPS displayed on B2 *E coli*, the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to LamB displayed on B2 *E coli*, and the third type of particle comprises a second adhesion moiety that is capable of
30 recognising and binding to Tsx displayed on B2 *E coli*.

For example, each particle comprises a phage capsid containing a said nucleic acid; wherein at least 4 different types of transduction particle are administered to the subject; and wherein a first type of
35 transduction particle, a second type of transduction particle, a third type of transduction particle and a fourth type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to LPS displayed on B2 *E coli*, the second type of particle comprises a second adhesion moiety that is capable of recognising

and binding to LamB displayed on B2 *E coli*, the third type of particle comprises a second adhesion moiety that is capable of recognising and binding to Tsx displayed on B2 *E coli*, and the fourth type of particle comprises a second adhesion moiety that is capable of recognising and binding to LPS displayed on B2 *E coli*, wherein the adhesion moieties of said particles are different from each other.

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For example, each particle comprises a phage capsid containing a said nucleic acid; wherein at least 4 different types of transduction particle are administered to the subject; and wherein a first type of transduction particle, a second type of transduction particle, a third type of transduction particle and a fourth type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to LPS displayed on B2 *E coli*, the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to LamB displayed on B2 *E coli*, the third type of particle comprises a second adhesion moiety that is capable of recognising and binding to Tsx displayed on B2 *E coli*, and the fourth type of particle comprises a second adhesion moiety that is capable of recognising and binding to LamB displayed on B2 *E coli*, wherein the adhesion moieties of said particles are different from each other.

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For example, each particle comprises a phage capsid containing a said nucleic acid; wherein at least 4 different types of transduction particle are administered to the subject; and wherein a first type of transduction particle, a second type of transduction particle, a third type of transduction particle and a fourth type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to LPS displayed on B2 *E coli*, the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to LamB displayed on B2 *E coli*, the third type of particle comprises a second adhesion moiety that is capable of recognising and binding to Tsx displayed on B2 *E coli*, and the fourth type of particle comprises a second adhesion moiety that is capable of recognising and binding to Tsx displayed on B2 *E coli*, wherein the adhesion moieties of said particles are different from each other.

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13. The composition or method of Embodiment 12, wherein the first and second cognate moieties are different from each other.

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In an alternative, the first and second cognate moieties are identical. In an alternative, the first and second cognate moieties are LPS. In an alternative, the first and second cognate moieties are Tsx. In an alternative, the first and second cognate moieties are LamB.

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14. The composition or method of any preceding Embodiment, wherein the nucleic acid of each particle comprises a nucleotide sequence (N1) encoding said nuclease, wherein each particle is a synthetic T-even phage (optionally a T4 phage) comprising an insertion of N1 into the genome of

the phage, wherein the region is between the *pin* (protease inhibitor) gene and the *iPPI* (internal protein) gene.

Optionally, the phage is

- 5 (a) a synthetic T-even (eg, a T4) phage that comprises a deletion of DNA from, and/or an insertion of heterogeneous DNA into, a region of the genome of the phage corresponding to a region between coordinates
- (i) 1887 and 8983;
 - (ii) 2625 and 8092;
 - 10 (iii) 1904 and 8113;
 - (iv) 2668 and 7178;
 - (v) 7844 and 11117;
 - (vi) 8643 and 10313;
 - (vii) 9231 and 13383;
 - 15 (viii) 9480 and 12224;
 - (ix) 8454 and 17479; or
 - (x) 9067 and 16673;

wherein coordinates are with reference to wild-type T4 phage genome (SEQ ID NO: 5);

- 20 15. The composition or method of any preceding Embodiment, wherein said nuclease is a guided nuclease, optionally a Cas, meganuclease, zinc finger nuclease or TALEN.

16. The composition or method of any preceding Embodiment, wherein the nuclease is a Type I, II, III, IV, V or VI nuclease, optionally a Cas9 or a Cas3.

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17. The composition or method of any preceding Embodiment, wherein at least 1×10^7 PFU of particles are administered to the subject.

In an example, 1×10^8 to 1×10^{13} PFU of particles are administered to the subject. In an example, 1×10^8 to 1×10^{12} PFU of particles are administered to the subject. In an example, 1×10^{10} to 1×10^{12} PFU of particles are administered to the subject.

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18. The composition or method of any preceding Embodiment, wherein the particles are administered to the subject at an MOI (multiplicity of infection) of at least 0.01.

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Optionally, the particles are administered to the subject at an MOI of no more than 1. Optionally, the particles are administered to the subject at an MOI from 0.001 to 1. Optionally, the particles are

administered to the subject at an MOI from 0.01 to 1. Optionally, the particles are administered to the subject at an MOI from 0.1 to 1.

19. The composition or method of any preceding Embodiment, wherein the strain or at least one
5 strain is an antibiotic-resistant or MDR strain; and/or wherein the strain or at least one strain is a B2-I strain.

For example, the strain or at least one strain is a strain selected from B2-I (STc131), B2-II, B2-IX, and B2-VI.

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For example, at least one MDR strain is resistant to fluoroquinolone and the strain is a beta-lactamase (ESBL)-producing *E coli*.

20. The composition or method of Embodiment 19, wherein the antibiotic is fluoroquinolone
15 (optionally levofloxacin), carbapenem or vancomycin; and/or wherein the *E coli* are beta-lactamase (ESBL)-producing *E coli*.

Optionally, the antibiotic is selected from ciprofloxacin (eg, Cipro™), gemifloxacin (eg, Factive™), levofloxacin (eg, Levaquin™), moxifloxacin (eg, Avelox™), and ofloxacin.

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For example, the *E coli* produce CTX-M-15. CTX-M-15 is the most abundant enzyme in ESBL-producing *E. coli* causing human infections.

- Preferably, the antibiotic is fluoroquinolone (FQ). For example, the FQ is levofloxacin.
25 Levofloxacin, sold under the brand name Levaquin™ among others, is an antibiotic medication. It is used to treat a number of bacterial infections including acute bacterial sinusitis, pneumonia, urinary tract infections, chronic prostatitis, and some types of gastroenteritis. Levofloxacin prophylaxis is recommended to prevent gram-negative bloodstream infections (BSIs) in patients with prolonged chemotherapy-induced neutropenia. However, increasing fluoroquinolone resistance may decrease
30 the effectiveness of this approach (eg, Clin Infect Dis. 2021 Oct 5;73(7):1257-1265. doi: 10.1093/cid/ciab404, "Colonization With Fluoroquinolone-Resistant Enterobacterales Decreases the Effectiveness of Fluoroquinolone Prophylaxis in Hematopoietic Cell Transplant Recipients, Michael J Satlin *et al*). This study found that in the patients tested, nearly one-third of hematopoietic cell transplantation (HCT) recipients with pretransplant fluoroquinolone-resistant *Enterobacterales*
35 (FQRE) colonization developed gram-negative bloodstream infections (BSIs) while receiving levofloxacin prophylaxis, and infections were typically caused by their colonizing strains. In contrast, levofloxacin prophylaxis was highly effective in patients not initially colonized with FQRE. The

authors found that 23% of patients admitted for HCT were colonized with FQRE and *E. coli* was the predominant species. Patients with hematologic malignancies who receive intensive chemotherapy, including those undergoing hematopoietic cell transplantation (HCT), frequently develop severe neutropenia and gastrointestinal mucositis, placing them at high risk of developing bloodstream infections (BSIs) from gram-negative enteric bacteria (*Enterobacterales*). Neutropenic patients often suffer severe consequences from BSIs caused by *Enterobacterales*, with mortality rates as high as 15%–20%. Moreover, many fluoroquinolone-resistant *Enterobacterales* (FQRE) also harbor extended-spectrum β -lactamases (ESBLs); thus, breakthrough infections that occur despite fluoroquinolone prophylaxis may be resistant to first-line antimicrobial therapies for fever and neutropenia. Finally, adverse effects of fluoroquinolones have become increasingly apparent, including *Clostridioides difficile* infection, aortic dissection and rupture, dysglycemia, tendinopathy, QT interval prolongation, and mental status changes. Thus, fluoroquinolones should only be administered to patients when they are likely to provide clinical benefit to justify these potential adverse effects. Thus, Although fluoroquinolones may decrease the risk of gram-negative BSI in many patients, those who are colonized with FQRE may not benefit from fluoroquinolone prophylaxis.

The high rate and absence of risk factors for FQRE colonization suggest that FQRE are prevalent in the community. Indeed, a study of urinary isolates among outpatients in the United States demonstrated that 12% of *E. coli* isolates from young women and 29% of *E. coli* isolates from elderly women were fluoroquinolone resistant. A surveillance study of 1831 urinary *E. coli* isolates from 2017 found that one-quarter were FQ resistant. Furthermore, 13%–16% of men undergoing transrectal prostate biopsies were found to be colonized with fluoroquinolone-resistant *E. coli*. Nearly one-half of fluoroquinolone-resistant *E. coli* isolates in the study were ST131, a common sequence type that has spread throughout the world and whose isolates are frequently fluoroquinolone resistant and ESBL producers.

Bacteraemia caused by extended-spectrum β -lactamase (ESBL)-producing *Enterobacteriaceae* (ESBL-E), such as *E. coli*, is associated with inadequate empirical therapy and substantial mortality in neutropenic patients (see eg, “Colonization With Levofloxacin-resistant Extended-spectrum β -Lactamase-producing *Enterobacteriaceae* and Risk of Bacteremia in Hematopoietic Stem Cell Transplant Recipients”, Satlin MJ et al, Clin Infect Dis. 2018 Nov 13;67(11):1720-1728. doi: 10.1093/cid/ciy363). The study found that HSCT recipients who are colonized with levofloxacin-resistant ESBL-E pre-transplant and receive levofloxacin prophylaxis have high rates of bacteraemia from their colonizing strain during neutropenia. In this single-centre study of 312 HSCT recipients, it was found that 10% of patients were colonized with ESBL-E prior to their transplant. Nearly one-third of patients with pre-transplant ESBL-E colonization developed subsequent ESBL-E bacteraemia while neutropenic after their transplant, compared to <1% of patients who were not initially colonized

with ESBL-E. Furthermore, the bloodstream and gastrointestinal ESBL-E had identical MLST and PFGE profiles in all cases, suggesting that these patients developed bacteraemia from their colonizing isolates.

- 5 In an example, the composition or method of the invention is for preventing the translocation of B2 phylogroup *E coli* from the gastrointestinal tract to the blood stream of the subject, thereby preventing or reducing bacteraemia in the patient.

- 10 In an example, the composition or method of the invention is for preventing the translocation of B2 phylogroup *E coli* from the urinary tract to the blood stream of the subject, thereby preventing or reducing bacteraemia in the patient.

- In an example, the *E coli* are comprised by the gastrointestinal tract of the subject. Optionally, in these examples, the composition is administered orally to the subject.

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In another example, the *E coli* are comprised by the urinary tract of the subject. For example, the infection is a kidney, bladder or urethra infection. Optionally, in these examples, the composition is administered to the urinary tract of the subject, such as by a catheter.

- 20 21. The composition or method of any preceding Embodiment, wherein
 (i) the nuclease is a Cas,
 (ii) each particle comprises a phage capsid containing the nucleic acid; and
 (iii) wherein a first type of transduction particle and a second type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety
 25 that is capable of recognising and binding to a first cognate moiety selected from the group LPS, LamB and Tsx displayed on B2 strain *E coli*, and the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to a second cognate moiety selected from said group, wherein the first and second adhesion moieties different from each other.
- 30 22. A method for treating or preventing an infection by *E coli* cells in a human or animal subject, the method comprising administering to the subject a plurality of transduction particles, wherein
 (a) each particle comprises a nucleic acid encoding a crRNA or guide RNA that is operable with a Cas nuclease for chromosomal targeting in the cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the crRNA or guide RNA is
 35 expressed and guides the Cas nuclease wherein the nuclease cuts the chromosomes of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
 (b) the *E coli* cells are cells of *E coli* phylogroup B2; and

(c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.

Each nucleic acid preferably encodes a plurality of different crRNAs comprising spacer sequences that target *E coli* chromosomal genes. For example, Each nucleic acid preferably encodes a plurality of different crRNAs comprising spacer sequences that target 2, 3 or 4 *E coli* chromosomal genes selected from fimH, bolA, rpoH, lptA and murA.

Optionally, each crRNA or guide RNA comprises a spacer that targets an *E coli* gene selected from the group fimH, bolA, rpoH, lptA and murA. Optionally, each nucleic acid encodes a plurality of different crRNAs or guide RNAs, wherein the crRNAs or guide RNAs target at least 2, 3 or 4 (or targets all of) *E coli* genes selected from the group fimH, bolA, rpoH, lptA and murA. Optionally, each nucleic acid encodes a plurality of different crRNAs or guide RNAs, wherein the crRNAs or guide RNAs target fimH and bolA. Optionally, each nucleic acid encodes a plurality of different crRNAs or guide RNAs, wherein the crRNAs or guide RNAs target rpoH and lptA. Optionally, each nucleic acid encodes a plurality of different crRNAs or guide RNAs, wherein the crRNAs or guide RNAs target fimH and murA. Optionally, each crRNA or guide RNA comprises a spacer sequence that is complementary to an *E coli* gene selected from the group fimH, bolA, rpoH, lptA and murA. Optionally, each crRNA or guide RNA comprises a spacer sequence that is at least 80, 90 or 95% identical to a nucleotide sequence selected from the group SEQ ID NO: 6-10.

Optionally, each nucleic acid encodes a first crRNA, second crRNA, third crRNA, fourth crRNA and fifth crRNA, wherein the crRNAs are different from each other and each crRNA targets a B2 phylogroup *E coli* gene. Optionally, each nucleic acid encodes a first crRNA, second crRNA, third crRNA, fourth crRNA and fifth crRNA, wherein the crRNAs are different from each other and each crRNA is complementary to a B2 phylogroup *E coli* gene. Optionally, each nucleic acid encodes a first crRNA, second crRNA, third crRNA, fourth crRNA and fifth crRNA, wherein the crRNAs comprise respectively SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10. Optionally, each nucleic acid encodes a first crRNA, second crRNA, third crRNA, fourth crRNA and fifth crRNA, wherein the crRNAs comprise respectively a nucleotide sequence that is at least 80% identical to SEQ ID NO: 6, a nucleotide sequence that is at least 80% identical to SEQ ID NO: 7, a nucleotide sequence that is at least 80% identical to SEQ ID NO: 8, a nucleotide sequence that is at least 80% identical to SEQ ID NO: 9 and a nucleotide sequence that is at least 80% identical to SEQ ID NO: 10. Optionally, each nucleic acid encodes a first crRNA, second crRNA, third crRNA, fourth crRNA and fifth crRNA, wherein the crRNAs comprise respectively a nucleotide sequence that is at least 90% identical to SEQ ID NO: 6, a nucleotide sequence that is at least 90% identical to SEQ ID NO: 7, a nucleotide sequence that is at least 90% identical to SEQ ID NO: 8, a

- nucleotide sequence that is at least 90% identical to SEQ ID NO: 9 and a nucleotide sequence that is at least 90% identical to SEQ ID NO: 10. Optionally, each nucleic acid encodes a first crRNA, second crRNA, third crRNA, fourth crRNA and fifth crRNA, wherein the crRNAs comprise respectively a nucleotide sequence that is at least 95, 96, 97, 98 or 99% identical to SEQ ID NO: 6, a
- 5 nucleotide sequence that is at least 95, 96, 97, 98 or 99% identical to SEQ ID NO: 7, a nucleotide sequence that is at least 95, 96, 97, 98 or 99% identical to SEQ ID NO: 8, a nucleotide sequence that is at least 95, 96, 97, 98 or 99% identical to SEQ ID NO: 9 and a nucleotide sequence that is at least 95, 96, 97, 98 or 99% identical to SEQ ID NO: 10.
- 10 23. The method of Embodiment 22, wherein the method is according to any one of Embodiments 1-20, optionally except that the nuclease is an endogenous nuclease of the cells and is not encoded by the nucleic acid comprised by the particles.
24. A composition comprising a plurality of transduction particles for use in a method of treating or
- 15 preventing an infection by *E coli* cells in a human or animal subject according to Embodiment 22 or 23, wherein
- (a) each particle comprises a nucleic acid encoding a crRNA or guide RNA that is operable with a Cas nuclease for chromosomal targeting in the cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the crRNA or guide RNA is
- 20 expressed and guides the Cas nuclease wherein the nuclease cuts the chromosomes of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
- (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
- (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.
- 25
25. A method of detecting the presence of a B2 phylogroup *E coli* in a sample, the method comprising contacting the sample comprising B2 phylogroup *E coli* with a composition comprising a plurality of transduction particles, wherein
- (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of said *E*
- 30 *coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
- (b) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells;
- 35 and
- (c) the method comprises detecting that B2 phylogroup *E coli* cells have been killed or the growth

or proliferation thereof has been reduced.

26. A method of detecting the presence of a B2 phylogroup *E coli* in a sample, the method comprising contacting the sample comprising B2 phylogroup *E coli* with a composition comprising a plurality of transduction particles, wherein
- 5 (a) each particle comprises a nucleic acid comprising or encoding a detectable label, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein optionally the label is expressed in the cells;
- 10 (b) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells; and
- (c) the method comprises detecting B2 phylogroup *E coli* cells comprising the label.
27. The method of Embodiment 26 or 27, wherein the composition comprises the features of the composition of any one of Embodiments 1, 2-21 24 and 25.
28. The method of any one of Embodiments 26-28, wherein the *E coli* comprise one or more *E coli* strains selected from the group ST131, ST1193, ST648, ST315, ST405, ST361, ST88 and ST453.
29. The method of any one of Embodiments 26-29, wherein the sample is a patient sample (eg, blood, urine, stool or saliva sample), wherein the subject is a transplant or cancer patient (optionally a haematological cancer patient), or wherein the patient is suffering from or at risk of a urinary tract infection (UTI); and optionally wherein the transplant is a solid organ or stem cell transplant (optionally a haematopoietic cell transplant).
30. The method of any one of Embodiments 26-30, wherein the nucleic acid of each particle comprises a nucleotide sequence (N1) encoding said nuclease, or comprising or encoding the label wherein each particle is a synthetic T-even phage (optionally a T4 phage) comprising an insertion of N1 into the genome of the phage, wherein the region is between the *pin* (protease inhibitor) gene and the *iPII* (internal protein) gene.
31. The method of any one of Embodiments 26-31, wherein at least 1×10^7 PFU of particles are contacted with the sample.
32. The method of any one of Embodiments 26-32, wherein the particles are contacted with the sample at an MOI (multiplicity of infection) of at least 0.01.

Labelling for detection methods is routine for the skilled addressee. The label may, for example, be a fluorescence label, eg, GFP. The sample may be a blood, spit, sputum or cell sample.

CONCEPTS

- 5 The invention also provides the following Concepts, which are fully supported by Example 2. All Concepts can be combined with any other features disclosed herein.

Patients with hematological malignancies frequently develop bloodstream infections due to translocation of *E. coli* and other bacteria from the gut. Antibiotic treatment to prevent these
 10 infections has detrimental effects on the microbiome and immune tonus and is further hampered by increasing antibiotic resistance, in particular towards fluoroquinolones. As described in Example 2, compositions of the Concepts may target bacteria in biofilms, reduce the emergence of phage-tolerant *E. coli* and out-compete their ancestral WT phages in co-culture experiments. Compositions are provided with broad host-range across the *E. coli* phylogeny, including multi-drug resistant strains
 15 (B2 phylogroup). Compositions comprising different particles as per the Concepts may surprisingly reduce *E. coli* load in the murine gut better than individual constituent particles. The compositions, methods and doses of the Concepts find utility to selectively kill *E. coli* which may cause fatal infections in hematological cancer, transplant or UTI patients. The compositions, methods and doses also find utility for treating or preventing *E. coli* blood stream infection.

20

To this end, there is provided:-

Concept A:

- A composition comprising a plurality of different types of transduction particles, wherein each of said
 25 particles comprises a nucleic acid and said particles are capable of contacting *E. coli* cells and introducing therein the nucleic acid, wherein
- (a) said nucleic acid of each particle comprises a nucleotide sequence encoding a product of interest (POI), wherein the nucleic acid is capable of expressing the POI in an *E. coli* cell;
 - (b) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety
 30 selected from a LPS, LamB and Tsx (optionally selected from LPS and Tsx) displayed on the surface of *E. coli* cells; and
 - (c) said plurality of different types of transduction particles comprises (i) a first type of particles that comprise a LPS adhesion moiety; and (ii) a second type of particles that comprise a Tsx adhesion moiety.

35

Concept B:

A composition comprising a plurality of transduction particles for use in a method of treating or preventing an infection by *E coli* cells in a human or animal subject, wherein the method comprises administering the particles to the subject, wherein

- 5 (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
- (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
- 10 (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx (optionally selected from LPS and Tsx) displayed on the surface of the phylogroup B2 *E coli* cells.

Concept C:

- 15 A composition comprising a plurality of transduction particles for use in a method of treating or preventing an infection by *E coli* cells (optionally B2 phylogroup *E coli* cells) in a human or animal subject, wherein the method comprises administering the particles to the subject, wherein (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid,
- 20 wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject; and
- (b) each particle comprised by the composition is a T-even phage capsid; optionally a capsid of a T2 phage, T2-like phage, RB69 phage or a RB69-like phage.

25

The following features are optional features that are combinable with the Concepts above or any other Configuration, example, embodiment or option herein.

Optionally,

- 30 A: each particle of the first type comprises a LPS adhesion moiety and a LamB adhesion moiety;
- B: each particle of the first type comprises a LPS adhesion moiety and a Tsx adhesion moiety; or
- C: each particle of the second type comprises a Tsx adhesion moiety but is devoid of LamB and LPS adhesion moieties.

Optionally, the composition comprises particles according to A, B and C.

35

Optionally, each particle comprises a phage capsid containing the nucleic acid. In an embodiment, the capsid comprises capsid proteins of a T-even (optionally T2) or lambda phage.

Optionally, the capsid of each particle comprised by the composition is a T-even phage capsid;

5 optionally a capsid of a T2 phage, T2-like phage, RB69 phage or a RB69-like phage.

Optionally, each particle is a phage (optionally a lytic phage) or packaged phagemid.

Optionally, the composition comprises at least 3 or 4 different types of transduction particles. In an
10 embodiment the composition has 4 (but no more than 4) different types of transduction particles.

Optionally, the nucleic acid of each particle comprises at least one nucleotide sequence (N1) encoding said POI, wherein each particle is a synthetic T-even phage comprising an insertion of N1 into a Modification Permissive Region (MPR) of the genome of the phage, wherein the MPR is immediately
15 after *gene 49* and to *gene E* when compared to a reference wild-type T2 phage. The nucleic acid may comprise a DNA deletion of phage DNA in the MPR. The insertion may comprise up to 5000, 6000, 7000 or 8000bp of DNA and/or the deletion comprises up to 5000, 6000, 7000 or 8000bp of DNA. The MPR may comprise contiguous DNA between said *gene 49* and *gene E*, wherein the contiguous DNA is at least 1000bp in length; or wherein the MPR comprises at least 100bp of DNA between said
20 *gene 49* and *gene E*.

Optionally a T-even phage herein is a phage selected from T2, T4 or T6 phage; or comprises a genome that is at least 95% identical to the genome of a said selected phage. This percentage may be at least 96, 97, 98 or 99%.

25

The composition of any one of claims 8-11, wherein the synthetic phage genome comprises said insertion between coordinates 9000 and 21000, wherein the coordinates are the nucleotide positions counted from the nucleotide (coordinate number 1) immediately after *gene 49* towards *gene E* when compared to a reference wild-type T2 phage. Optionally, the insertion is between coordinates 10300
30 and 19800, eg, between 10359 and 19810 with reference to the T2 genome.

Optionally, the insertion is in a window selected from the following windows (numbers being coordinates with reference to the T2 genome):-

9000-21000; 10000-21000, 10100-21000, 10200-21000, 10300-21000, 10400-21000, 10500-21000, 11000-21000, 15000-21000;
35 9000-20000; 10000-20000, 10100-20000, 10200-20000, 10300-20000, 10400-20000, 10500-20000, 11000-20000, 15000-20000;

9000-19500; 10000-19500, 10100-19500, 10200-19500, 10300-19500, 10400-19500, 10500-19500, 11000-19500, 15000-19500;

9000-19000; 10000-19000, 10100-19000, 10200-19000, 10300-19000, 10400-19000, 10500-19000, 11000-19000, 15000-19000;

5 9000-18000; 10000-18000, 10100-18000, 10200-18000, 10300-18000, 10400-18000, 10500-18000, 11000-18000 and 15000-18000.

Optionally, the nuclease is a dsDNA nuclease, ie, is capable of cutting dsDNA. Optionally, the nuclease is a nickase, eg, a Cas9 nickase.

10

In an embodiment, the POI is a protein. In an embodiment, the POI is an RNA, eg, a crRNA.

Optionally, the POI comprises

- 15 (a) a nuclease for targeting the DNA of an *E coli* cell, wherein the nuclease is capable of being expressed in the cell for cutting the DNA of the cell, thereby modifying or killing the cell; or
(b) a dead Cas (dCas) for targeting the DNA of an *E coli* cell, wherein the dCas is capable of being expressed in the cell and targeting the DNA of the cell, thereby modifying the cell.

Optionally, when (a) applies, said nuclease is a guided nuclease, optionally a Cas, meganuclease, zinc finger nuclease or TALEN; or when (b) applies, the dCas is a dCas9.

20

The nuclease may be a Type I, II, III, IV, V or VI Cas nuclease, optionally a Cas9 or a Cas3.

In a preferred embodiment, the nucleic acid comprises (optionally in 5' to 3' order) a *cas3* gene (*ygcb*) and a cognate cascade gene complex comprising *casA* (*ygcl*, *cas8e*), *casB* (*ygck*, *cas11*), *casC* (*ygci*, *cas7*), *casD* (*ygcl*, *cas5*), and *casE* (*ygch*, *cas6*), and optionally a CRISPR array or nucleotide
25 sequence encoding a guide RNA that targets an *E. coli* genome.

In a preferred embodiment, POI comprises at least one crRNA or guide RNA that is operable with a Cas for DNA targeting in *E coli* cells. Optionally, each crRNA or guide RNA comprises a spacer
30 sequence that is complementary to an *E coli* protospacer sequence; optionally a protospacer of a B2 phylogroup *E coli* cell or an *E coli* cell of a strain selected from the group ST131, ST1193, ST648, ST315, ST405, ST361, ST88 and ST453.

Each nucleic acid preferably encodes a plurality of different crRNAs comprising spacer sequences that
35 target *E coli* chromosomal genes. For example, Each nucleic acid preferably encodes a plurality of different crRNAs comprising spacer sequences that target 2, 3 or 4 *E coli* chromosomal genes selected from *fimH*, *bolA*, *rpoH*, *lptA* and *murA*. In an embodiment, the composition of the invention

comprises a type of transduction particle that targets *E coli* genes *bolA*, *rpoH* and *fimH*. Additionally or alternatively, the composition of the invention comprises a type of transduction particle that targets *E coli* genes *lptA* and *murA*. Optionally, the composition comprises a first type of transduction particle that targets *E coli* genes *bolA*, *rpoH* and *fimH*; and a second type of transduction particle that targets *E coli* genes *lptA* and *murA*. Optionally, the first type of particle is according any other first type of particle herein which comprises a LPS adhesion moiety (eg, comprises LPS and Tsx adhesion moieties). Optionally, the second type of particle is according any other first type of particle herein which comprises a Tsx adhesion moiety. Optionally, the second type of particle comprises a LPS adhesion moiety (eg, comprises LPS and LamB adhesion moieties).

10

The protospacer sequence may comprised by a gene selected from *E coli* genes *fimH*, *bolA*, *rpoH*, *lptA* and *murA*.

15 In an embodiment, the particles of the composition target all of *E coli* genes *fimH*, *bolA*, *rpoH*, *lptA* and *murA*.

The nucleotide sequence encoding the POI may comprise a stress-phase active (SPA) promoter for expression of the POI in *E coli* cells. The promoter may be an *E coli bolA* promoter. The promoter may comprise SEQ ID NO: 13.

20

In an embodiment, the *E coli* cells comprise a strain of *E coli* that causes sepsis, septicaemia or diarrhoea in humans or animals.

25 Optionally, the *E coli* cells are GI tract cells. Optionally, the *E coli* cells are comprised by a gut microbiome. Optionally, the *E coli* cells are comprised by blood of the subject. Optionally, the *E coli* cells are comprised by a urinary tract of the subject. Optionally, the *E coli* cells are comprised by a microbiome selected from a GI tract (eg, stomach), blood, urinary tract, mouth, nose, eye, ear, skin, anus or hair microbiome.

30

In an embodiment, the composition is for use in a method of treating or preventing infection of by *E coli* cells in a human or animal subject, wherein the method comprises administering the particles to the subject.

Optionally, the method is for preventing the translocation of B2 phylogroup *E coli* from the gastrointestinal or urinary tract to the blood stream of the subject, thereby preventing or reducing bacteriaemia in the subject.

- 5 Optionally, the method is for preventing *E coli* infection in a subject at risk of febrile neutropenia. The subject may be a cancer patient, such as a haematological cancer patient. The infection may be a bloodstream infection in the subject. Thus, in an embodiment, the method is for preventing a bloodstream *E coli* infection in a subject at risk of febrile neutropenia, wherein the subject is a human cancer patient, such as a haematological cancer patient.

10

There is provided:-

A method of treating or preventing sepsis, septicaemia or diarrhoea in a human or animal subject, the method comprising administering to the subject the composition described herein (eg, the composition of Concept A, B or C), wherein the *E coli* cells comprise a strain of *E coli* that causes sepsis,

- 15 septicaemia or diarrhoea in humans or animals.

A method of treating or preventing infection of by *E coli* cells in a human or animal subject, wherein the method comprises administering the composition described herein (eg, the composition of Concept A, B or C) to the subject, wherein the infection is treated or prevented.

20

The infection may be reduced or eliminated. The infection may be reduced by at least 20, 30, 40, 50, 60, 70, 80 or 90%.

- The subject may be a transplant or cancer patient (optionally a haematological cancer patient), or
25 wherein the patient is suffering from or at risk of a urinary tract infection (UTI). Optionally, the transplant is a solid organ or stem cell transplant (optionally a haematopoietic cell transplant) or wherein the transplant is a transplant of a medical device. The subject may be suffering from a haematological cancer, eg, leukaemia. The subject (eg, cancer patient) may be at risk of neutropenia or may be suffering from neutropenia. Preferably, the subject is suffering from a haematological
30 cancer, eg, leukaemia, and is at risk of neutropenia or suffering from neutropenia.

Optionally, the method is carried out prior to the subject receiving a or said transplant. The transplant may be a stem cell transplant, eg, when the subject is a cancer patient.

The composition or method may be for preventing haemolytic uremic syndrome (HUS), a UTI infection, sepsis, septicaemia or diarrhoea in a human subject.

In the composition for use in a method, or any method described herein, at least 1×10^7 PFU of particles are administered to the subject. Also provided is a dose of a composition described herein, wherein the dose is a dose of at least 1×10^7 PFU of said particles. For example, the dose is at least 1×10^7 , 1×10^8 , 1×10^9 , 1×10^{10} or 1×10^{11} PFU of said particles. For example, the dose is 1×10^7 , 2×10^9 or 2×10^{11} PFU of said particles. For example, the dose is from 1×10^7 to 2×10^{11} PFU of said particles. For example, the dose is 1×10^7 to 2×10^{11} PFU of said particles per gram body weight of the subject. In the method, *E coli* may be reduced by at least a 3 or 4 log₁₀ CFU/g of body weight of the subject.

The dose may be comprised by a medical device or container, eg, for oral or intravenous administration. The device may be an IV device, syringe or comprise an injection needle. The device or container may be sterile.

Optionally, the particles are administered to the subject at an MOI (multiplicity of infection) of at least 0.01. For example, the MOI is at least 0.1 or 1.

Preferably, the *E coli* cells comprise at least one strain that is an antibiotic-resistant or MDR strain; and/or at least one B2-I strain. Optionally, the antibiotic is fluoroquinolone (optionally levofloxacin), carbapenem or vancomycin; and/or wherein the *E coli* cells comprise beta-lactamase (ESBL)-producing *E coli*.

In an embodiment,

(i) the composition comprises first and second types of particles according to A, B and C

A: each particle of the first type comprises a LPS adhesion moiety and a LamB adhesion moiety;

B: each particle of the first type comprises a LPS adhesion moiety and a Tsx adhesion moiety; or

C: each particle of the second type comprises a Tsx adhesion moiety but is devoid of LamB and LPS adhesion moieties;

(ii) the POI comprises a guided nuclease for targeting the DNA of an *E coli* cell, wherein the nuclease is capable of being expressed in the cell for cutting the DNA of the cell, thereby modifying or killing the cell;

- (iii) each particle comprises a phage capsid containing the nucleic acid; and
- (iv) the particles of the composition target a plurality of different *E coli* genes, optionally selected from essential and virulence genes.

5 There is provided:-

A method of detecting the presence of *E coli* (optionally B2 phylogroup *E coli* cells) in a sample, the method comprising

- (a) contacting the sample with a composition described herein (eg, according to Concept A, B or C); and

10 (b) detecting that *E coli* cells have been killed or the growth or proliferation thereof has been reduced.

A method of detecting the presence of *E coli* (optionally B2 phylogroup *E coli* cells) in a sample, the method comprising

- (a) contacting the sample with a a composition described herein (eg, according to Concept A, B or
- 15 C); wherein particles of the composition comprise a nucleic acid comprising or encoding a detectable label, wherein the said particles contact the cells and introduce therein the nucleic acid, wherein optionally the label is expressed in the cells; and
- (b) detecting that *E coli* cells comprising the label.

20 A method for modifying the genomes of *E coli* cells, the method comprising contacting the cells with a composition described herein (eg, according to Concept A, B or C), wherein nucleic acid encoding the POI is introduced into the cells thereby modifying the genomes of the cells.

25 Where the POI is a nuclease, the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject.

A method for treating or preventing an infection by *E coli* cells in a human or animal subject, the method comprising administering to the subject a composition described herein (eg, according to Concept A, B or C).

30

Optionally, the sample is a patient sample (eg, blood, urine, stool or saliva sample), wherein the subject is a transplant or cancer patient (optionally a haematological cancer patient), or wherein the patient is suffering from or at risk of a urinary tract infection (UTI); and optionally wherein the transplant is a solid organ or stem cell transplant (optionally a haematopoietic cell transplant).

35

A method herein, in an embodiment, may be carried out *in vitro*. A method herein, in an embodiment, may be carried out *ex vivo*.

Optionally, at least 1×10^7 PFU of particles are contacted with the sample. Optionally the PFU of particles described above is contacted with the sample.

- 5 Optionally, the particles are contacted with the sample at an MOI (multiplicity of infection) of at least 0.01.

- Optionally, each particle comprised by the composition comprises a T-even phage capsid; optionally a capsid of a T2 phage, T2-like phage, RB69 phage or a RB69-like phage. Each particle may comprise
10 a capsid of a *Tevenvirinae* phage. Each particle may be a modified *Tevenvirinae* phage that comprises a genomic insertion of a nucleotide sequence encoding POI or said nuclease (and optionally a cognate crRNA when the nuclease is a Cas).

- Each particle may be a phage or packaged phagemid. Preferably, the phage is a lytic phage. In
15 another embodiment, the phage may be a non-lytic phage. . In another embodiment, the phage may be a temperate phage.

- Optionally the phage is a modified T2 phage, T2-like phage, RB69 phage or a RB69-like phage. Preferably the phage is a T2- or RB69-like phage. Preferably, each particle is a modified first phage,
20 wherein the genome of the first phage is at least 95, 96, 97, 98 or 99 % identical (by nucleotide sequence identity) to the genome of wild-type T2 or RB69. Preferably, said percentage is at least 95%. Preferably, percentage identity between phage genomes is determined by Mash analysis using a kmer size of 17 and 1000.

- 25 Optionally, the composition comprises at least 3 or 4 different types of transduction particles, wherein the types have different adhesion moieties for recognising and binding to a cognate moieties comprised by *E coli* cells.

- Optionally the cells comprise B2 phylogroup *E coli*. The cells are preferably pathogenic cells. The
30 cells are preferably pathogenic to the subject. The cells may mediate a disease or condition in the subject.

In an embodiment,

- (i) the nuclease is a guided nuclease for targeting the DNA of an *E coli* cell, wherein the nuclease is
35 capable of being expressed in the cell for cutting the DNA of the cell, thereby killing the cell;
(ii) each particle comprises a phage capsid containing the nucleic acid; and
(iii) the particles of the composition target a plurality of different *E coli* genes, optionally selected

from essential and virulence genes; and

(iv) optionally the composition comprises

A: particles which comprise a LPS adhesion moiety and a LamB adhesion moiety (and is devoid of Tsx);

5 B: particles which comprise a LPS adhesion moiety and a Tsx adhesion moiety; or

C: : particles which comprise a Tsx adhesion moiety but is devoid of LamB and LPS adhesion moieties.

The nuclease may be a Cas nuclease and the nucleic acid encodes at least one crRNA or guide RNA
10 that is operable with the Cas for DNA targeting in *E. coli* cells. Each crRNA or guide RNA may comprise a spacer sequence that is complementary to an *E. coli* protospacer sequence; optionally a protospacer of a B2 phylogroup *E. coli* cell or an *E. coli* cell of a strain selected from the group ST131, ST1193, ST648, ST315, ST405, ST361, ST88 and ST453. Each protospacer sequence may be comprised by a gene selected from *E. coli* genes *fimH*, *bolA*, *rpoH*, *lptA* and *murA*.

15 Optionally, the particles of the composition target at least one virulence gene and at least one essential gene. Optionally, the particles of the composition targets at least three genes selected from virulence and essential genes. Optionally, the particles of the composition target all of *E. coli* genes *fimH*, *bolA*, *rpoH*, *lptA* and *murA*.

20 Expression of the POI or nuclease or crRNA may be under the control of a stress-phase active (SPA) promoter. Optionally, the promoter is an *E. coli bolA* promoter or comprises SEQ ID NO: 13. Additionally or alternatively, expression of the POI or nuclease or crRNA may be under the control of an *E. coli* promoter PJ23100 (SEQ ID NO: 14), for example a promoter having a nucleotide sequence
25 that is at least 80, 90, 95, 96, 97, 98 or 99% identical to SEQ ID NO: 14. For example, the promoter has the nucleotide sequence of SEQ ID NO: 16.

A spacer herein may be adjacent a direct repeat sequences in the nucleic acid comprised by the particles. For example, the repeat has the sequence of SEQ ID NO: 15 (or said sequence with up to 5,
30 4, 3, or 2 nucleotide changes compared with SEQ ID NO: 15).

In an embodiment, expression of the POI or nuclease or crRNA may be under the control of a first promoter and expression of the adhesion moiety/ies is under the control of a second promoter that is different from the first promoter.

35 An adhesion moiety herein is on the outer surface of the cognate particle. In an embodiment, the moiety is comprised by a tail fibre, spike or capsid of the particle, preferably a tail fibre.

There is provided the following Paragraphs.

5 **PARAGRAPHS:**

1. A composition comprising a plurality of transduction particles for use in a method of treating or preventing an infection by *E coli* cells in a human or animal subject, wherein the method comprises administering the particles to the subject, wherein
 - 10 (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
 - (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
 - 15 (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.
2. A method for treating or preventing an infection by *E coli* cells in a human or animal subject, the method comprising administering to the subject a plurality of transduction particles, wherein the method comprises administering the particles to the subject, wherein
 - 20 (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
 - 25 (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
 - (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.
3. The composition or method of Paragraph 1 or 2 respectively, wherein the method is for treating or preventing infection of the subject by an *E coli* strain selected from the group ST131, ST1193, ST648, ST315, ST405, ST361, ST88 and ST453.
4. The composition or method of any preceding Paragraph, wherein the method is for treating or preventing infection of the subject by a plurality of different phylogroup B2 strains of *E coli*;
35 optionally wherein the plurality comprises *E coli* ST131 and ST1193 cells.

5. The composition or method of any preceding Paragraph, wherein the subject is a transplant or cancer patient (optionally a haematological cancer patient), or wherein the patient is suffering from or at risk of a urinary tract infection (UTI); and optionally wherein the transplant is a solid organ or stem cell transplant (optionally a haematopoietic cell transplant) or wherein the transplant is a transplant of a medical device.
6. The composition or method of any preceding Paragraph, wherein the method is carried out prior to the subject receiving a transplant.
7. The composition or method of any preceding Paragraph, wherein the B2 *E coli* cells comprise a strain of *E coli* that causes sepsis, septicaemia or diarrhoea in humans.
8. The composition or method of any preceding Paragraph for preventing haemolytic uremic syndrome (HUS), a UTI infection, sepsis, septicaemia or diarrhoea in the subject.
9. The composition or method of any preceding Paragraph, wherein each particle comprises a phage capsid containing the nucleic acid; optionally wherein the capsid comprises capsid proteins of a T-even (optionally T4) or lambda phage.
10. The composition or method of any preceding Paragraph, wherein each particle is a phage (optionally a lytic phage) or packaged phagemid.
11. The composition or method of any preceding Paragraph, wherein at least 2, 3 or 4 different types of transduction particle are administered to the subject.
12. The composition or method of any preceding Paragraph, wherein a first type of transduction particle and a second type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to a first cognate moiety selected from the group LPS, LamB and Tsx displayed on B2 *E coli*, and the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to a second cognate moiety selected from said group, wherein the first and second adhesion moieties different from each other.
13. The composition or method of Paragraph 12, wherein the first and second cognate moieties are different from each other.

14. The composition or method of any preceding Paragraph, wherein the nucleic acid of each particle comprises a nucleotide sequence (N1) encoding said nuclease, wherein each particle is a synthetic T-even phage (optionally a T4 phage) comprising an insertion of N1 into the genome of the phage, wherein the region is between the *pin* (protease inhibitor) gene and the *iPII* (internal protein) gene.
15. The composition or method of any preceding Paragraph, wherein said nuclease is a guided nuclease, optionally a Cas, meganuclease, zinc finger nuclease or TALEN.
16. The composition or method of any preceding Paragraph, wherein the nuclease is a Type I, II, III, IV, V or VI nuclease, optionally a Cas9 or a Cas3.
17. The composition or method of any preceding Paragraph, wherein at least 1×10^7 PFU of particles are administered to the subject.
18. The composition or method of any preceding Paragraph, wherein the particles are administered to the subject at an MOI (multiplicity of infection) of at least 0.01.
19. The composition or method of any preceding Paragraph, wherein the strain or at least one strain is an antibiotic-resistant or MDR strain; and/or wherein the strain or at least one strain is a B2-I strain.
20. The composition or method of Paragraph 19, wherein the antibiotic is fluoroquinolone (optionally levofloxacin), carbapenem or vancomycin; and/or wherein the *E coli* are beta-lactamase (ESBL)-producing *E coli*.
21. The composition or method of any preceding Paragraph, wherein
- (i) the nuclease is a Cas,
 - (ii) each particle comprises a phage capsid containing the nucleic acid; and
 - (iii) wherein a first type of transduction particle and a second type of transduction particle are administered to the subject, wherein the first type of particle comprises a first adhesion moiety that is capable of recognising and binding to a first cognate moiety selected from the group LPS, LamB and Tsx displayed on B2 strain *E coli*, and the second type of particle comprises a second adhesion moiety that is capable of recognising and binding to a second cognate moiety selected from said group, wherein the first and second adhesion moieties different from each other.

22. A method for treating or preventing an infection by *E coli* cells in a human or animal subject, the method comprising administering to the subject a plurality of transduction particles, wherein
(a) each particle comprises a nucleic acid encoding a crRNA or guide RNA that is operable with a Cas nuclease for chromosomal targeting in the cells, wherein the said administered particles
5 contact the cells and introduce therein the nucleic acid, wherein the crRNA or guide RNA is expressed and guides the Cas nuclease wherein the nuclease cuts the chromosomes of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
(b) the *E coli* cells are cells of *E coli* phylogroup B2; and
(c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety
10 selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.
23. The method of Paragraph 22, wherein the method is according to any one of Paragraphs 1-20, optionally except that the nuclease is an endogenous nuclease of the cells and is not encoded by the nucleic acid comprised by the particles.
15
24. A composition comprising a plurality of transduction particles for use in a method of treating or preventing an infection by *E coli* cells in a human or animal subject according to Paragraph 22 or 23, wherein
(a) each particle comprises a nucleic acid encoding a crRNA or guide RNA that is operable with a
20 Cas nuclease for chromosomal targeting in the cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the crRNA or guide RNA is expressed and guides the Cas nuclease wherein the nuclease cuts the chromosomes of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
(b) the *E coli* cells are cells of *E coli* phylogroup B2; and
25 (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.
25. The composition or method of any preceding Paragraph for preventing the translocation of B2 phylogroup *E coli* from the gastrointestinal or urinary tract to the blood stream of the subject,
30 thereby preventing or reducing bacteraemia in the patient.
26. A method of detecting the presence of a B2 phylogroup *E coli* in a sample, the method comprising contacting the sample comprising B2 phylogroup *E coli* with a composition comprising a plurality of transduction particles, wherein
35 (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of said *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells,

thereby killing the cells or reducing growth or proliferation of the cells in the subject;

(b) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells; and

5 (c) the method comprises detecting that B2 phylogroup *E coli* cells have been killed or the growth or proliferation thereof has been reduced.

27. A method of detecting the presence of a B2 phylogroup *E coli* in a sample, the method comprising contacting the sample comprising B2 phylogroup *E coli* with a composition comprising a plurality
10 of transduction particles, wherein

(a) each particle comprises a nucleic acid comprising or encoding a detectable label, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein optionally the label is expressed in the cells;

(b) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety
15 selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells; and

(c) the method comprises detecting B2 phylogroup *E coli* cells comprising the label.

28. The method of Paragraph 26 or 27, wherein the composition comprises the features of the
20 composition of any one of Paragraphs 1, 2-21 24 and 25.

29. The method of any one of Paragraphs 26-28, wherein the *E coli* comprise one or more *E coli* strains selected from the group ST131, ST1193, ST648, ST315, ST405, ST361, ST88 and ST453.

25 30. The method of any one of Paragraphs 26-29, wherein the sample is a patient sample (eg, blood, urine, stool or saliva sample), wherein the subject is a transplant or cancer patient (optionally a haematological cancer patient), or wherein the patient is suffering from or at risk of a urinary tract infection (UTI); and optionally wherein the transplant is a solid organ or stem cell transplant (optionally a haematopoietic cell transplant).

30 31. The method of any one of Paragraphs 26-30, wherein the nucleic acid of each particle comprises a nucleotide sequence (N1) encoding said nuclease, or comprising or encoding the label wherein each particle is a synthetic T-even phage (optionally a T4 phage) comprising an insertion of N1 into the genome of the phage, wherein the region is between the *pin* (protease inhibitor) gene and
35 the *iPII* (internal protein) gene.

32. The method of any one of Paragraphs 26-31, wherein at least 1×10^7 PFU of particles are contacted with the sample.

33. The method of any one of Paragraphs 26-32, wherein the particles are contacted with the sample
5 at an MOI (multiplicity of infection) of at least 0.01.

It will be understood that particular embodiments described herein are shown by way of illustration and not as limitations of the invention. The principal features of this invention can be employed in
10 various embodiments without departing from the scope of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine study, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the claims. All publications and patent applications mentioned in the specification are indicative of the level of skill of those skilled in the art to which this invention
15 pertains. All publications and patent applications and all US equivalent patent applications and patents are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. Reference is made to the publications mentioned herein and equivalent publications by the US Patent and Trademark Office (USPTO) or WIPO, the disclosures of which are incorporated herein by reference
20 for providing disclosure that may be used in the present invention and/or to provide one or more features (eg, of a vector) that may be included in one or more claims herein.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more,"
25 "at least one," and "one or more than one." The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the method being employed to determine the value, or the variation
30 that exists among the study subjects.

As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"),
"including" (and any form of including, such as "includes" and "include") or "containing" (and any
35 form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

The term "or combinations thereof" or similar as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, MB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of items or terms in any combination, unless otherwise apparent from the context.

Any part of this disclosure may be read in combination with any other part of the disclosure, unless otherwise apparent from the context.

All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

The present invention is described in more detail in the following non-limiting Examples.

EXAMPLES

EXAMPLE 1: Particle Composition for surprisingly targeting a plurality of different *E coli* strains, including B2 phylogroup

Overview

Patient samples containing various different *E coli* strains were challenged with a particle composition. The composition comprised a plurality of transduction particles bearing adhesion moieties that are able to bind to LPS, LamB or Tsx present on *E coli* cells. The particles comprised capsids comprising phage capsid proteins, with the capsids containing nucleic acids encoding CRISPR/Cas systems for chromosomal targeting in *E coli*. Each nucleic acid encoded a plurality of different crRNAs comprising spacer sequences that target *E coli* chromosomal genes. The ability to kill or reduce growth of *E coli* in the samples was determined using a plaque assay as described

below. Whole genome sequencing and genome assembly were used to assign susceptible *E coli* strains to phylogroups. It was surprisingly seen that use of such a particle composition was able to very effectively and extensively target a plurality of different *E coli* strains (these strains being clinically relevant from actual patient samples). Furthermore, advantageously a large number of different strains of the B2 phylogroup were targeted and killed. This is significant, because B2 *E coli* strains often display antibiotic resistance (such as MDR), eg, resistance to fluoroquinolone, that causes potentially life-threatening infections in patients, such as in cancer, transplant and UTI patients. In addition to the B2 group, we surprisingly also were able to successfully kill or inhibit the growth of multiple strains of *E coli* phylo groups B1, D, F and G.

Patient Sampling

The samples tested (n=71) were obtained from two prospective observational studies enrolling adult (≥ 18 years) patients who were admitted to hospital for an autologous or allogenic hematopoietic cell transplantation (HCT) and who received levofloxacin (a fluoroquinolone, FQ) prophylaxis starting the day before the transplantation (Day -1) (Satlin 2021 and Satlin 2018). Trimethoprim-sulfamethoxazole (TMP-SMX) was administered to allogenic HCT recipients from 2 to 4 days prior to the HCT. The antibiotic treatments do not eradicate all *E coli* in the patients; for example, fluoroquinolone-resistant *E coli* would persist and thus sampling obtained *E coli* from patients. *E coli* isolates were derived from either anal swabs or fecal samples obtained upon admission for transplantation. The timing of sampling varied from Day -7 (7 days before transplantation) to Day 0 (the day of transplantation).

General Plaque Assay: Spotting for Coverage

This procedure describes the method to assess the coverage of phage particle lysates on a panel of bacterial strains.

Section	Procedure
Step 1	Prepare bacterial indicator strains by inoculating 5 μ l of a frozen stock into 250 μ l of LB broth on a 96 well plate. Incubate overnight at 37°C and 250 rpm. This step is done the day before the assay is performed.
Step 2	Pre-acclimatize the LB plates to room temperature. Melt and acclimatize the top agar to 55°C and add CaCl ₂ and MgSO ₄ to a final concentration of 5 mM.
Step 3	In a culture tube mix 100 ml of the overnight indicator strain with 3 ml of pre-warmed top agar. When working with many strains, the top agar can be distributed to culture tubes and kept at 60 °C before use.

Step 4	Pour the mixture on top of the room temperature LB plate and distribute evenly by swirling. Let plates solidify in a biosafety cabinet or lab bench for 5-10 minutes. Keep the lids closed. While solidifying avoid stacking plates to keep the layer of the top agar even across the plate surface
Step 5	In the meantime, dilute the sample(s) or the stock of phage to the required dilutions (10-fold dilutions). Use PBS for dilutions.
Step 6	Spot dilutions 0 to -9, 5µl of each on top of your overlays and let them dry for 20 minutes (or until the spots are completely absorbed by the agar) on a bench with the lids open. Incubate plates upside down at 37°C overnight.
Step 7	Evaluation of the results: 1. If visible plaques appear, they are counted, and phage concentration is calculated. The result is recorded as YES. Calculation of phage concentration: number of plaques x 200 (to make it into ml) x dilution where you counted. i.e: If counted 5 plaques on dilution - 6: $5 \times 200 \times 1e6 = 1e9$ pfu/ml 2. If there are no visible plaques, but inhibition of growth is observed the result is recorded as LZ (lysis zone) and the lowest dilution of inhibition is noted. 3. If no plaques or inhibition is observed, the result is recorded as NO

Spotting was performed according to the above general plaque assay. Bacterial strains were prepared by inoculating 5µl of a frozen stock into 250µl of LB broth in a 96 well plate. The plate was incubated at 37°C and 250 rpm overnight. Next day, in a culture tube 100 ml of the overnight strain was mixed with 3 ml of pre-warmed top agar (at 55°C) containing 5mM CaCl₂ and 5mM MgSO₄. The mixture was poured on top of pre-acclimatized LB plate and distributed evenly by swirling. The plates were left on the lab bench for 5-10 minutes to solidify. In the meantime, the particle composition was diluted in PBS buffer from 10⁰ to 10⁻⁹ and 5µL of each serial dilution were spotted on top of the overlays. Plates were left on the bench with the lid open for 20 minutes or until the spots are completely absorbed by the agar, and then incubated upside down at 37°C overnight.

Evaluation of the results:

If visible plaques appeared, the results were recorded as positive, plaques were counted, and phage concentration was calculated. Calculation of phage concentration: number of plaques x 200 x dilution where plaques were observed. i.e: If counted 5 plaques on dilution - 6: $5 \times 200 \times 1e6 = 1e9$ pfu/ml

If there were no visible plaques, but inhibition of growth was observed, the result was recorded as lysis zone and the lowest dilution of inhibition is noted.

If no plaques or inhibition was observed, the results were recorded as negative.

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Whole genome sequencing

DNA extraction was performed using Omega Bio-tek, Mag-Bind Bacterial DNA 96 Kit. The protocol was followed, and samples were eluted in 100µL Elution Buffer.

Sequencing libraries were generated using Illumina Nextera XT, and sequencing was performed with paired ends on an Illumina MiSeq instrument with a V2 flow cell (300 cycles). The average sequencing depth for all samples was 48x (range: 31-72x)

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Genome assembly and phylogenetic tree reconstruction

Raw data was trimmed for adaptor sequences and low-quality bases using fastp 0.22.0 (Chen et al 2018). Genomes were assembled using SKESA 2.4.0 (Souvorov et al 2018). The phylogroup of each sample was determined using EzClermont 0.7.0 (<https://github.com/nickp60/EzClermont>). Genomic distances were estimated using Mash 1.1 (Ondov et al 2016) with a kmer size of 17 and 1000 sketches. A neighbour joining tree was constructed using rapidnj 2.3.2 (Simonsen et al 2008). The final tree visualization was generated in Interactive Tree Of Life (iTOL) version 6.5.3 (Letunic et al 2021). Strain phylotyping was carried out in silico using the method disclosed in Microb Genom. 2018 Jul; 4(7): e000192, Published online 2018 Jun 19. doi: 10.1099/mgen.0.000192, PMID: PMC6113867, PMID: 29916797, "ClermonTyping: an easy-to-use and accurate in silico method for Escherichia genus strain phylotyping, Johann Beghain *et al.* The set of primer sequences described in Table S1 of that reference (available in the online version of this article) was used.

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Results

It was surprisingly seen that use of such a particle composition was able to very effectively and extensively target a plurality of different *E coli* strains (these strains being clinically relevant from actual patient samples), see Figure 1. Furthermore (see Figure 2), advantageously a large number of different strains of the B2 phylogroup were targeted and killed. This is significant, because B2 *E coli* strains often display antibiotic resistance (such as MDR), eg, resistance to fluoroquinolone, that causes potentially life-threatening infections in patients, such as in cancer, transplant and UTI patients. More than 10 different ST131 strains were killed (plaques formed) and more than 10 different ST1193 strains were killed (plaques formed). Sequence type 1193 has recently emerged as a new, virulent and resistant lineage among fluoroquinolone resistant *E coli*. *Escherichia coli* ST131 is a globally dominant multidrug resistant clone associated with high rates of rUTI. Uropathogenic *E coli* (UPEC) are the primary cause of urinary tract infection (UTI), being responsible of ~90% of all cases. UPEC

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strains largely belong to the *E. coli* phylogenetic groups B2 or D and are often clonal, with the most common sequence types (STs) isolated worldwide being ST69, ST73, ST95 and ST1312. The recently emerged and globally disseminated ST131 clone is a major contributor to hospital- and community-acquired UTI, as well as bloodstream infections and infections in companion animals and poultry.

- 5 Originally identified in 2008, ST131 is associated with the worldwide spread of the CTX-M-15 extended spectrum β -lactamase (ESBL) resistance gene. Most ST131 strains are now strongly associated with multidrug resistance (MDR), including resistance to fluoroquinolones. Recent reports have also identified strains that are resistant to last-line carbapenems.
- 10 Significantly, we included clinical samples from patients that went on to develop *E. coli* bacteraemia (despite pre-treatment with FQ/TMP-SMX). The composition could kill or reduce growth of *E. coli* strains in these samples (and this is indicative of the possibility of using the composition for prophylaxis of bacteraemia in subjects). This included strains of the following Multi-Locus Sequence Typing (MLST) types (see Figure 2): ST648, ST315, ST405, ST361, ST88, ST453, ST1193 and
- 15 ST131.

In addition to the B2 group, we surprisingly also were able to successfully kill or inhibit the growth of multiple strains of *E. coli* phylo groups B1, D, F and G.

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EXAMPLE 2: Phage-derived CRISPR therapeutic designed to reduce *E. coli* in hematological cancer patients

ABSTRACT

Patients with hematological malignancies frequently develop bloodstream infections due to translocation of *E. coli* and other bacteria from the gut. Antibiotic treatment to prevent these infections has detrimental effects on the microbiome and immune tonus and is further hampered by increasing antibiotic resistance, in particular towards fluoroquinolones. We screened a library of 162 wild-type (WT) phages identifying 8 phages with broad coverage of *E. coli*. Selected phages were engineered with novel tail fibers and a CRISPR-Cas machinery targeting clinically relevant *E. coli*. Engineered phages target bacteria in biofilms, reduce the emergence of phage-tolerant *E. coli* and out-compete their ancestral WT phages in co-culture experiments. SNIPR001 comprises four engineered bacteriophages with broad host-range across the *E. coli* phylogeny, including multi-drug resistant strains. SNIPR001 is well-tolerated in animals and reduces *E. coli* load in the murine gut better than its constituent components. SNIPR001 represents a novel CRISPR-Cas-therapeutic designed to selectively target *E. coli* which may cause fatal infections in hematological cancer patients.

INTRODUCTION

Cancer treatment continues to advance and survival rates for people with hematological malignancies are increasing¹. However, the chemotherapeutic regimens that are frequently used in this immunocompromised population cause bone marrow suppression and gastrointestinal mucositis with associated increased intestinal permeability²⁻⁴. Translocation of gut bacteria, including *E. coli*, from the gastrointestinal tract is a frequent cause of bloodstream infections⁵.

The mortality related to bloodstream infections caused by enteric bacteria such as *E. coli* is 15-20%⁶; thus, antimicrobial prophylaxis is applied in people at risk of febrile neutropenia⁷. There are no approved therapies for the prevention of bloodstream infections in patients with hematological cancers, yet fluoroquinolones are used off-label in the United States based on two randomized trials that demonstrated that they decrease bacterial infections during neutropenia^{7,8,9}. Beyond the side effects of fluoroquinolones, including safety warnings and precautions, bacterial resistance is rising in oncology patients and approaching 60% in *E. coli* bloodstream infections in the USA¹⁰. In immunocompromised patients with hematological malignancies who develop chemotherapy-induced neutropenia, *E. coli* is responsible for 25.1-30% of all bacteremia cases^{11,12}. Moreover, up to 65% of *E. coli* isolated as the causative pathogen from bloodstream infections⁵ in patients with hematological cancers undergoing hematopoietic stem cell transplantation were resistant to fluoroquinolones¹³. Accordingly, novel narrow-spectrum prophylactic options that would also cover fluoroquinolone resistant *E. coli* are needed to prevent infections in these vulnerable patients.

Bacteriophage therapy has been used prior to the broad availability of antibiotics¹⁴, but has now regained interest¹⁵ due to the rise in bacterial antimicrobial resistance combined with several successful individual case reports¹⁶⁻¹⁸. Still, few clinical trials with wild-type (WT) phages have been conducted¹⁹⁻²² and, although several have been directed towards *E. coli*, these have failed to produce convincing results in larger randomized controlled trials likely due to incomplete coverage of the target strains by the phage cocktail²³. Recent efforts have involved more extensive characterization of phages ($n = 41$) targeting *Klebsiella pneumoniae* strains ($n = 17$)²⁴ and phages ($n = 248$) targeting *Vibrio* strains ($n = 294$)²⁵ suggesting that better coverage of target strains can be achieved with large scale systematic screening. Synthetic biology has also been used to engineer T3 phage tailfibers augmenting the spectrum of strains targeted by the engineered phage²⁶. Finally, clustered regularly interspaced short palindromic repeats (CRISPR)-Cas systems can contribute to efficacy as a complementary killing modality to the lytic activity of the phage. CRISPR-associated nucleases (Cas) and a CRISPR-RNA form a complex, which, in some CRISPR-Cas systems, can bind to a homologous DNA target sequence and result in DNA degradation^{27,28}. Since prokaryotes lack error-prone non-homologous end-joining and rely solely on homologous recombination to repair DNA damage, they are prone to cell death following DNA degradation. This vulnerability has been exploited by using CRISPR-Cas as an antimicrobial modality for several bacteria, including *Staphylococcus aureus*, *E. coli* or *Clostridium difficile*²⁹⁻³⁵.

To address the significant unmet medical need for new prophylactic agents for patients with hematological malignancies we report on the development of SNIPR001. Our research process for

designing SNIPR001 includes several steps (**Fig. 3**). In short, a library ($n = 162$) of WT phages were tested *in vitro* on a panel of phylogenetically diverse *E. coli* strains representing the biology of the target bacterium *E. coli*. WT phages with the broadest and most complementary target strain coverage were selected for further engineering. Selected WT phages were subjected to both tail fiber engineering and

5 CRISPR-Cas-arming to create a library of CRISPR-Cas-Armed Phages (CAPs). The CAP library was assessed for manufacturability, *in vitro* stability, spectrum of efficacy, *in vivo* pharmacokinetics, and efficacy. A combination of four CAPs was selected to create the development candidate SNIPR001, which has now entered clinical development (ClinicalTrials.gov ID NCT05277350).

10 **RESULTS**

Wild-type lytic phages $\alpha 15$, $\alpha 17$, $\alpha 20$, $\alpha 48$ and $\alpha 51$ (all members of *Tevenvirinae*) were the starting point for phage engineering to produce synthetic phage types (CRISPR-armed phage; CAPs) $\alpha 15.2$, $\alpha 20.4$, $\alpha 48.4$ or $\alpha 51.5$. Tail fibre specificities for cognate *E. coli* surface ligands TSX, LPS and LamB were as follows:-

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$\alpha 15.2$ binds LPS and Tsx

$\alpha 20.4$ binds LPS and LamB

$\alpha 48.4$ binds Tsx

$\alpha 51.5$ binds Tsx

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A cocktail comprising these phage types targeting LPS, LamB and Tsx was made (herein the cocktail termed SNR001).

CRISPR-Cas-arming of phages to target *E. coli*

25 To CRISPR-Cas-arm the selected lytic phages and generate a library of CAPs, the type I-E CRISPR-Cas system of *E. coli*³⁹ was engineered to target phylogenetically diverse *E. coli* strains. A CRISPR-Guided Vector (CGVTM) was generated, containing the *cas3* gene (*vgcB*) and a downstream cascade gene complex composed of *casA* (*vgcL*, *cas8e*), *casB* (*vgcK*, *cas11*), *casC* (*vgcJ*, *cas7*), *casD* (*vgcI*, *cas5*), and *casE* (*vgcH*, *cas6*), and a CRISPR array targeting a plurality of different genes of the *E. coli*

30 genome (CGV-EcCas). To evaluate the killing efficiency of the CRISPR-Cas system, the CGV-EcCas

was conjugated to *E. coli* strain b52, showing an average reduction of 3.5 log₁₀ CFU/mL, compared to the empty vector. As expected, no effect was observed after conjugating the CGV-EcCas to a non-target *E. coli* strain. The killing efficiency of CGV-EcCas was further assessed on an abbreviated panel of 82 *E. coli* strains. Conjugative delivery of the empty vector was accomplished in 75% of the isolates. For all strains where the CGV-EcCas was delivered, bacterial counts were reduced below the limit of detection (LOD, 200 CFU/mL) corresponding to a reduction of 1-6 log₁₀, highlighting the potent CRISPR-Cas-mediated killing (Fig. 3A).

In addition to promoter *P_{bolA}*, the CRISPR-Cas systems was engineered to express from a synthetic constitutively expressed *E. coli* promoter (*P_{J23100}*). CRISPR arrays were designed to target multiple virulence (spacer 1, 2 and 3) or essential genes (spacer 4 and 5) (SEQ ID NOs: 6-10), as targeting multiple regions has been shown to prevent resistance evolution⁴³. To confirm the CRISPR-Cas activity in the CAPs, we measured the *cas3* transcripts in samples obtained at 5-, 15- and 30-min following a synchronized infection with the equal MoI of CAP α15.2 in comparison to WT α15 using RT-qPCR and observed increasing levels of *cas3* RNA only upon CAP α15.2 infection. Next, we extended this assay to all four CAPs (α15.2, α20.4, α48.4, α51.5) and demonstrated increasing levels of *cas3* transcripts highlighting that the CAPs expressed the CRISPR-Cas system during infection of a target strain.

To demonstrate the competitive superiority of the CAPs, we performed competition experiments in which CAPs (α20.4 and α15.2) and their WT ancestral phages were co-cultured with *E. coli* strain b230, serving as target for both competing phages. Approximate initial ratios of 1 CAP to 9 WT phages were co-cultured and passaged four times on fresh target cells in liquid cultures. After each passage, the relative abundance of CAP and WT phage particles was evaluated. Both CAPs outcompeted their WTs within four rounds; CAP α20.4 reached 68% after four rounds, and CAP α15.2 reached 86% after two rounds (Fig. 3B-C), demonstrating an improved fitness compared to the WT phages.

CAP cocktail SNIPR001

The activity of CAPs was tested against the *E. coli* panel (*n* = 429) using the growth kinetics assay. To maximize our coverage, we combined CAPs, resulting in a composition (SNR001) comprising phage types α15.2, α20.4, α48.4, and α51.5.

The ancestors of CAPs $\alpha 15.2$, $\alpha 20.4$, $\alpha 48.4$, and $\alpha 51.5$ are classified under the *Tevenvirinae* subfamily. Specifically, ancestors $\alpha 15$, $\alpha 48$, $\alpha 20$ and $\alpha 51$ have sequence similarity to *E. coli* phage T2, T4 and RB69 phages as shown below (as determined by Mash analysis). *In silico* analyses of the genomes of SNIPR001 showed that the CAPs encode no known transposase or integrase genes, indicating that the phages are not temperate, and thus not predicted to be capable of inserting their DNA in bacterial cells.

Similarity Matrix:

	T2	T4	RB69
a15	96.56%	96.41%	82.60%
a20	82.60%	83.20%	96.70%
a48	96.74%	96.57%	81.82%
a51	96.23%	96.13%	81.86%

This similarity was calculated wherein the distance between genomes was calculated using Mash (v 1.1) with a k-mer size of 21 and a sketch size of 10 000.

See Ondov, B.D., Treangen, T.J., Melsted, P. *et al.*, “Mash: fast genome and metagenome distance estimation using MinHash”, *Genome Biol* 17, 132 (2016). <https://doi.org/10.1186/s13059-016-0997-x> for further discussion of Mash

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SNIPR001 does not affect other gut associated bacteria

Ideally, a phage-based therapy should not disturb the non-targeted genera of the microbiome, thus the specificity of SNIPR001 towards *E. coli* was assessed by investigating its effects on a panel of strain which includes non-*E. coli* species which are *E. coli* relatives, as well as a range of families associated with the commensal bacterial community in the gut bacteria (and *E. coli* as a positive control). The bacteria were cultured without CAPs, with the SNIPR001 cocktail, or with individual SNIPR001 CAPs ($n = 4$). The growth in CFU/mL was evaluated over a 4-hour period (DCFU/mL_{4h-0h}). In parallel, *E. coli* b2480 was grown under the same conditions as a positive control (Fig. 4). We observed no significant effect ($p > 0.05$, Student's t-test, FDR-corrected with Holm's method) of the SNIPR001 cocktail or any of the SNIPR001 CAPs on non-*E. coli* strains, while the growth of *E. coli*

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was significantly inhibited ($p < 0.05$, Student's t-test, FDR-corrected with Holm's method). Thus, SNIPR001 is not expected to impact the gut microbiome beyond the target *E. coli*.

***In vitro* host-range of SNIPR001 in clinical target population**

- 5 To understand the potential effect in strains relevant to hematological cancer patients the coverage of SNIPR001 was tested against our internal *E. coli* panel (429 strains) and a set of 382 clinical *E. coli* strains (JMI Laboratories, North Liberty, IA, USA). These JMI strains originated from patients with bloodstream infections hospitalized in hemato-oncology units across four different regions from 2018-2020 (Asia-Pacific 54 isolates, Europe 161 isolates, Latin America 26 isolates, and North America 141
- 10 isolates). The genotypic distribution of *E. coli* strains in the patient population was determined using whole genome sequencing and was found to be diverse, representing nine phylogroups and 118 multi-locus sequence types (MLSTs) (**Fig. 5A**). Using a spotting assay, we recorded phage infectivity against the panel of JMI strains. Visible single plaques were differentiated from lysis zones in cases where single plaques could not be verified. All spotting assays were run in duplicates. We observed overall
- 15 coverages of $90.4 \pm 1.6\%$ of SNIPR001 in the 382 JMI *E. coli* panel, and of $95.6 \pm 0.3\%$ of SNIPR001 on the internal *E. coli* panel (429 strains). Furthermore, we observed plaques in $53.1 \pm 7.7\%$ and lysis zones in $37.3 \pm 6.1\%$ of the JMI panel strains, and similarly plaques in $60.5 \pm 6.6\%$ and lysis zones in $35.1 \pm 6.3\%$ of the internal panel strains (**Fig. 5B**). SNIPR001 showed 100% coverage in the B2 phylogroup, representing 53% of the JMI panel. This phylogroup is correlated with multi-drug
- 20 resistance and virulence. Additionally, we observed that SNIPR001 covered 91.7% ($n = 55$) of strains classified as multi-drug resistant, 100% ($n = 5$) of carbapenem-resistant strains, 92.2% ($n = 95$) of extended-spectrum β -lactamases producing strains, and 88.9% ($n = 176$) of strains that are resistant to fluoroquinolones, such as ciprofloxacin and levofloxacin (**Fig. 5C**).
- 25 Finally, we validated SNIPR001 on a clinical panel ($n = 72$) of fluoroquinolones-resistant *E. coli* strains that were isolated from either a fecal sample or a perianal swab from hematological cancer patients. This population represents the expected clinical target patient population being pursued (SNIPR001 has been designated fast-track status by the FDA). A subset of these strains gave rise to a bloodstream infection (**Fig. 5D**). 82% of the *E. coli* strains ($n = 72$) were susceptible to at least two or more of the
- 30 CAPs in SNIPR001, and 93% of the strains were susceptible to the whole SNIPR001 cocktail (**Fig. 5E**). This data demonstrates the benefit of SNIPR001 compared to the individual CAPs with regards to improving the spectrum of efficacy.

Tolerability and gastrointestinal recovery of SNIPR001 in minipigs

The tolerability and gastrointestinal recovery of SNIPR001 were evaluated in Göttingen minipigs. Blood and feces were sampled over 7 days following oral administration of 2×10^{12} PFU of SNIPR001 or vehicle. No CAPs were recovered from plasma, indicating no systemic exposure, while CAPs were recovered in the feces up to 7 days after SNIPR001 administration with a peak of 2×10^7 PFU 24h post-dosing (Fig. 6A). The minipigs exhibited no clinical signs and no significant changes were observed in hematology or biochemistry parameters, in particular, no changes were seen in any immune cells, compared to vehicle treatment, supporting that SNIPR001 was well tolerated. Similar recoveries were obtained with the individual CAPs (Fig. 6B). In conclusion, SNIPR001 appears to be well tolerated in Göttingen minipigs with gastrointestinal recovery.

Efficacy of SNIPR001 and constituent CAPs in a mouse colonization model

To assess the *in vivo* efficacy of the four selected CAPs in reducing *E. coli*, we adapted a mouse gut colonization model from Galtier et al.⁴⁴ for *E. coli* strain b17. Streptomycin was administered for 3 days to reduce Gram-negative bacteria from the mouse gastrointestinal tract, after which streptomycin administration was stopped and animals were inoculated once perorally with *E. coli* b17 (1×10^7 CFU). This allowed stable colonization for 3 to 4 days. Aiming at assessing the efficacy of CAPs on an established colonization, treatment was started 2 days after inoculation and the study was terminated on day 4 after inoculation, as the colonization starts to drop. To ensure maximum exposure of CAPs, mice were treated with 3 daily doses, administered 8h apart, for a total of 6 doses over 2 days.

Mice were treated by oral gavage with a high, medium, or low dose (2×10^{11} PFU, 2×10^9 PFU, 1×10^7 PFU, respectively) of SNIPR001, vehicle (negative control), or gentamicin (positive control). CAP recovery in the feces ranged from 3×10^7 PFU/g in the low dose to 1×10^{10} PFU/g in the high dose, confirming successful GI passage (Fig. 6C). These levels of CAPs were associated with a significant ($p < 0.05$, Mann-Whitney U test, FDR corrected) dose-dependent reduction in the target *E. coli* population compared to vehicle treated mice, after 24h of treatment (Day 3). At the high dose, SNIPR001 led to a 4 log₁₀ CFU/g reduction (Fig. 6D). Despite an increased variability in bacterial recovery on day 4, possibly due to clearance of the colonizing strain as illustrated in the vehicle group, similar reductions were observed after 2 days of treatment (Day 4). While the medium dose did not reach statistical significance ($p < 0.05$, Mann-Whitney U test), there was nevertheless a numerical reduction in comparison to the vehicle group. Subsequently, the efficacies of the individual CAPs were compared to the SNIPR001 cocktail in this model. In this experiment a greater reduction in the

colonization of the target strain was observed with SNIPR001 compared to any single CAP (which showed a numerical, but not statistically significant reduction) highlighting a benefit in efficacy from the combination (Fig. 6E). We also assayed the resistance profile of randomly sampled surviving bacteria and found no isolates that were resistant to the SNIPR001 cocktail. Overall, this data demonstrates the ability of SNIPR001 to decrease the target *E. coli* in the GI tract of colonized mice.

DISCUSSION

Here we describe the development of SNIPR001 designed to target gut *E. coli* that frequently translocate in the bloodstream to cause bloodstream infections in patients with hematological cancers who are neutropenic. While fluoroquinolones are being used off-label, these patients continue to have a high morbidity and mortality. The use of traditional antibiotics has yielded important health benefits over the past century. However, in parallel we are now experiencing significant bacterial resistance development, and the number of deaths attributable to bacterial antimicrobial resistance in 2019 has been estimated to 1.27 million, with *E. coli* being the leading pathogen⁴⁵. Accordingly, new antibiotic modalities are needed to address both the unmet medical need of antimicrobial resistant infections in this vulnerable population. In this study we describe the development of SNIPR001 a novel development candidate with the potential to address these challenges.

Confirmation of the efficacy of SNIPR001 on a large and clinically relevant strain panels supports the clinical potential of the SNIPR001. The observed 4 log₁₀ reduction of *E. coli* in our *in vivo* model is a clear improvement over the previous studies⁴⁹⁵⁰.

SNIPR001 is an orthogonal antimicrobial approach as it has shown activity in multi-drug resistant strains. In addition, there is emerging evidence that maintaining a normal microbiome is important for upholding immunological tonus and potentially benefiting the outcome of oncology treatments⁵¹, and this has also been recognized in the most recent guidance on prophylactic management of patients at risk of febrile neutropenia⁷. In this context *in vitro* studies with SNIPR001 have shown specificity towards *E. coli* with no off-target effects toward any of the tested non-*E. coli* strains, thereby having a less detrimental effect on the microbiome. In the future, individualized combinations of narrow-spectrum antibiotics such as SNIPR001 may be used first line rather than use in addition to broad-spectrum antibiotics such as fluoroquinolones.

A clinical study to evaluate the ability of SNIPR001 to ascertain safety and its ability to reduce *E. coli* in the gut without perturbing the overall gut microbiome is currently ongoing in the US (NCT05277350). We believe that SNIPR001 exemplifies a potentially significant therapeutic advance in the field of antimicrobials for high-risk patient populations and can serve as a blueprint for narrow-spectrum therapies for other life-threatening antimicrobial resistant pathogens in high-risk patient populations.

Table 1a: Overview of the four CAPs making up SNIPR001. The *E. coli* genes targeted by the five individual spacers and the sequence are listed in Table 1b.

- 10 α 15.2 comprises spacers 1, 2, 3
 α 20.4 comprises spacers 4, 5
 α 48.4 comprises spacers 4, 5
 α 51.5 comprises spacers 4, 5

15 **Table 1b: The *E. coli* genes targeted by the five individual spacers comprised by SNR001 CAPs, and the spacer sequences used in the CAPs.**

spacer	Target gene	Spacer Sequence
1	<i>bolA</i>	AGTGGGAAGGGTTGCAGGACACCGTCTTTGCC
2	<i>rpoH</i>	CCGATGTTACCTTCCTGAATCAAATCCGCCTG
3	<i>fimH</i>	CGAATGACCAGGCATTTACCGACCAGCCCATC
4	<i>lptA</i>	TGATTGACGGCTACGGTAAACCGGCAACGTTC
5	<i>murA</i>	GCTGTTAACGTACGTACCGCGCCGCATCCGGC

METHODS

- 20 Phage isolation was carried out by using *E. coli* strain panels. In brief, 100 μ L of overnight cultures of each *E. coli* strain were mixed with 100 μ L of each phage cocktail or wastewater samples. Following 6

minutes incubation at RT (in this period infection should occur), 3 mL of pre-warmed top agar containing Ca^{2+} were added to the *E. coli*/phage or wastewater mixtures and poured immediately on a LB plate. Alternatively, 10-fold dilutions of each cocktail were spotted on lawns prepared with isolation strains. After drying, plates were incubated at 37 °C overnight. Plaques were picked from each plate and resuspended in 500 µL of SM buffer, vortexed and stored at 4 °C. Ten-fold dilutions were spotted on the isolation strain which the plaque was originally picked from. To increase the likelihood of obtaining plaques corresponding to single phages, the procedure was repeated at least three times. Lysates were prepared from single plaques picked at the previous round of propagation, DNA was extracted, and their genomes were sequenced.

E. coli panels and isolation procedures

Three *E. coli* panels, one in-house panel and two clinically relevant panels were included in this study. The in-house panel consisted of 429 phylogenetically diverse *E. coli* strains, isolated from the blood of patients with bloodstream infections and urinary tract infections, from feces of humans with no known disease, from animals and the environment. The strains cover seven different phylogroups (A, B1, B2, C, D, E, F), 114 multi-locus sequence typing (MLST) groups, serotypes (K- and O-type), antibiotic resistance profiles, and different geographical locations of isolation.

The JMI panel comprises of 382 strain *E. coli* clinical collection obtained from JMI Laboratories (North Liberty, IA, USA). These strains were isolated from patients with bloodstream infections hospitalized in hematology and oncology units across four different regions (Asia-Pacific 54 isolates, Europe 161 isolates, Latin America 26 isolates, and North America 141 isolates), sourced through the SENTRY Antimicrobial Surveillance Program (2018–2020), which is composed of a network of more than 150 medical centers in more than 28 countries worldwide (<https://www.jmilabs.com/sentry-surveillance-program>).

Finally, the panel comprising of 72 fluoroquinolone-resistant *E. coli* strains are isolated from either fecal samples or perianal swabs of hematological cancer patients hospitalized for hematopoietic cell transplantation^{53,54}.

E. coli strains were cultivated at 37 °C in lysogeny broth (LB) at 250 rpm in liquid media or on agar plates containing 1.5% (w/v) agar. When necessary, cultures were supplemented with ampicillin (100 µg/mL), kanamycin (50 µg/mL), gentamicin (15 µg/mL) or amikacin (50 µg/mL). All media for the

growth of conjugation donor *E. coli* JKE201⁵⁵ and its derivatives were supplemented with 1,6-diaminopimelic acid (DAP) (80 µg/mL) to complement their auxotrophy.

- Both *E. coli* strain b52, which was used to produce α15.2, α48.4 and α51.5, and *E. coli* strain b2479, which was selected to produce α20.4, belong to phylogroup A. Strain *E. coli* b17 was used as colonizing strain in the *in vivo* efficacy models as the strain is susceptible to all SNIPR001 CAPs and is part of the SNIPR Biome strain bank.

Phage screening by growth kinetics

- In vitro* susceptibility of the intin-house *E. coli* panel ($n = 429$) to the 162 WT phages was evaluated using a growth kinetics assay. The assay measures the metabolic activity of a bacteria by tracking the reduction of a tetrazolium dye to a purple compound that aggregates during bacterial growth. The colorimetric reading was recorded every 15 min over a 24-h period by using the OmniLog® (Biolog, Hayward, CA, USA) – adapted from Henry *et al.*, 2012⁵⁶. The inhibitory area under the curve (iAUC) was calculated from the kinetic curves over the course of the experiment and was defined as the ratio between the normalized AUC of the phage-treated bacterial growth curve and the bacteria-only control. Susceptibility was defined at iAUC values ≥ 0.2 .

Calculation of bacterial growth inhibition using iAUC

- The growth inhibitory effect of SNIPR001 was determined using growth kinetic curves constructed using the OmniLog® apparatus. To limit technical variability in measurement between timepoints, a cubic smoothing spline function was applied to the data in Scala using the “umontreal.ssj.functionfit” package. To identify appropriate ρ and weight variables, every combination of ρ and weight 0.1 and 0.5 was applied in 0.1 increments (i.e., 0.1, 0.2, ... 0.5). The spline with the lowest mean absolute error was chosen for AUC calculation. The initial cumulative amount of fluorescent dye at the initial timepoint varies slightly from well to well, leading to an artificial inflation of the AUC of certain wells. Using the best smoothed square spline, the mean signal for the first 1.5 h, prior to any measurable growth, was removed from all growth curves to approximate a zero-growth signal intercept. The total incremental AUC (iAUC) was calculated as the sum of the Riemann midpoint sums for each timepoint along the smoothed square spline. Lastly, we calculated the iAUC as $iAUC = 1 - AUC_{\text{Sample}} / AUC_{\text{Control}}$, where AUC_{Sample} is the AUC of the spline created by a given bacteria and SNIPR001, while AUC_{Control} refers to the AUC of the spline created with a given bacteria without a given phage or CAP, or a combination

of those. Thus, iAUC values usually lie between 0 and 1, where 0 indicates no growth inhibition and 1 indicates complete growth inhibition. Some biological and technical noise does result in iAUC values outside these bounds on occasion but is considered negligible.

- 5 Host-range was calculated as the fraction of a panel that had an iAUC<0.2 for each repeat. Reported standard deviations were calculated as the deviance in the number of strains with an iAUC<0.2, and then normalized to the size of the panel, by dividing the standard deviation with the size of the panel.

CRISPR-Cas-arming phages to target E. coli

- 10 Phages were CRISPR-Cas-armed by using homologous recombination. We inserted the payload in the region immediately after *gene 49* and to *gene E* when compared to a reference wild-type T2 phage. Thus, the the synthetic phage genomes comprised the insertion between coordinates 9000 and 21000, wherein the coordinates are the nucleotide positions counted from the nucleotide (coordinate number 1) immediately after *gene 49* towards *gene E* when compared to a reference wild-type T2
- 15 phage. Recombination was carried out in bacterial cells during phage propagation. Cells carried a plasmid that served as a recombination template. Recombination template plasmids carried the sequences that were aimed to be inserted into the phage genome between ~200-700 bp flanking sequences that were homologous to the phage sequences at the insertion site. For each phage, we inserted the Type-I-E CRISPR-Cas system endogenous to *E. coli* (Genbank CP032679.1), i.e., the *cas3*
- 20 gene (*vgcB*) and the downstream genes encoding the cascade complex, *casA* (*vgcL*), *casB* (*vgcK*), *casC* (*vgcJ*), *casD* (*vgcI*) and *casE* (*vgcH*), as well as a CRISPR array targeting selected *E. coli* sequences. For all CAPs selected, the *cas* genes originating from *E. coli* are identical. Insertion of the CRISPR-Cas system resulted in deletion of ~7 kbp deletion of phage DNA in the *gene 49 – gene E*. The sequences of the resulting CAPs were verified by NGS (BaseClear, Leiden, The Netherlands).

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Transduction of CGVs in biofilms

- E. coli* b52 cells were grown in 96-well plates, and biofilms were allowed to develop on peg lids. Each well contained 180 μ L M9 medium (Sigma, M6030) supplemented with 20 mM Glucose, 2 mM MgSO_4 , 0.1 mM CaCl_2 , 0.1% Amicase (Sigma), and 0.1% mannitol. Wells were inoculated with 1 μ L
- 30 of overnight b52 culture. The peg lid was inserted, and the microtiter plate was incubated static for 24 hours at 37 °C. Next, the peg lid was transferred to a new plate with fresh media without washing, and the plate was incubated for an additional 24 hours. After incubation, a new plate was prepared with 100

μL media and 100 μL of CGV transducing particles (~10⁸ particles) in each well (3 replicates). Biofilms grown on the pegs were rinsed 3 times in sterile H₂O (200 μl) before transferring them to the new plate. The plate was incubated static for 5 hours at 37 °C.

To assay the metabolic activity of cells in the biofilms, lids were rinsed three times in sterile H₂O (200 μL) before placing them in a plate with 20 μL Alamarblue stain (ThermoFisher) and 180 μl media in each well. Plates were incubated for 1.5 hours at 37 °C and moved to a microplate reader (Synergy H1, Biotek). Fluorescence (excitation: 560 nm; emission 590 nm) and absorbance (600 nm) was recorded for each well.

The metabolic activities of the biofilms treated with CGVs carrying the promoter P_{bolA} were reported relative to the metabolic activities of biofilms treated with a CGV not carrying a promoter transcribing the *cas* genes.

Plasmid and strain construction

Plasmids were constructed by InFusion HD cloning using PCR-generated DNA fragments. To construct CGV-EcCas, *cas3* and cascade genes from *E. coli* were amplified and cloned into a ColE1-type plasmid, pZE21⁵⁷. Additionally, a 3-spacer array targeting genes in *E. coli* was included in the vector under the control of constitutive promoter J23100. The array contains nucleotides from the genome of *E. coli* per target locus separated by direct repeats (repeat sequence, SEQ ID NO: 15). The protospacer adjacent motif (PAM) is located adjacent to the selected target sequences in the genome of *E. coli*.

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Transformation assays

Overnight cultures were diluted (1:100) in fresh LB medium and grown to mid-exponential phase (OD₆₀₀ ≈ 0.6). Subsequently, cells were prepared for electroporation and concentrated 50-fold in ice-cold MilliQ water. Cells were then electroporated with appropriate plasmids, allowed to recover for 1 h at 37 °C in super optimal broth (SOB), and plated on LB plates supplemented with antibiotics.

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Conjugation assays

Conjugation experiments assessing the transfer and killing efficiency of CGV-EcCas were established using *E. coli* JKE201 as the donor and *E. coli* clinical isolates as recipients (including target and non-target and *E. coli* strains as controls). Plasmids were conjugated into *E. coli* recipients by liquid mating. Briefly, overnight cultures were diluted (1:100) in fresh LB medium, grown to OD₆₀₀ ≈ 0.4, washed,

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and suspended in fresh LB to $OD_{600} \approx 0.25$. 125 μ L of donor and 25 μ L of recipient cell suspensions were mixed for 5:1 mating in a 96-well microplate and incubated for 16 h at 37°C. The conjugation efficiency was determined by plating a dilution series of conjugation reactions onto LB agar supplemented with antibiotics (to select for the transconjugants). The specific killing efficiency was quantified by plating 90 μ L of the conjugation reactions on selective plates. The CGV-EcCas plasmid encodes kanamycin, gentamycin, and amikacin resistance to enable selection for transconjugants. Viability was calculated by counting CFUs on the plates, and data were recorded as viable cell concentration (CFU/mL).

10 *Synchronized CAP infection and cas3 expression assay*

An overnight culture of the test strain in LB was 100-fold diluted and incubated to stationary phase in LB at 37 °C with shaking, and 10-mL aliquots were subsequently separated into 50-mL falcon tubes. Each aliquot was then seeded with 50 μ L of high-titer lysate of the individual CAPs, and incubation was continued under the same conditions. Additionally, a mock 10 mL LB volume for each CAPs were also seeded with 50 μ L of CAP lysates and used for 0 min phage enumeration. At 5 min, 15 min and 30 min post-seeding, aliquots were collected for total RNA extraction and phage enumeration. Phage enumeration aliquots were syringe filtered (0.2 μ m, Sartorius, Göttingen, Germany) and subjected to an efficiency of plating assay. For total RNA extraction, 1 mL aliquots of individual cultures were centrifuged at $13.3k \times g$ using a table-top centrifuge for 15s, and supernatants were discarded. Then, pellets were immediately resuspended in cold RNA Later (Thermo Fischer Scientific, AM7020) and stored at -20 °C until extraction. Total RNA was extracted using a GeneElute Total RNA kit (Sigma-Aldrich, St. Louis, MO, USA) following the manufacture's protocol for extraction of RNA from bacteria. After first elution, 1 μ L of Dnase I (1U/ μ L) was added and incubated overnight at 37°C. The reaction was terminated by incubation at 70 °C for 15 min. The RNA was re-purified on a GeneElute column and eluted in 35 μ L of kit elution buffer. Total RNA concentration was estimated on a NanoDrop instrument (Thermo Scientific, One/OneC), and 0.5 to 2 μ g of RNA was added to a cDNA synthesis reaction containing SuperScriptIII RT enzyme (Thermo Fisher, Waltham, MA, USA) and random decamers to prime synthesis in a 20- μ L reaction volume. The cDNA reaction was diluted to 100 μ L in water. Real-Time PCR was conducted in triplicate using 5 μ L of cDNA as template, 10 μ L of Power SYBR Green PCR Master mix (Thermo Fisher) and 0.2 μ M of each PCR primer. PCRs were performed on an AB QuantStudio5 system (Applied Biosystems, Foster City, CA, USA) using the standard two-step thermocycling protocol for Power SYBR Green PCR Master Mix with 60 °C annealing/extension. The forward and reverse primers for *gapA* (reference gene) were 5'-cgctaactcgacaaatgtgctggc-3' (SEQ ID NO: 17), and 5'-aggacgggatgatgttctggga-3' (SEQ ID NO: 18), and for *cas3* were 5'-caagtatgctaccaacggctaaag-3' (SEQ ID NO: 19) and 5'-ccaatcaaatcaacgtcgagtga-

3' (SEQ ID NO: 20). Single PCR products were confirmed for these primer pairs by melting curve analysis. Relative levels of transcripts were estimated using 10-fold dilutions of purified PCR products as standards, and values were expressed as the ratio of *cas3* to *gapA* transcripts.

5 *Phage competition assay*

Lysates of the two phages were mixed at 9:1 (WT:CAP) ratio and the phage mixtures were added to 10 ml 2xYT medium containing 10 mM CaCl₂ and 20 mM MgCl₂, and 100 µL overnight of *E. coli* strain b230, serving as target for both competing phages. After 2h incubation in a 37 °C shaking incubator, the cultures were centrifuged and 1 µL of the supernatant was added to a new b230 culture. The same steps were repeated twice.

The ratio of phages was assessed by PCR with three primers, resulting in two specific products, one for the WT phage and one for the CAP. PCR products were separated on a 1% agarose gel and DNA bands were stained by SYBRsafe and visualized and quantified by the ChemiDoc XRS+ System (Model 1708265, Biorad). The background-corrected intensity of the band corresponding to the WT phage was divided by the intensity of the band corresponding to the CAP in the same lane, to obtain the ratio of the two band intensities (WT/CAP). The fraction of CAP compared to the total phage content (WT+CAP) were determined based on the calibration curve, which was made by using a set of different mixtures of the two phages, and fitting a curve to the measured band intensity ratios (WT/CAP). The estimated error of the reported values is less than 20%.

Lawn killing assay

An overnight culture of the test strain in LB was adjusted to 10⁹ CFU/mL. Hundred µL aliquots of CFU/mL adjusted strain was mixed with 100 µL of 10⁹ PFU/mL to achieve a multiplicity of infection of 1 of either CAP α15.2 or WT α15 in 15 mL falcon tubes, mixed with 3 mL of molten and pre-tempered top agar and spread on LB plates. After lawns were solidified, plates were incubated at 37 °C overnight, and the total number of surviving colonies were counted for CAP α15.2 or WT α15 groups the next day. Assays were performed as independent biological duplicates where each experiment comprised of ten technical replicates. Statistical significance was established using both replicates using a Mann-Whitney U test.

Generalized transduction assay

The transduction ability of each CAP was evaluated via the generalized transduction assay. Briefly, transducing lysates were prepared by propagating each CAP on *E. coli* MG1655 *lamB::Cm*. This strain was modified from the WT MG1655 (Catalog number 700926, American Type Culture Collection, Manassas, VA, USA) to carry a chloramphenicol selection marker. Experiments were conducted in parallel with the well characterized lytic T4 phage (negative control), and its transducing mutant T4GT7⁵⁸ (positive control). Following this step, the WT *E. coli* MG1655 strain was infected at an OD₆₀₀ of 0.3 with each transducing lysate at a MoI of 0.5, 0.1 and 0.01, and spread on LB plates containing chloramphenicol. Next day, the number of transductant colonies were recorded for each CAP, controls and different MOIs. The frequency of transduction was calculated as the number of transductants divided by the titer of the transducing lysate.

Sequence analysis of CAPs

Sequences of the individual SNIPR001 CAPs were analyzed for the presence of antibiotic resistance, virulence genes and lysogeny associates genes (transposases and integrases) using databases (Table 2). Furthermore, phage samples were analyzed using whole genome sequencing. This typically results in >1000x coverage of the whole phage genome. Assemblies are constructed by down-sampling the data to 1000x average coverage for the phage and assemble using SKESA. To detect differences between samples and to detect non-majority mutations the raw reads were mapped back to the assembly using BWA (version 0.7.17).

Table 2: List of databases used for the analysis of SNIPR001 CAP sequences

Database	Source	Analysis
ResFinder 4.1	https://cge.cbs.dtu.dk/services/ResFinder	Identification of acquired genes and/or chromosomal mutations mediating antimicrobial resistance
VirulenceFinder-2.0	https://cge.cbs.dtu.dk/services/VirulenceFinder/	Detection of virulence genes including exotoxins
PHASTER Prophage/Virus DB	https://phaster.ca/	Detection of potential transposases and integrases

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Phage specificity assay using liquid killing assay

SNIPR001 CAPs (α 15.2, α 20.4, α 48.4 and α 51.5) and SNIPR001 killing specificity was evaluated via a biopotency assay against a panel of human-relevant, aerobic ($n = 6$) and anaerobic ($n = 3$) bacterial strains. An *E. coli* strain b2480, was included as positive control for phage-mediated killing (**Table 3**).

In brief, overnight cultures were adjusted to 10^6 CFU/mL in LB broth. SNIPR001 CAPs or SNIPR001 (in which each CAP was combined in equal ratio) were added at a MOI of 1 prior to incubation for 4 h. Untreated bacteria were cultured in parallel as controls for bacterial growth. CFU counts were recorded at 0 and 4 h post phage-treatment, and data are represented as $\Delta \log_{10}$ CFU/mL by subtracting the initial inoculum (0 h) from the assay end point CFU/mL (4 h).

Table 3: Panel of bacterial strains (Aerobic: $n = 6$, Anaerobic: $n = 3$, Aerobic/Anaerobic: $n = 1$) tested via a biopotency assay, showing Gram-type classification, growth conditions and source/ID

Strain/name	Gram type	Growth condition	Source/ID
<i>Acinetobacter baylyi</i>	Negative	Aerobic	ATCC 33304
<i>Klebsiella pneumonia</i>	Negative	Aerobic	SNIPR Biome ID b2951
<i>Enterococcus faecalis</i> (Andrewes and Horder) Schleifer and Kipper-Balz	Positive	Aerobic	ATCC 47077
<i>Streptococcus thermophilus</i> Orla-Jensen	Positive	Aerobic	ATCC 19258
<i>Bacillus coagulans</i> Hammer	Positive	Aerobic	ATCC 7050

<i>Staphylococcus aureus</i> subsp. <i>Aureus</i> Rosenbach	Positive	Aerobic	ATCC 12600
<i>Eubacterium limosum</i> Eggerth	Positive	Anaerobic	ATCC 8486
<i>Bacteroides vulgatus</i> Eggerth and Gagnon	Negative	Anaerobic	ATCC 8482
<i>Bacteroides thetaiotaomicron</i> (Distaso) Castellani and Chalmers	Negative	Anaerobic	ATCC 29148
<i>E. coli</i>	Negative	Aerobic/Anaerobic	Takara Cat. #636763

Spotting assay and efficiency of plating (EoP)

For counting of phage titers, phage lysates or the equal volume mix of SNIPR001 CAPs were serially diluted 10-fold in SM buffer or PBS, respectively. Bacterial lawns were prepared by adding 100 or 300 μ L of bacterial overnight culture to 3 or 10 mL of 0.5% top agar (containing Ca^{2+} and Mg^{2+}), which was vortexed briefly and poured onto a round or square LB plate. Five μ L of the dilution series of test phages were then spotted onto lawns and left to dry at room temperature with an open lid prior to incubation at 37°C overnight. The strains b52, b2479 and b17 were used as controls of the assay and included in each round of assays.

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The next day, results were assessed (**Table 4**). In this assay, a susceptible strain is defined as one producing plaques that are countable in PFU/mL as well as one without visible plaques but demonstrating impairment of bacterial growth (i.e., lysis zones). Coverage defines the percentage of the total number of susceptible strains. Images of all plates were recorded. Figures illustrating efficiency of plating results first had titers $\log_{10}$ transformed and then standard deviances and averages were calculated subsequently. The clinical panels and control strains were tested in two independent experiments.

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Table 4 Criteria used to evaluate results of the spot assay and define strain susceptibility following standards^{46,59}

Spot assay categories	Observation	Strain susceptibility to SNIPR001
Plaques	Visible plaques were counted, and PFU/mL was calculated by multiplying with volume and dilution	Susceptible strain
Lysis zone	Impairment of bacterial growth observed as lysis zones. No plaques are visible; the highest dilution of visible zones is recorded	Susceptible strain
Negative	Neither plaques nor lysis zones are detected	Non-susceptible strain

5 Animals and housing

Mouse studies were performed with female CD-1® IGS mice (approximately 6–7 weeks of age upon arrival) from Charles River (Freiburg, Germany). The animals were housed in groups of 3 to 5 mice per cage within a climate-controlled room (temperature 20–23°C; relative humidity 30–70%) under a 12:12 h light-dark cycle (illuminated 07:00–19:00). Standard pelleted chow and tap water were available *ad libitum*. Animals were allowed an acclimatization period of at least 7 d before the start of the experimental procedures. 30 female Göttingen minipigs (approximately 4–7 months of age upon arrival) from Ellegaard Göttingen minipigs A/S, Denmark was used for tolerability and kinetic studies. Animals were allowed an acclimatization period of at least 14 days before the start of experiments. Pigs were housed in groups of 2 to 3 animals and given standard pig diet twice daily and tap water was available *ad libitum*. All procedures were conducted in accordance with guidelines from the Danish Animal Experiments Inspectorate, Ministry of Environment and Food of Denmark and in accordance with the institutional license (BioAdvice, animal license no. 2015-15-0201-00540).

Mouse gut colonization model

The mouse gut colonization model was adapted from *Galtier et al.* (2016)⁴⁴. Briefly, pre-treatment with streptomycin (5 g/L) in the drinking water was given 3 days prior inoculation with *E. coli* b17 to decrease the level of native bacteria. On day 0, an inoculum of 3×10^7 CFU of *E. coli* b17 was prepared from a frozen glycerol stock and administered to all mice in 0.25mL by oral gavage.

Treatment was administered three times daily for 2 days starting 2 days after inoculation. Right before each administration, the 4 CAPs were mixed in a 1:1:1:1 ratio to form SNIPR001 at a high, medium, or low concentration resulting in dose levels of 2×10^{11} , 2×10^9 and 1×10^7 PFU. At the time of treatment, mice were administered 0.1 mL of 10% sodium bicarbonate by oral gavage followed by the oral administration of 0.3 mL of SNIPR001, saline (vehicle) or 43.5 mg/kg gentamicin.

CAP recovery and tolerability studies

Göttingen minipigs were first given a cocktail of antibiotic comprising neomycin (60 mg/kg, orally, once daily for 4 days) and cefquinome (2 mg/kg, intramuscular once daily for 3 days) before SNIPR001 or single CAP administration to decrease the level of Gram-negative bacteria in the GI tract and therefore limiting phage replication. Animals were then fasted overnight and lightly sedated before administration of a single CAP, or SNIPR001 cocktail, once orally at 2×10^{12} PFU in 100 mL, following an oral administration of 50 mL of 10% sodium bicarbonate. Fecal samples were collected daily for CAPs quantification by plaque assay. In addition, for the tolerability study, blood samples were collected for hematology and blood chemistry analysis, including C-reactive protein, and plaque assay. Animals were closely monitored following SNIPR001 administration, and their body temperature was recorded regularly.

*Quantification of *E. coli* b17 and CAPs in feces*

Fecal samples were homogenized and serially diluted in SM buffer. Triplicates of 10 µl of each dilution were then spotted on McConkey agar plates (Sigma, M7408) supplemented with streptomycin (1 mg/mL) and incubated for 12 to 16h at 37 °C for *E. coli* enumeration.

Plaque assays were performed for enumeration of CAPs in feces samples. Briefly, homogenized samples were centrifuged at 10,000g for 10min, and the supernatant was serially diluted. Triplicates of 10 µl of each dilution were spotted on an *E. coli* b17 overlay and incubated for 12 to 16h at 37°C.

- 5 To quantify the presence of *in vivo* resistors, three colonies from each mice fecal sample in the medium dose group at 3 different time points were picked from the McConkey agar plates. Colonies were incubated for 12 to 16h at 37 °C in LB broth and used to make top agar overlays on LB agar plates. Then, plates were dried for 15 minutes in the LAF bench. The SNIPR001 cocktail, as well as the 4 individual CAPs, were spotted as a dilution series from 1×10^5 PUF/mL stocks. As a control, a top agar
- 10 overlay of colonization strain *E. coli* b17 was spotted in the same way. Plates were left to dry in the LAF bench with the lid on and subsequently incubated upside down for 12 to 16h at 37°C.

Whole Genome Sequencing of E. coli strains from JMI

- Total genomic DNA was extracted and purified using the KingFisher Cell and Tissue DNA kit (Thermo Scientific, Waltham, MA, USA) in a robotic KingFisher™ Flex Magnetic Particle Processor (Thermo Scientific) workstation.
- 15

Total genomic DNA was used as input material for library construction. DNA libraries were prepared using the Nextera XT™ library construction protocol and index kit (Illumina, San Diego, CA, USA) and sequenced on a MiSeq Sequencer (Illumina) using MiSeq Reagent Kits v3 (600 cycle).

- 20 *Resistance Phenotype Definitions*
- The extended-spectrum β-lactamase (ESBL)-phenotype was defined for *Escherichia coli* as a minimum inhibitory concentration (MIC) value ≥ 2 mg/L for ceftriaxone, ceftazidime and/or aztreonam (<https://clsi.org/>).
- Carbapenem-resistant *Enterobacterales* (CRE) was defined as any isolate displaying imipenem,
- 25 doripenem, and/or meropenem resistance with MIC > 2 mg/L (<https://clsi.org/>).

Assembly of whole genome sequencing data

- Raw sequencing reads were trimmed using Trimmomatic⁶⁰ (version 0.39) with the settings “LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36”. Trimmed reads were assembled
- 30 using SPAdes⁶¹ (version 3.14.1) with default settings. Contigs shorter than 500 bp or with a sequencing depth below 2x were removed from the final assemblies.

Comparative genomic methods for clinical E. coli strains

Multi-locus sequence typing (MLST) was performed using MLST2⁶² on the assembled genomes of the *E. coli* bacteria using default settings, with the MLST database downloaded on July 1, 2021, from the MLST2 repository (https://bitbucket.org/genomicepidemiology/mlst_db/src/master/). Phylogroup classification was conducted using ClermonTyping⁶³ on the assembled *E. coli* genomes using default settings. Distance matrices for phylogenetic tree construction were generated using MASH⁶⁴ with a k-mer size of 21 and 10,000 sketches per genome. Sketches were then compared to create the MASH-distance in a pairwise manner to create a distance matrix of *E. coli* genomes.

10

Phage synteny analysis

To generate the synteny plot, wild type sequences of the four phages included in the final cocktail, plus the two closely related and well-known reference phages (RB69 AY303349.1, and T2 NC_054931.1) were annotated with RAST to extract predicted protein sequences. All protein sequences for each phage were queried against all other phage genomes using tblastn (v 2.12.0), with an E-value cutoff of 1e-10. The synteny plot was then generated using a custom Python script (see Data Availability), using the drawSvg library (v 1.9.0). The plot shows the phage genomes in order of similarity and displays all tblastn hits as synteny blocks shaded by their protein identity. The proteins of the two reference phages were manually classified as belonging to each of the functional groups “DNA metabolism”, “Structure” or “Other”, and colored accordingly.

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Data processing and visualization

Figures and key statistics were generated using R version 4.1.0. For figure generation the following packages were used: RcolorBrewer v. 1.1-2, ape v. 5.5, ggsignif v. 0.6.2, ggpubr v. 0.4.0, matrixStats v. 0.59, reshape2 v. 1.4.4, ggimage v. 0.3.0, here v. 1.0.1, purr v. 0.3.4, ggtree⁶⁵ v. 3.0.2, systemfonts v. 1.0.2, Cairo v. 1.5-12.2, cowplot v. 1.1.1, reaxxl v. 1.3.1, and ggplot2 v.3.3.3. Averages and standard deviations are calculated after transforming the values to the scale shown on a given figure, e.g. when a log₁₀ scale is used, the averages and standard deviations are calculated after log₁₀ transformation.

30

DATA AVAILABILITY

Data and results that were generated during this study is deposited at https://github.com/sniprbiome/SNIPR001_paper. Phage genome sequences are deposited at Genbank under access numbers OQ067373 - 76

5 CODE AVAILABILITY

All code needed to produce this study is available at https://github.com/sniprbiome/SNIPR001_paper.

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SEQUENCES

Amino acid sequences are written in N- to C-terminal direction and DNA sequences are written in 5' to 3' direction.

PROTEIN

5 >LamB (SEQ ID NO: 1)

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20

DNA

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>NC_000866.4 Enterobacteria phage T4, complete genome (SEQ ID NO: 5)

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Spacer targeting *E. coli* fimH (SEQ ID NO: 6)

40 CGAATGACCAGGCATTTACCGACCAGCCCATC

Spacer targeting *E. coli* bolA (SEQ ID NO: 7)

AGTGGGAAGGGTTGCAGGACACCGTCTTTGCC

5 Spacer targeting *E. coli* rpoH (SEQ ID NO: 8)

CCGATGTTACCTTCCTGAATCAAATCCGCCTG

Spacer targeting *E. coli* lptA (SEQ ID NO: 9)

TGATTGACGGCTACGGTAAACCGGCAACGTTT

10

Spacer targeting *E. coli* murA (SEQ ID NO: 10)

GCTGTTAACGTACGTACCGCGCCGCATCCGGC

Escherichia phage T2 DNA, complete sequence (NCBI Reference Sequence: NC_054931.1)**15 (SEQ ID NO: 11)**

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Enterobacteria phage RB69, complete genome (GenBank: AY303349.1) (SEQ ID NO: 12)

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TGTTGAAATGTCGAAGTAATCGGCAATTTCTTTACGAGTCCATTTGCCTGATTCAACCAGGCCGTAAGCT
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5

bolA promoter sequence (SEQ ID NO: 13)

AACCTAAATATTTGTTGTTAAGCTGCAATGGAAACGGTAAAAGCGGCTAGTATTTAAAG
GGATGGATGACATCTCAGCGTTGTCG

10 **P_{J23100} promoter sequence (SEQ ID NO: 14)**

TTGACGGCTAGCTCAGTCCTAGGTACAGTGCTAGC

Repeat Sequence (SEQ ID NO: 15)

CGGTTTATCCCCGCTGGCGCGGGGAAGTC

15

Promoter sequence (SEQ ID NO: 16)

TTGACGCGTAGCTCAGAGGTAGGTATAATGCTAGAAC

CLAIMS:

1. A composition comprising a plurality of different types of transduction particles, wherein each of said particles comprises a nucleic acid and said particles are capable of contacting *E coli* cells and introducing therein the nucleic acid, wherein
 - 5 (a) said nucleic acid of each particle comprises a nucleotide sequence encoding a product of interest (POI), wherein the nucleic acid is capable of expressing the POI in an *E coli* cell;
 - (b) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS and Tsx displayed on the surface of *E coli* cells; and
 - (c) said plurality of different types of transduction particles comprises (i) a first type of particles
 - 10 that comprise a LPS adhesion moiety; and (ii) a second type of particles that comprise a Tsx adhesion moiety.
2. The composition of claim 1, wherein
 - A: each particle of the first type comprises a LPS adhesion moiety and a LamB adhesion moiety;
 - 15 B: each particle of the first type comprises a LPS adhesion moiety and a Tsx adhesion moiety; or
 - C: each particle of the second type comprises a Tsx adhesion moiety but is devoid of LamB and LPS adhesion moieties.
3. The composition of claim 2, wherein the composition comprises particles according to claims 2A,
 - 20 2B and 2C.
4. The composition or method of any preceding claim, wherein each particle comprises a phage capsid containing the nucleic acid.
5. The composition of claim 4, wherein the capsid of each particle comprised by the composition is
 - 25 a T-even phage capsid; optionally a capsid of a T2 phage, T2-like phage, RB69 phage or a RB69-like phage.
6. The composition of any preceding claim, wherein each particle is a phage (optionally a lytic
 - 30 phage) or packaged phagemid.
7. The composition of any preceding claim, wherein the composition comprises at least 3 or 4 different types of transduction particles.
8. The composition of any preceding claim, wherein the nucleic acid of each particle comprises at
 - 35 least one nucleotide sequence (N1) encoding said POI, wherein each particle is a synthetic T-even phage comprising an insertion of N1 into a Modification Permissive Region (MPR) of the genome

of the phage, wherein the MPR is immediately after *gene 49* and to *gene E* when compared to a reference wild-type T2 phage.

9. The composition of claim 8, wherein the nucleic acid comprises a DNA deletion of phage DNA in the MPR.
10. The composition of claim 8 or 9, wherein the insertion comprises up to 8000bp of DNA and/or the deletion comprises up to 8000bp of DNA.
11. The composition of any one of claims 8-10, wherein the MPR comprises contiguous DNA between said *gene 49* and *gene E*, wherein the contiguous DNA is at least 1000bp in length; or wherein the MPR comprises at least 100bp of DNA between said *gene 49* and *gene E*.
12. The composition of any one of claims 8-11, wherein the synthetic phage genome comprises said insertion between coordinates 9000 and 21000, wherein the coordinates are the nucleotide positions counted from the nucleotide (coordinate number 1) immediately after *gene 49* towards *gene E* when compared to a reference wild-type T2 phage.
13. The composition of any preceding claim, wherein the POI comprises
 - (a) a nuclease for targeting the DNA of an *E coli* cell, wherein the nuclease is capable of being expressed in the cell for cutting the DNA of the cell, thereby modifying or killing the cell; or
 - (b) a dead Cas (dCas) for targeting the DNA of an *E coli* cell, wherein the dCas is capable of being expressed in the cell and targeting the DNA of the cell, thereby modifying the cell.
14. The composition of claim 13(a), wherein said nuclease is a guided nuclease, optionally a Cas, meganuclease, zinc finger nuclease or TALEN; or the composition of claim 13(b) wherein the dCas is a dCas9.
15. The composition of claim 13 or 14, wherein the nuclease is a Type I, II, III, IV, V or VI Cas nuclease, optionally a Cas9 or a Cas3.
16. The composition of any preceding claim, wherein the POI comprises at least one crRNA or guide RNA that is operable with a Cas for DNA targeting in *E coli* cells.
17. The composition of claim 16, wherein each crRNA or guide RNA comprises a spacer sequence that is complementary to an *E coli* protospacer sequence; optionally a protospacer of a B2 phylogroup *E coli* cell or an *E coli* cell of a strain selected from the group ST131, ST1193,

ST648, ST315, ST405, ST361, ST88 and ST453.

18. The composition of claim 17, wherein the protospacer sequence is comprised by a gene selected from *E coli* genes *fimH*, *bolA*, *rpoH*, *lptA* and *murA*.
- 5
19. The composition of claim 18, wherein the particles of the composition target all of *E coli* genes *fimH*, *bolA*, *rpoH*, *lptA* and *murA*.
20. The composition of any preceding claim, wherein the nucleotide sequence encoding the POI comprises a stress-phase active (SPA) promoter for expression of the POI in *E coli* cells; optionally wherein the promoter is an *E coli bolA* promoter or comprises SEQ ID NO: 13.
- 10
21. The composition of any preceding claim, wherein the *E coli* cells comprise a strain of *E coli* that causes sepsis, septicaemia or diarrhoea in humans or animals.
- 15
22. The composition of any preceding claim, wherein the composition is for use in a method of treating or preventing infection of by *E coli* cells in a human or animal subject, wherein the method comprises administering the particles to the subject.
- 20
23. A method of treating or preventing sepsis, septicaemia or diarrhoea in a human or animal subject, the method comprising administering to the subject the composition of any preceding claim, wherein the *E coli* cells comprise a strain of *E coli* that causes sepsis, septicaemia or diarrhoea in humans or animals.
- 25
24. A method of treating or preventing infection of by *E coli* cells in a human or animal subject, wherein the method comprises administering the composition of any one of claims 1-21 to the subject, wherein the infection is treated or prevented.
- 30
25. The composition or method of any one of claims 22-24, wherein the subject is a transplant or cancer patient (optionally a haematological cancer patient), or wherein the patient is suffering from or at risk of a urinary tract infection (UTI); and optionally wherein the transplant is a solid organ or stem cell transplant (optionally a haematopoietic cell transplant) or wherein the transplant is a transplant of a medical device.
- 35
26. The composition or method of claim 25, wherein the method is carried out prior to the subject receiving a transplant.

27. The composition or method of any preceding claim for preventing haemolytic uremic syndrome (HUS), a UTI infection, sepsis, septicaemia or diarrhoea in a human subject.
28. The composition or method of any one of claims 22-27, wherein at least 1×10^7 PFU of particles
5 are administered to the subject; or a dose of a composition according to any one of claims 1-22 and 25-27 wherein the dose is a dose of at least 1×10^7 PFU of said particles.
29. The composition or method of any one of claims 22-28, wherein the particles are administered to the subject at an MOI (multiplicity of infection) of at least 0.01.
10
30. The composition, method or dose of any preceding claim, wherein the *E coli* cells comprise at least one strain that is an antibiotic-resistant or MDR strain; and/or at least one B2-I strain.
31. The composition, method or dose of claim 30, wherein the antibiotic is fluoroquinolone
15 (optionally levofloxacin), carbapenem or vancomycin; and/or wherein the *E coli* cells comprise beta-lactamase (ESBL)-producing *E coli*.
32. The composition, method or dose of any preceding claim, wherein
20 (i) the composition comprises first and second types of particles according to claims 2A, 2B and 2C;
(ii) the POI comprises a guided nuclease for targeting the DNA of an *E coli* cell, wherein the nuclease is capable of being expressed in the cell for cutting the DNA of the cell, thereby modifying or killing the cell;
(iii) each particle comprises a phage capsid containing the nucleic acid; and
25 (iv) the particles of the composition target a plurality of different *E coli* genes, optionally selected from essential and virulence genes.
33. A method of detecting the presence of *E coli* (optionally B2 phylogroup *E coli* cells) in a sample, the method comprising
30 (a) contacting the sample with a composition according to any preceding claim; and
(b) detecting that *E coli* cells have been killed or the growth or proliferation thereof has been reduced.
34. A method of detecting the presence of *E coli* (optionally B2 phylogroup *E coli* cells) in a sample, the method comprising
35 (a) contacting the sample with a composition according to any preceding claim; wherein particles of the composition comprise a nucleic acid comprising or encoding a detectable label, wherein the

said particles contact the cells and introduce therein the nucleic acid, wherein optionally the label is expressed in the cells; and

(b) detecting that *E coli* cells comprising the label.

- 5 35. A method for modifying the genomes of *E coli* cells, the method comprising contacting the cells with a composition of any preceding claim, wherein nucleic acid encoding the POI is introduced into the cells thereby modifying the genomes of the cells.
36. The method of any one of claims 33-35, wherein the sample is a patient sample (eg, blood, urine, stool or saliva sample), wherein the subject is a transplant or cancer patient (optionally a haematological cancer patient), or wherein the patient is suffering from or at risk of a urinary tract infection (UTI); and optionally wherein the transplant is a solid organ or stem cell transplant (optionally a haematopoietic cell transplant).
- 10 37. The method of any one of claims 33-36, wherein at least 1×10^7 PFU of particles are contacted with the sample.
38. The method of any one of claims 33-37, wherein the particles are contacted with the sample at an MOI (multiplicity of infection) of at least 0.01.
- 20 39. A composition comprising a plurality of transduction particles for use in a method of treating or preventing an infection by *E coli* cells in a human or animal subject, wherein the method comprises administering the particles to the subject, wherein
- (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby killing the cells or reducing growth or proliferation of the cells in the subject;
- 25 (b) the *E coli* cells are cells of *E coli* phylogroup B2; and
- (c) each particle comprises an adhesion moiety for recognising and binding to a cognate moiety selected from a LPS, LamB and Tsx displayed on the surface of the phylogroup B2 *E coli* cells.
- 30 40. The composition of claim 39, wherein each particle comprised by the composition comprises a T-even phage capsid; optionally a capsid of a T2 phage, T2-like phage, RB69 phage or a RB69-like phage.

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41. The composition of any claim 39 or 40, wherein each particle is a phage (optionally a lytic phage) or packaged phagemid.
42. A composition comprising a plurality of transduction particles for use in a method of treating or preventing an infection by *E coli* cells (optionally B2 phylogroup *E coli* cells) in a human or animal subject, wherein the method comprises administering the particles to the subject, wherein
- 5 (a) each particle comprises a nucleic acid encoding a nuclease for targeting the genomes of *E coli* cells, wherein the said administered particles contact the cells and introduce therein the nucleic acid, wherein the nuclease is expressed in the cells and cuts genomic DNA of the cells, thereby
- 10 killing the cells or reducing growth or proliferation of the cells in the subject; and
- (b) each particle comprised by the composition is a T-even phage capsid; optionally a capsid of a T2 phage, T2-like phage, RB69 phage or a RB69-like phage.
43. The composition of any one of claims 39-42, wherein the composition comprises at least 3 or 4
- 15 different types of transduction particles, wherein the types have different adhesion moieties for recognising and binding to a cognate moieties comprised by *E coli* (optionally B2 phylogroup *E coli*) cells.
44. The composition according to any one of claims 39-43, wherein
- 20 (i) the nuclease is a guided nuclease for targeting the DNA of an *E coli* cell, wherein the nuclease is capable of being expressed in the cell for cutting the DNA of the cell, thereby killing the cell;
- (ii) each particle comprises a phage capsid containing the nucleic acid; and
- (iii) the particles of the composition target a plurality of different *E coli* genes, optionally selected
- 25 from essential and virulence genes; and
- (iv) optionally the composition comprises
- A: particles which comprise a LPS adhesion moiety and a LamB adhesion moiety;
- B: particles which comprise a LPS adhesion moiety and a Tsx adhesion moiety; or
- C: : particles which comprise a Tsx adhesion moiety but is devoid of LamB and LPS adhesion
- 30 moieties.
45. The composition according to any one of claims 39-43, wherein the nuclease is a Cas nuclease and the nucleic acid encodes at least one crRNA or guide RNA that is operable with the Cas for DNA targeting in *E coli* cells.
- 35
46. The composition of claim 45, wherein each crRNA or guide RNA comprises a spacer sequence that is complementary to an *E coli* protospacer sequence; optionally a protospacer of a B2

phylogroup *E coli* cell or an *E coli* cell of a strain selected from the group ST131, ST1193, ST648, ST315, ST405, ST361, ST88 and ST453.

47. The composition of claim 46, wherein the protospacer sequence is comprised by a gene selected
5 from *E coli* genes *fimH*, *bolA*, *rpoH*, *lptA* and *murA*.
48. The composition of claim 47, wherein the particles of the composition target all of *E coli* genes
fimH, *bolA*, *rpoH*, *lptA* and *murA*.
- 10 49. The composition of any one of claims 39-48, wherein the nucleotide sequence encoding
comprises a stress-phase active (SPA) promoter for expression of the nuclease in *E coli* cells;
optionally wherein the promoter is an *E coli bolA* promoter or comprises SEQ ID NO: 13 (or a
promoter sequence that is at least 80, 90, 95, 96, 97, 98 or 99% identical to SEQ ID NO: 13).
- 15 50. The composition of any one of claims 39-49, wherein the *E coli* cells comprise a strain of *E coli*
that causes sepsis, septicaemia or diarrhoea in humans or animals.
51. The composition of any preceding claim, wherein the composition is for use in a method of
treating or preventing infection of by *E coli* cells in a human or animal subject, wherein the
20 method comprises administering the particles to the subject.
52. A method of treating or preventing sepsis, septicaemia or diarrhoea in a human or animal subject,
the method comprising administering to the subject the composition of any one of claims 39-51,
wherein, wherein the *E coli* cells comprise a strain of *E coli* that causes sepsis, septicaemia or
25 diarrhoea in humans or animals.
53. A method of treating or preventing infection of by *E coli* cells in a human or animal subject,
wherein the method comprises administering the composition of any one of claims 39-51 to the
subject, wherein the infection is treated or prevented.
30
54. The composition or method of any one of claims 51-53, wherein the subject is a transplant or
cancer patient (optionally a haematological cancer patient), or wherein the patient is suffering
from or at risk of a urinary tract infection (UTI); and optionally wherein the transplant is a solid
organ or stem cell transplant (optionally a haematopoietic cell transplant) or wherein the
35 transplant is a transplant of a medical device.

55. The composition or method of claim 54, wherein the method is carried out prior to the subject receiving a transplant.
56. The composition or method of any one of claims 39-55, for preventing haemolytic uremic syndrome (HUS), a UTI infection, sepsis, septicaemia or diarrhoea in a human subject.
57. The composition or method of any one of claims 52-56, wherein at least 1×10^7 PFU of particles are administered to the subject; or a dose of a composition according to any one of claims 1-22 and 25-27 wherein the dose is a dose of at least 1×10^7 PFU of said particles.
58. The composition or method of any one of claims 52-57, wherein the particles are administered to the subject at an MOI (multiplicity of infection) of at least 0.01.
59. The composition, method or dose of any one of claims 39-58, wherein the *E coli* cells comprise at least one strain that is an antibiotic-resistant or MDR strain; and/or at least one B2-I strain.
60. The composition, method or dose of claim 59, wherein the antibiotic is fluoroquinolone (optionally levofloxacin), carbapenem or vancomycin; and/or wherein the *E coli* cells comprise beta-lactamase (ESBL)-producing *E coli*.