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Bacteriophages to reduce gut carriage of antibiotic resistant uropathogens with low impact on microbiota composition

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Summary

Uropathogenic *Escherichia coli* (UPEC) is the leading cause of urinary tract infections (UTIs) worldwide, causing over 150 million clinical cases annually. There is currently no specific treatment addressing the asymptomatic carriage in the gut of UPEC before they initiate UTIs. This study investigates the efficacy of virulent bacteriophages to decrease carriage of gut pathogens. Three virulent bacteriophages infecting an antibiotic-resistant UPEC strain were isolated and characterized both *in vitro* and *in vivo*. A new experimental murine model of gut carriage of *E. coli* was elaborated and the impact of virulent bacteriophages on colonization levels and microbiota diversity was assessed. A single dose of a cocktail of the three bacteriophages led to a sharp decrease in *E. coli* levels throughout the gut. We also observed that microbiota diversity was much less affected by bacteriophages than by antibiotics. Therefore, virulent bacteriophages can efficiently target UPEC strains residing in the gut, with potentially profound public health and economic impacts. These results open a new area with the possibility to manipulate specifically the microbiota using virulent bacteriophages, which could have broad applications in many gut-related disorders/diseases and beyond.

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Introduction

Urinary tract infections (UTIs) are the most common type of bacterial infection worldwide, with more than 150 million clinical cases per year (Harding and Ronald, 1994; Totsika *et al.*, 2012). Extra-intestinal pathogenic *Escherichia coli* such as uropathogenic *E. coli* (UPEC) are reported to colonize the human gut asymptomatically for months, or even years, a situation referred to as 'carriage'. The use of antibiotics has been shown to increase the relative abundance of drug-resistant UPEC strains in feces, whilst increasing the occurrence of UTIs in women by a factor of 10 (Tosh and McDonald, 2012; Ruppe *et al.*, 2013).

UPEC carriage has yet to be successfully addressed, despite the major public health and economic impacts of UTIs (Brumbaugh and Mobley, 2012). Non-specific treatments such as cranberry juice and probiotics are moderately effective, and prophylactic antibiotic treatment is no longer recommended due to increasing prevalence of multidrug-resistant bacteria as well as major disturbances in the microbiota (Grin *et al.*, 2013; Ruppe *et al.*, 2013; Takahashi *et al.*, 2013; Keeney *et al.*, 2014). No vaccine specifically targeting UPEC strains has yet been developed.

Bacteriophages – viruses infecting bacteria – are highly abundant and present in diverse ecological niches such as the natural microflora of humans (Minot *et al.*, 2013). Bacteriophages have a limited species-specific host range and consequently, they are of particular interest as a potential agent capable of targeting bacterial pathogens in a specific manner, without massive effects on the resident microflora. In the last century, bacteriophages were exploited in a medical approach, known as phage therapy, before this method was supplanted by the discovery of antibiotics (Abedon *et al.*, 2011; Viertel *et al.*, 2014; Wittebole *et al.*, 2014). Reports of experimental phage therapy and a few clinical trials targeting various multidrug-resistant pathogens, including *E. coli* strains, have been published or are currently underway as the European-funded phagoburn

project (NCT02116010, NCT00937274) (Häussler, 2006; Wright *et al.*, 2009; Sarker *et al.*, 2012; Dufour *et al.*, 2015; Sarker *et al.*, 2016).

We used a multidrug-resistant UPEC strain to investigate whether bacteriophages could efficiently target an extra-intestinal pathogen during its carriage in the gut. Three new virulent bacteriophages were isolated, characterized and assembled into a cocktail, a single administration of which led to a sharp decrease in the levels of the UPEC strain throughout the entire gut. The composition of fecal microbiota, assessed on the basis of 16S rRNA sequences, was much less severely affected by bacteriophages than by antibiotic regimens. This approach could therefore be used as a new method for decreasing the intestinal carriage of UPEC strains but also potentially other drug-resistant pathogens and, more broadly, to manipulate the gut microbiota.

Results

Three virulent bacteriophages infect UPEC strain AL505 both in vitro *and* in vivo

We isolated three bacteriophages, AL505_P1, AL505_P2 and AL505_P3 using *E. coli* strain AL505, which is clinical isolate resistant to multiple antibiotics obtained from a collection of UTI strains (Archambaud *et al.*, 1988). These bacteriophages displayed distinct host range on the *E. coli* Collection of Reference (ECOR) and respectively belonged to *Siphoviridae*, *Myoviridae* and *Podoviridae* subfamilies of viruses (Fig. 1A and see Supporting Information Table S1). Genomic sequences of these bacteriophages and their host revealed that these viruses are closely related to bacteriophages K1H, RB49 and K1E respectively and that strain AL505 belonged to the B2 phylogenetic group and is closely related to strain S88 (see Supporting Information Table S1 and Fig. S1) (Johnson *et al.*, 2007). When the three bacteriophages were assembled into a cocktail (containing equal amounts of each of them) suspended cells of AL505 were lysed and in contrast to individual bacteriophages, no subsequent increase in optical density was observed, even after 15 h of incubation and no bacteriophage insensitive mutants were detected (Fig. 1B and methods). We also determined the efficacy of this cocktail to infect strain AL505 cells growing as a biofilm showing that a concentration of 1×10^4 pfu/ml led to 70% reduction of biofilm compared with control (Fig. 1C). In a murine model of gut colonization, in which strain AL505 remains at high levels by using a constant antibiotic pressure (Fig. 2A), we administrated a single dose of each of the three bacteriophages individually, or the cocktail, and observed that the active replication of these viruses persisted for more than three weeks (Fig. 2B), with no major change in the fecal level of strain AL505. These data are similar to those obtained previously with the same protocol

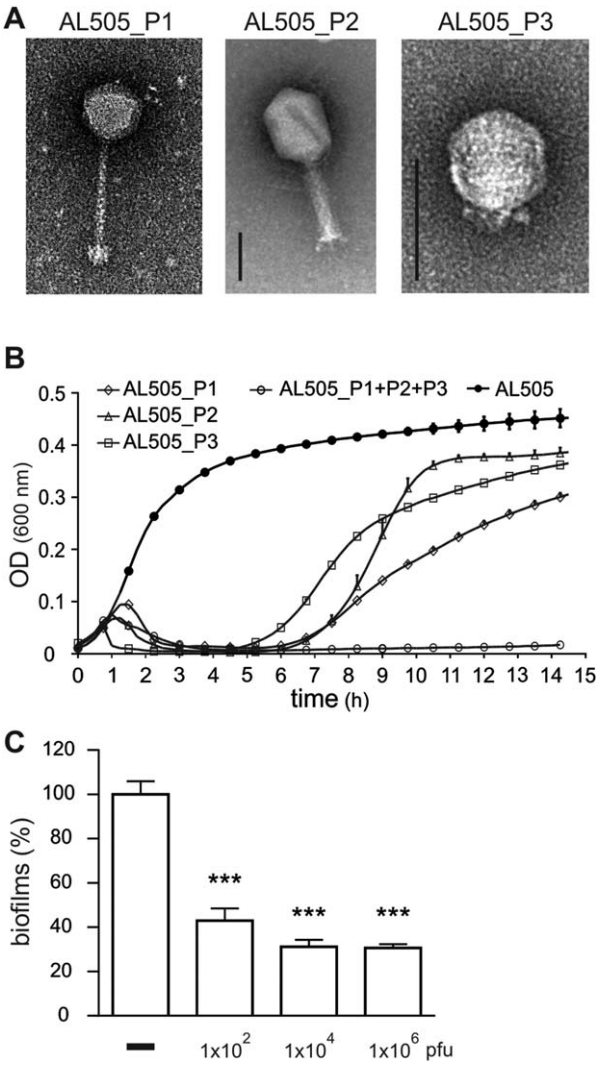

Fig. 1. Characteristics of bacteriophages AL505_P1, AL505_P2 and AL505_P3.
A. Electron micrographs of bacteriophages AL505_P1, AL505_P2 and AL505_P3. The scale bar represents 100 nm.
B. Lysis kinetics of UPEC strain AL505 infected with bacteriophages. The UPEC strain AL505 was incubated alone (black circles), or with AL505_P1 (diamonds), AL505_P2 (triangles), AL505_P3 (squares), or the cocktail of the three bacteriophages (white circles), at 37°C and $OD_{600\ nm}$ readings were taken every 15 min. A multiplicity of infection of 1×10^{-3} was used for all conditions (the values indicated are the means of two independent experiments, with $n = 6$ for each condition).
C. Infection of UPEC strain AL505 biofilms *in vitro* with the cocktail of bacteriophages. 48-hour-old biofilms of UPEC strain AL505 were exposed for 24 h to LB (-) or to various amounts (1×10^2, 1×10^4, and 1×10^6 pfu/ml) of a cocktail consisting of equal proportions of the AL505_P1, AL505_P2 and AL505_P3 bacteriophages. Biofilm biomass was quantified by crystal violet staining and the results are expressed as a ratio relative to the control (LB). Experiments were performed in duplicate, with $n = 10$ per set of conditions, $^{***}P < 0.001$.

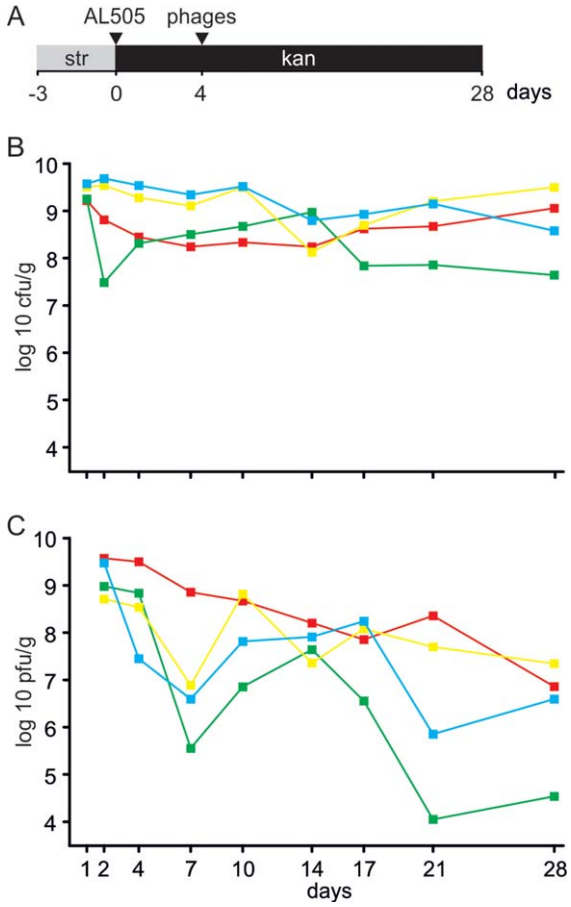

Fig. 2. *In vivo* replication of bacteriophages in AL505-colonized mice.
A. Scheme of the model of gut colonization with strain AL505 in the presence of continuous antibiotic pressure.
B. C. Following the administration of streptomycin in drinking water for 3 days, eight mice received drinking water with kanamycin, which was replaced every 5 days, throughout the experiment. One day before the per os administration of bacteriophage solutions (2×10^7 pfu of each bacteriophage; AL505_P1, blue; AL505_P2, yellow; AL505_P3, green; cocktail, red) the UPEC strain AL505 was administered per os and the mice were split into four groups ($n = 2$). Feces were collected at the indicated time points for the counting of AL505 cells (B) and bacteriophages (C).

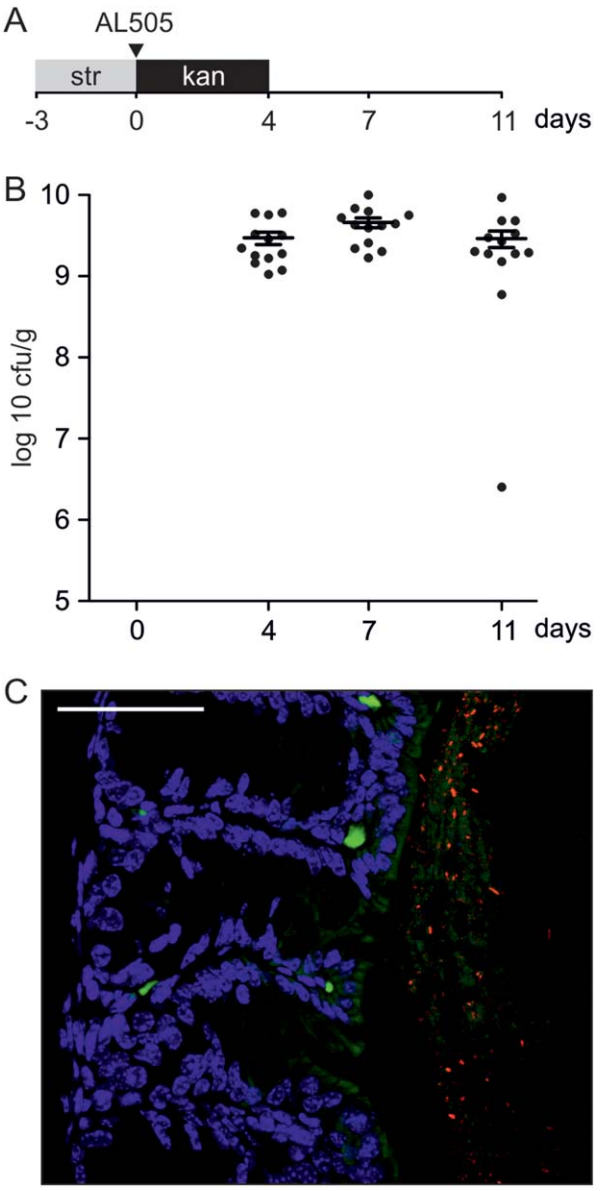

Fig. 3. Characterization of the model of gut colonization with UPEC strain AL505.
A. Scheme of the model of gut colonization with strain AL505 in the absence of continuous antibiotic pressure.
B. Kinetics of fecal levels of strain AL505 in mice in the absence of antibiotic pressure.
C. *In situ* localization of strain AL505 in the colonic segment of mice. *E. coli*-specific EC1531 – Cy3 probe (red) and DAPI (blue) were used to visualize strain AL505 and DNA, respectively ($n = 5$; a representative image is shown). Scale bar = 50 μm.

but using a different set of three bacteriophages infecting enteroaggregative strain 55989 (O104:H4) (Maura *et al.*, 2012a,b). A significant but temporary decrease in the colonization of strain 55989 was reported in the intestinal tract or feces using a dose of 10^8 pfu/ml or 10^{10} pfu/ml respectively (Maura *et al.*, 2012a).

A single dose of bacteriophages strongly decrease strain AL505 level in the gut

The continuous antibiotic pressure used on the above model could confound the results of the impact of bacteriophages as antibiotics favor the colonization while bacteriophages aim at decreasing it. We then developed a new gut colonization protocol which would not request a continuous antibiotic pressure (Fig. 3A; methods). First, streptomycin was added in drinking water for 3 days. Then, mice were force-fed UPEC strain AL505 and immediately streptomycin was replaced by kanamycin in drinking water and kept during 4 days. Last, kanamycin

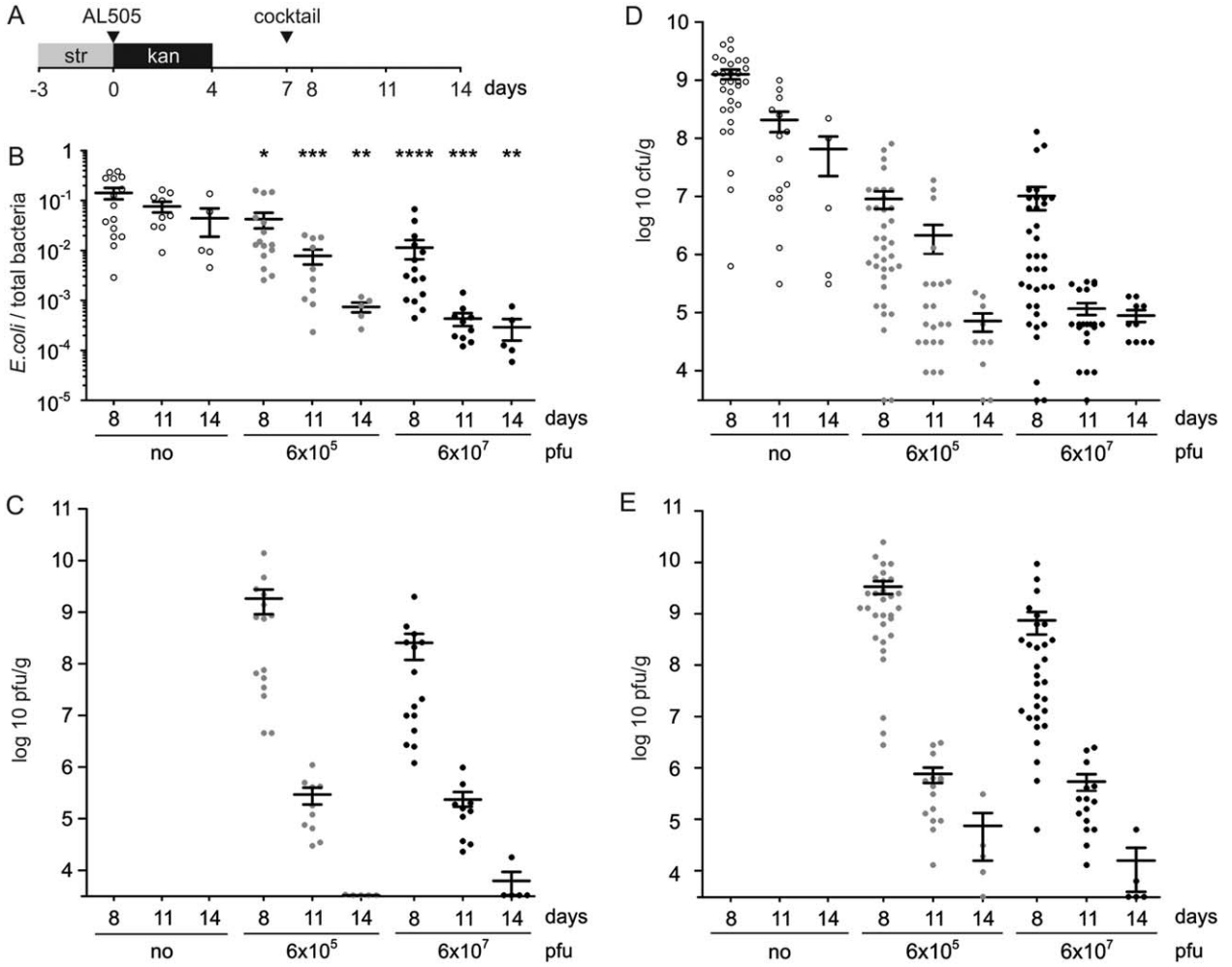

Fig. 4. A single dose of the three-bacteriophage cocktail strongly and gradually decreased the level of carriage of UPEC strain AL505. Scheme of the model of gut colonization with the UPEC strain AL505 in the absence of continuous antibiotic pressure (A). Three groups of mice colonized with strain AL505 received, on day 7, a single dose of buffer (white circles) or 6×10^5 (gray circles) or 6×10^7 (black circles) pfu of the three-bacteriophage cocktail. Quantification of strain AL505 by qPCR (expressed as the *E. coli*/total bacteria ratio) (B) and bacteriophages by direct plating (C) was performed on colonic segments collected at day 8, 11 and 14. Fecal samples were also directly plated for the quantification of strain AL505 (D) and bacteriophages (E) in all animals at each indicated time point. Statistical analyses were carried out to compare the bacteriophage-treated groups with the corresponding untreated group (n=5 to 15 animals per time point; *, $P < 0.5$; **, $P < 0.01$; ***, $P < 0.005$; ****, $P < 0.001$).

was removed from drinking water and we observed that colonization levels remained high and stable during the following 7 days (Fig. 3B). In these conditions, strain AL505 was located mostly in the luminal part of colonic sections (Fig. 3C). The absence of histological signs of damage on epithelial cells supports asymptomatic carriage of the bacterium in the gut (Supporting Information Fig. S2).

Using this new colonization model, a single dose of either 6×10^5 or 6×10^7 pfu of the bacteriophage cocktail was introduced by oral gavage, 3 days after the removal of antibiotics from the drinking water, in two groups of mice; a third group of animals received a bacteriophage-free gavage solution (Fig. 4A). One, four and seven days after bacteriophage gavage, levels of strain AL505 were assessed in the cecum, colon and feces (Fig. 4B and D and see Supporting Information Fig. S3). We observed a progressive, dose-dependent decrease in the levels of strain AL505 over time, in all gut segments. Four days after administration of the single highest dose of bacteriophages, the levels of strain AL505 (day 11 with highest dose vs day 8 with PBS) were found to have decreased by a factor of more than 500 in the colon as assessed by qPCR (Fig. 4B) and by a factor of 10,000 in feces as assessed by direct counts of strain AL505 (Fig. 4D). Concomitantly, bacteriophage levels had decreased strongly by day four and, in most cases, they fell below the detection threshold seven days after treatment (Fig. 4C and E). Similar patterns were observed in the cecum (see

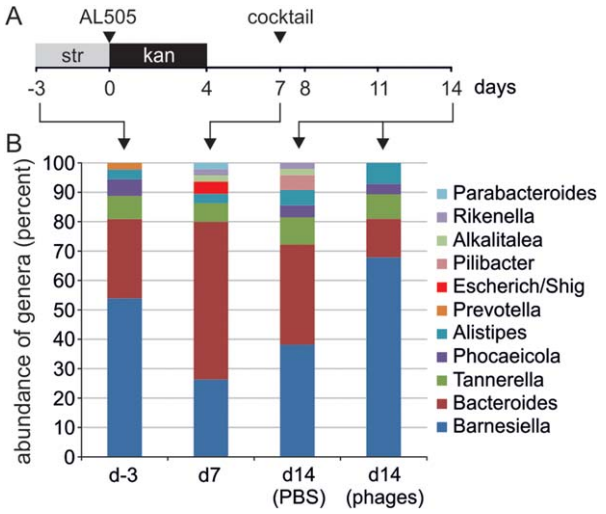

Fig. 5. Bacterial community structure profiles for the feces of mice subjected to antibiotic and bacteriophage treatments.
Schematic diagram of the model of gut colonization with the UPEC strain AL505 (A).
Two groups of five mice each colonized with strain AL505 received, on day 7, a single dose of PBS (mice 1–5) or 6×10^7 pfu (mice 6–10) of the three-bacteriophage cocktail. Feces were collected at day −3 before antibiotic treatment, on day 7 after antibiotic treatment and gavage with strain AL505 and on day 14, which correspond to 7 days following PBS or cocktail administration (B). Within each group of mice, the mean number of 16S rDNA V4 region reads for each genus was used to calculate and represent the relative abundance, in percent, of genera (only genera accounting for at least 2% of the community for each group of mice are indicated on the graph).

Supporting Information Fig. S3). A similar final efficacy was achieved with the lowest dose of bacteriophages, but after seven days rather than four (Fig. 4B and D).

Microbiota diversity is not directly affected by bacteriophages

One of the advantages of bacteriophages over antibiotics is their narrow host spectrum. Using fecal pellets from the previous experiments we assessed microbial diversity upon bacteriophage treatment. Region V4 of the 16S rRNA genes amplified from the fecal DNA of two groups of mice (from the above experiment: the PBS group and the high dose of cocktail, on days – 3, 7 and 14; Fig. 5A) was sequenced and analysed (Methods; see Supporting Information Tables S3 and S4). Across samples, most of the reads (around 75%) matched both *Barnesiella* and *Bacteroides* (Fig. 5B). However, the relative abundance of these two genera differed between treatments. The other main genera (accounting for at least 2% of total reads) also behaved differently following antibiotic and bacteriophage treatments, with the exception of *Tannerella* and *Alistipes*, the levels of which remained roughly stable (Fig. 5B; see Supporting Information Fig. S4 for a detailed representation of the data for each mouse). In addition, statistical analysis comparing the antibiotic and bacteriophage treatments revealed significant differences in the changes in abundance for some genera. After antibiotic treatment (day 7 vs day 3), 50 genera were found to have decreased in abundance, whereas 14 had increased (see Supporting Information Table S2). By contrast, when comparing the two groups that had received either PBS or bacteriophages (day 14 vs day 7), only 11 genera were found to have decreased in abundance, whereas 21 had increased (see Supporting Information Table S3).

Discussion

UPEC strains are the principal causal agents of UTIs worldwide and, as such, represent a major economic burden in terms of public health spending. Three bacteriophages were isolated and characterized to investigate their potential for decreasing UPEC levels in the gut.

Each of these three bacteriophages individually lysed the host rapidly in broth cultures, but the use of individual bacteriophages also favored the growth of bacteriophage-insensitive mutants (BIMs). By contrast, no BIMs were observed when the three bacteriophages were used as a cocktail, confirming the value of using several different bacteriophages together to minimize the selection of BIMs.

During the course of this study, we developed a new mouse gut colonization model in which continuous antibiotic pressure was not required to maintain high levels of colonization with the UPEC strain AL505 for at least 7 days. Our own experience of removing antibiotic pressure when streptomycin alone is used as selective agent, revealed that colonization levels of *E. coli* strains (55989 or AL505) decrease rapidly to undetectable levels within the next 2 days. The long term persistence of the high colonization level after removing antibiotics pressure (obtained from the sequential use of two antibiotics), most likely comes from a stronger microbiota alteration leading for a greater availability of nutrients for strain AL505.

Looking at bacteriophage efficacy using constant antibiotic pressure model, our previous work with *E. coli* strain 55989 revealed that despite the lack of strong effect on bacterial fecal titers, we found that bacteriophages significantly reduced bacterial colonization in intestinal sections (Maura *et al.*, 2012a,b). While such in-depth experiments were not performed with mice colonized by AL505 in presence of constant antibiotic pressure, we are expecting that similar results would be obtained, since we observed both a long-term replication of bacteriophages over several weeks and *ex-vivo* replication of bacteriophages in intestinal sections as previously with strain 55989.

Using our new colonization model, we observed that a single dose of a cocktail of three bacteriophages led to a strong and sustainable (within the limit of the period

observed) effect on the level of carriage of the UPEC strain. Indeed, as little as 6×10^5 pfu administered only once decreased AL505 levels by four orders of magnitude (from 1×10^9 at day 8 in PBS to 1×10^5 at day 14 cfu/g of feces) within seven days. A 100-times greater dose of bacteriophages had the same effect more rapidly, in only four days. The fecal level of the UPEC strain AL505 in this model is higher than *E. coli* level in human intestine (around 10^7 to 10^8 cfu per gram of feces) (Ruppe *et al.*, 2013). Therefore, bacteriophages efficacy in humans might be weaker as the targeted population would be lower than our animal model. Murine models displaying stable moderate *E. coli* colonization levels in absence of any antibiotics pressure or animals with natural asymptomatic *E. coli* carriage should now be used to address more adequately the ability of bacteriophages to replicate on lower abundant population (Payros *et al.*, 2014).

The concomitant decrease in the levels of bacteria and bacteriophages over time provides direct evidence for the amplification of bacteriophages in the gut at the expense of their host. In the mice receiving the highest dose of bacteriophages, levels of strain AL505 were similar on days 11 and 14, whereas bacteriophage levels continued to decrease, suggesting that host levels had become too low or that the bacteria were located in difficult-to-reach sites, making it hard to sustain further bacteriophage replication. Alternatively, these bacteria could represent BIMs, which is less likely as bacterial counts from these samples were inconsistent, a trademark of bacteriophage sensitive colonies. Nevertheless, as host levels remained stable between days 11 and 14, whereas bacteriophage levels fell to the detection threshold, we can hypothesize that this low abundance prevented the bacterial population from expanding again or that the natural resident flora prevented recolonization with strain AL505, or both.

While many studies have demonstrated that microbiota composition is altered by antibiotic treatments, such analysis with bacteriophage treatment has been reported only three times for human volunteers receiving a bacteriophages cocktail (Antonopoulos *et al.*, 2009; Sarker *et al.*, 2012; McCallin *et al.*, 2013; Sarker *et al.*, 2016). Our results confirmed that antibiotic treatment had a greater effect on microbiota diversity than bacteriophage administration. Furthermore, the change in diversity observed during bacteriophage treatment was, for 99.9% of reads, related to genera from the phyla Bacteroidetes and Firmicutes. These changes therefore cannot be directly linked to the infectivity of bacteriophages (*E. coli* belongs to the Proteobacteria phylum) and are probably an indirect consequence of the decrease in the abundance of the *E. coli* AL505 strain together with the recovery of the resident flora following initial antibiotic treatment. Interestingly, in the group of mice treated by bacteriophages we observed a significant increase of the Barnesiella genus, which was previously found to reduce vancomycin resistant *Enterococcus* intestinal colonization (Ubeda *et al.*, 2013). This observation supports the idea that bacteriophages could act in concert with 'favorable' bacteria for re-establishing normal microbiota balance. It is therefore expected that future research will associate narrow-spectrum agents, such as bacteriophages with broader strategies such as probiotics.

Our results may have broad applications and a strong economic impact worldwide, as decreasing the carriage of pathogens in the gut may decrease the frequency of gut-derived infections, such as UTIs. This approach may also limit the diffusion of multidrug-resistant epidemic clones, such as *E. coli* ST131 O25b (Nicolas-Chanoine *et al.*, 2014). Beyond phage therapy, bacteriophages may be of broader potential use in the domain of human health. Indeed, as many studies link microbiota composition to human diseases/disorders, we can envisage the use of bacteriophages to gently manipulate this microbiota composition to maintain an environment favoring human health (Nicholson *et al.*, 2012; Norman *et al.*, 2015; Parekh *et al.*, 2015; Sampson and Mazmanian, 2015). Therefore, given the current 'phage therapy 2.0' stage, with implementation of clinical trials and synthetic biology approaches, research should now engage in 'phage therapy 3.0' by taking into consideration a third partner, the eukaryotic cell (epithelial or immune) (Debarbieux, 2014; Young and Gill, 2015).

Experimental procedures

Bacterial strains and growth conditions

The UPEC strain AL505 was originally isolated from a patient with pyelonephritis (Archambaud *et al.*, 1988). Strains were routinely cultured in lysogeny broth (LB), or on LB agar or Drigalski agar plates, at 37°C. When required, streptomycin (100 μg/ml) or kanamycin (50 μg/ml) (Sigma, St. Louis, MO) was added.

Bacteriophage isolation, preparation and characterization

Strain AL505-specific bacteriophages were isolated from wastewater by an enrichment technique, and large-scale preparations were carried out, as previously described (Morello *et al.*, 2011). The host range and efficiency of plaquing (EOP) of each bacteriophage were determined with standard techniques (see details in the Supporting Information Table S4). The three-bacteriophage cocktail contained equal numbers of each of the three bacteriophages. Uranyl acetate was used as a contrast agent for the visualization of bacteriophages on electron microscopy (Debarbieux *et al.*, 2010).

Genome sequencing and analysis

Strain AL505 and its bacteriophages were sequenced with Illumina technology. The assembly of the strain AL505

genome resulted in 116 contigs covering a total of 5,283,278 bp (Bioproject PRJEB9469). Sequence analysis was performed at the MicroScope platform (Genoscope, Evry, France) (Vallenet *et al.*, 2013).

Strain AL505 is resistant to multiple antibiotics; kanamycin, tetracycline, chloramphenicol, streptomycin, erythromycin, ampicillin, ticarcillin and clavulanic acid in association with either ampicillin or ticarcillin. This resistance profile is consistent with the presence of a high-level penicillinase. The corresponding genetic markers (*acrD*, *aphA*, *bla*, *cat*, *emrE*, *strA*, *strB* and *tetA*) were found. Eleven of the 18 virulence determinants reported for pathogenic *E. coli* strains by Lefort *et al*. (*papC*, *papG*, *fyuA*, *irp2*, *iroN*, *iucC*, *ireA*, *neuC*, *ompT*, *traT*, *usp*) were found, resulting in the identification of three pathogenicity-associated islands (PAIs): PAI_{IV536}, PAI_{USP} and the high-pathogeny island (Lefort *et al.*, 2011).

For each bacteriophage genome, a single large contig was generated by reads assembly, and this contig was used to identify closely related homologs with the NCBI blastn tool (see Supporting Information Table S1).

In vitro lysis kinetics of strain AL505 and biofilm formation and quantification

An exponentially growing culture of strain AL505 was diluted in LB broth to an $OD_{600\ nm}$ of 0.1, and 50 μl of the resulting suspension were dispensed into each of the wells of 96-well flat-bottomed microplates (Microtest 96 plates, Falcon). We then added 50 μl of LB or bacteriophage suspension (diluted in LB to obtain a multiplicity of infection of 1×10^{-3}) to the plates, which were then incubated in a microplate reader, at 37°C, with orbital shaking (Glomax MultiDetection System, Promega, USA). OD_{600nm} was recorded automatically, at 15-min intervals, over a period of 15 h. For the quantification of BIMs, an aliquot was removed from each sample at the final time point and centrifuged at 8,000 *g* for 10 min. The pellets were washed twice in PBS, resuspended and spread on LB agar plates for colony isolation. We tested 40 colonies per set of conditions for susceptibility/resistance to bacteriophages, in the double-spot test described by Saussereau *et al.* (Saussereau *et al.*, 2014).

Biofilms were formed and quantified using a protocol based on 96-well microplates covered with lids equipped with pegs, described in a previous study (Maura *et al.*, 2012a) (see details in the Supporting Information).

Ethics statement

Mice (seven-week-old female BALB/cYJ mice) were supplied by Charles River Laboratories and housed in an animal facility in accordance with Institut Pasteur guidelines and European recommendations. Food and drinking water were provided *ad libitum*. Protocols were approved by the veterinary staff of the Institut Pasteur animal facility (approval ID 10.565) and the National Ethics Committee (approval number 2012-0018).

Murine model of intestinal colonization

Streptomycin sulfate (5 mg/ml) was added to the drinking water for three days to decrease the number of facultative aerobic/anaerobic resident bacteria (Croswell *et al.*, 2009). Before the introduction of strain AL505, we collected feces from the mice and plated them on Drigalski agar. None of the feces samples yielded any colonies. Following streptomycin treatment, mice were force-fed strain AL505 (1×10^7 cfu) in 200 μl of sterile 20% sucrose and 2.6% sodium bicarbonate, pH 8. At the same time, the drinking water was replaced with an aqueous 1 mg/ml kanamycin sulfate solution for 4 days. It was then replaced with plain water for assessment of the efficacy of the bacteriophage cocktail in the absence of antibiotic pressure, or with 1 mg/ml kanamycin sulfate in fresh water for the evaluation of replication for the individual bacteriophages in the presence of antibiotic pressure.

Histology and in situ *hybridization analyses*

Colonic samples of AL505-colonized mice were fixed by incubation in 4% formaldehyde for 24 h at 4°C and then embedded in paraffin. Serial 4 mm sections were stained with hematoxylin-eosin for histological analysis.

For *in situ* hybridization a detailed protocol is provided in the Supporting Information. EC1531 – Cy3 probe was used to detect *E. coli* cells and sections were stained with DAPI before examination under a fluorescence microscope.

Quantification of bacteria and bacteriophages from colonized mice

Freshly collected fecal samples were weighed and homogenized in PBS (0.08 g/ml final concentration), serially diluted in PBS and 4 μl of each dilution was spotted onto Drigalski agar supplemented with kanamycin for *E. coli* quantification. The plates were incubated for 18 h at 37°C. When necessary, large intestines were dissected and cut into two segments, corresponding to the cecum and colon. Each of these segments was weighed (0.2 to 1 g/sample), homogenized in 5 ml of PBS (Ultra Turrax T25, S25N-8G, IKA) and subjected to serial dilution and plating as described above. When necessary, mice received 200 μl of bacteriophage solution (either individual bacteriophages or the cocktail) containing 6×10^5 or 6×10^7 pfu of bacteriophages. Bacteriophages were quantified by spotting 10-fold serial dilutions on LB agar plates covered with a lawn of strain AL505. The detection limit was 5×10^2 cfu/g or pfu/g of tissue or feces.

Bacterial quantification by qPCR

For tissues homogenates, we found, as previously reported, that direct counts of *E. coli* colonies were not accurate, because of the abundance of bacteriophages, problem that can be overcome by using an indirect method based on qPCR (Maura *et al.*, 2012a). A detailed protocol is described in the Supporting Information. *E. coli* 16S rDNA-specific primers and bacterial 16S rDNA universal primers were used in parallel in each PCR (Furet *et al.*, 2009). Data are expressed as previously described (Maura *et al.*, 2012a).

Statistical analysis

One-way ANOVA and Mann–Whitney tests were carried out with Prism 6 software (Graphpad software, La Jolla, USA).

Assessment of microbiota diversity

Bacterial DNA was isolated from fecal pellets and primers 515F and 928R were used to amplify the V4 region of the 16S rRNA gene, which was subsequently sequenced by the GeT-Genotoul Platform, Toulouse (France) (Tamaki *et al.*, 2011). A detailed analysis protocol is described in the Supporting Information.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. The reference phylogenetic tree for the *E. coli/Shigella* core genome was reconstructed from the concatenated alignments of 1367 genes of the core genome of *E. coli/Shigella*.
Fig. S2. Colonization of UPEC strain AL505 is asymptomatic
Fig. S3. A single dose of the three-bacteriophage cocktail strongly and gradually decreased the level of UPEC strain AL505 carriage in the cecum.
Fig. S4. Bacterial community structure profiles for the feces of mice subjected to antibiotic and bacteriophage treatments.
Table S1. List of bacteriophages genomes with at least 90% identity at the nucleotide level to AL505 bacteriophages
Table S2. List of genera for which abundance changed significantly before and after antibiotics treatment.
Table S3. List of genera for which abundance changed significantly between PBS and bacteriophage cocktail groups.
Table S4. Host range in the ECOR collection of the 12 AL505-infecting bacteriophages initially isolated.