



HAL
open science

Bacteriophage LM33_P1, a fast-acting weapon against the pandemic ST131-O25b:H4 Escherichia coli clonal complex.

Nicolas Dufour, Olivier Clermont, Béatrice La Combe, Jonathan Messika, Sara Dion, Varun Khanna, Erick Denamur, Jean-Damien Ricard, Laurent Debarbieux

► **To cite this version:**

Nicolas Dufour, Olivier Clermont, Béatrice La Combe, Jonathan Messika, Sara Dion, et al.. Bacteriophage LM33_P1, a fast-acting weapon against the pandemic ST131-O25b:H4 Escherichia coli clonal complex.. Journal of Antimicrobial Chemotherapy, 2016, 71 (11), pp.3072-3080. 10.1093/jac/dkw253 . pasteur-01539016

HAL Id: pasteur-01539016

<https://pasteur.hal.science/pasteur-01539016>

Submitted on 14 Jun 2017

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - ShareAlike 4.0 International License

1 **Bacteriophage LM33_P1, a fast weapon against the pandemic ST131-O25b:H4**
2 ***Escherichia coli* clonal complex**

3
4 **Running title:** LM33_P1, a specific bacteriophage targeting the ST131-O25b:H4 clonal
5 complex.

6
7 **Authors list:**

8 Nicolas Dufour^{1,2,5}, Olivier Clermont^{2,3}, Béatrice La Combe^{1,2,3}, Jonathan Messika^{1,2}, Sara
9 Dion^{2,3}, Varun Khanna⁴, Erick Denamur^{2,3,5}, Jean-Damien Ricard^{1,2,3}, Laurent Debarbieux^{6*}

10

11 **Authors affiliation:**

12 ¹ AP-HP, Hôpital Louis Mourier, Service de Réanimation Médico-Chirurgicale, F-92700
13 Colombes, France

14 ² INSERM, IAME, UMR 1137, F-75018 Paris, France

15 ³ Univ Paris Diderot, IAME, UMR 1137, Sorbonne Paris Cité, F-75018 Paris, France

16 ⁴ Institut Pasteur, Hub Bioinformatique et Biostatistique – C3BI, USR 3756 IP CNRS, F-75015
17 Paris, France

18 ⁵ AP-HP, Hôpitaux Universitaires Paris Nord Val-de-Seine, Laboratoire de Génétique
19 Moléculaire, Site Bichat, F-75018 Paris, France

20 ⁶ Institut Pasteur, Department of Microbiology, Molecular Biology of Gene in Extremophiles,
21 F-75015 Paris, France

22

23 **Keywords:** ST131-O25b:H4, *Escherichia coli*, antibiotic resistance, phage therapy,
24 bacteriophage, extended spectrum beta-lactamase.

25

26 *** Corresponding author:** Laurent Debarbieux (Laurent.debarbieux@pasteur.fr), Institut
27 Pasteur, Département de Microbiologie, 25 rue du Dr Roux, 75015 Paris.

28

29 **Word count:** 3981.

30

31

32 **Abstract (253 words)**

33

34 **Background and objectives.** Amongst the highly diverse *Escherichia coli* population, the
35 ST131-O25b:H4 clonal complex is particularly worrisome as it is associated with a high level
36 of antibiotic resistance. The lack of new antibiotics, the worldwide continuous increase of
37 infections caused by multidrug resistant bacteria and the need for narrow-spectrum
38 antimicrobial agents have revived attention to phage therapy. In this article, we describe a
39 virulent bacteriophage, LM33_P1, which specifically infects O25b strains and provide data
40 related to its therapeutic potential.

41 **Methods.** A large panel of *E. coli* strains (n=283) were used to assess both the specificity and
42 the activity of bacteriophage LM33_P1. Immunology, biochemistry and genetic-based
43 methods confirmed this specificity. Virology methods and sequencing were used to
44 characterize this bacteriophage *in vitro* while three relevant mice models were employed to
45 show its *in vivo* efficacy.

46 **Results.** Bacteriophage LM33_P1 exclusively infects O25b *E. coli* strains with a 70% coverage
47 on ST131 and ST69. This specificity is due to an interaction with the lipopolysaccharide
48 mediated by an original tail fiber. LM33_P1 also has exceptional intrinsic properties with a
49 high adsorption constant and produces over 300 virions per cell in less than 10 minutes.
50 Using animal pneumonia, septicemia and urinary tract infection models, we showed the *in*
51 *vivo* efficacy of LM33_P1 to reduce the bacterial load in several organs.

52 **Conclusions.** Bacteriophage LM33_P1 represents the first weapon that specifically and
53 quickly kills O25b *E. coli* strains. Therapeutic approaches derived from this bacteriophage
54 could be developed to stop or slow down the spread of the ST131-O25b:H4 drug-resistant
55 clonal complex in humans.

56

57 **Introduction**

58 Amongst the highly diverse *Escherichia coli* population¹, the ST131-O25b:H4 clonal complex
59 is of particular concern. Since its first description in 2008 in a limited number of countries,
60 this clone has spread worldwide and shown an uncommon ability to propagate in humans.^{2, 3}
61 Moreover, ST131-O25b:H4 *E. coli* strains have a high pathogenic potential,⁴ they belong to
62 the B2 phylogroup where most extraintestinal-pathogenic *E. coli* are classified,⁵ they express
63 a large number of virulence factors⁶ and are lethal in a mouse model of sepsis.⁷ Involved in
64 community as well as hospital-acquired infections, ST131-O25b:H4 isolates are responsible
65 for a wide range of pathologies, from common cystitis to life threatening meningitis.² Finally,
66 these clones are also particularly worrisome as they are associated with a high level of
67 resistance to betalactams (mainly via the production of CTX-M-15 ESBL but also
68 carbapenemase⁸) and fluoroquinolones.^{9, 10} More recently described, the worrying plasmid-
69 mediated colistin resistance gene, *mcr-1*, has been found in an ST131 strain, highlighting the
70 propensity of this ST to carry antibiotic resistance genes.¹¹ To a lesser extent, O25b strains
71 may belong to another spreading antibiotic resistant clonal complex with a high
72 extraintestinal pathogenic potential, the ST69 (“clonal group A”).¹²
73 The lack of new antibiotics and the worldwide continuous increase of the infections caused
74 by multidrug resistant bacterial pathogens have revived attention to phage therapy,¹³
75 boosting the search for novel bacteriophages. Numerous experimental data have been
76 published demonstrating the proof of concept of this approach and clinical trials have been
77 reported or are ongoing (<http://www.clinicaltrials.gov>).
78 In this work, we characterized a novel virulent bacteriophage, LM33_P1, which only infects
79 *E. coli* O25b strains. The *in vitro* and *in vivo* efficacies of bacteriophage LM33_P1 are
80 reported, showing its value for therapeutic applications.

81

82

83 **Material and methods**

84

85 Bacterial strains and bacteriophages, susceptibility testing

86 The bacterial strains used in this work belong to previously published collections: human
87 commensal and extraintestinal *E. coli* gathered in France during the 2010s (n=83),¹⁴⁻¹⁶
88 Spanish extraintestinal *E. coli* (n=16),⁶ North American clinical *E. coli* (n=14),¹⁷ commensal

89 and pathogenic *E. coli* from various parts of the world (Africa, America, Australia, Europa
90 (n=18),^{18, 19} the ECOR collection (n=8)²⁰ as well as the unpublished ColoColi collection (n=144,
91 an ongoing French multicenter study collecting *E. coli* strains in the lower respiratory tract of
92 mechanically ventilated patients). The phylogroup and the ST belonging were determined as
93 described in.^{21, 22} The O-type and the *fimH* allele were determined by PCR-based assays as
94 previously described^{18, 23} and as reported in **Supplementary Data - Procedures**, respectively.
95 When needed, we confirmed the O25b phenotype using a monospecific O25 serum designed
96 for *E. coli* serotyping purpose (*E. coli* mono O25, Statens Serum Institut, Denmark).

97 All the strains were grown in lysogeny broth (LB) (Difco™ Bacto-Tryptone 10 g/L, Difco™
98 Yeast extract Difco 5 g/L, NaCl 5 g/L). Their antibiotic susceptibility was determined using the
99 disk diffusion method following the EUCAST guidelines.

100 Some *E. coli* strains, used for lipopolysaccharide (LPS) assays or bacteriophage susceptibility
101 testing, are detailed below:

- 102 - LM33, LM36, AVC02 (ST131-O25b:H4) and AVC03 (O25b, non-ST131) are clinical
103 strains responsible for ventilator-associated pneumonia,
- 104 - 536 (ST127-O6), LM02 (ST69-O17) and ECOR51 (ST73-O25a) have been used as a
105 source of their corresponding LPS,
- 106 - 81009 WT (ST131-O25b:H4) and its isogenic rough derivative (a mutant strain
107 obtained by deleting the gene encoding for the O-antigen ligase)²⁴ were used to
108 prove the LPS-dependent interaction of LM33_P1.

109 The bacteriophages were isolated from sewage, using specific host.²⁵ By convention,
110 bacteriophages are named as follows: “host bacteria_Px” (for example LM33_P1 represents
111 the first bacteriophage isolated using strain LM33). In all experiments, bacteriophage
112 solutions were obtained after purification by using ultracentrifugation on cesium chloride
113 gradient as previously described.²⁶

114 For bacteriophage susceptibility testing, we used the double spot test technique²⁷ as a
115 screening method and then we calculated the efficiency of plaquing (EOP) for all susceptible
116 strains. EOP was calculated as the ratio of the number of plaques formed by the
117 bacteriophage on the non-host strain to the number of plaques formed on its host, using the
118 same bacteriophage solution. More details are provided in **Supplementary Data –
119 Procedures**.

120

121

122 LPS extraction.

123 LPS extracts were obtained and purified from the same amount of bacteria using a phenol-
124 water-diethyl ether extraction²⁸ followed by extensive dialysis against sterile pyrolyzed
125 water. High purity LPS was confirmed by performing an agarose gel electrophoresis with
126 ethidium bromide staining (nucleic acids detection), an SDS-PAGE 12% followed by
127 Coomassie blue staining (proteins detection) and a silver staining to visualize the LPS O-
128 antigen pattern (SilverSNAP Stain Kit II, Pierce).

129

130 Plaque inhibition assays with LPS extracts

131 From a purified stock solution of bacteriophages in TN buffer (Tris-HCl 10 mM, NaCl 150 mM,
132 pH 7.5), 3 solutions of 10^6 , 10^5 and 10^4 pfu/mL in TN buffer were prepared. Each of these
133 working solutions was used to prepare final tubes with bacteriophages alone (100 μ L of
134 working solution + 100 μ L of pyrolyzed water) and tubes with bacteriophages + LPS (100 μ L +
135 100 μ L of undiluted LPS extract). Additional tubes containing bacteriophages and decreasing
136 amounts of LPS were also prepared (pyrolyzed water was used to reach an identical final
137 volume). Then, 10 μ L of each final bacteriophage tubes, with and without LPS, were spotted
138 in triplicate on an agar plate, previously overlaid by the bacteria to test. The plates were
139 incubated for 4 hours at 37°C before the plaque-forming units were counted in each
140 condition.

141

142 Characterization of bacteriophage LM33 P1

143 The adsorption assay and the one-step growth experiment were performed in triplicate as
144 described by Hyman and Abedon²⁹ and as detailed in **Supplementary Data - Procedures**.

145

146 Lysis kinetics (with and without LPS extracts) and aggregation assays with O25 antibody

147 The lysis kinetic was performed as detailed in **Fig. S4**. Briefly, the growth of LM33 with and
148 without LM33_P1 was followed overtime by recording optical density at 600 nm every 15
149 minutes. The aggregation assays were performed using an O25 *E. coli* anti-serum (see above)
150 and observed under light microscope as detailed in **Fig. S7**.

151

152

153 Sequencing of strain LM33 and bacteriophage LM33_P1

154 The sequencing of bacteriophage LM33_P1 and strain LM33 was performed using Illumina
155 sequencing technology (Illumina Inc., San Diego, CA). Genomes annotation was performed
156 by the MicroScope platform for strain LM33³⁰ and using the RAST server for bacteriophage
157 LM33_P1.³¹ See **Supplementary Data – Procedures** for more details.

158

159 Experimental murine infections models (additional details provided in **Supplementary Data -**
160 **Procedures**)

161 The primary experimental outcome was the decrease in bacterial load in relevant organs.

162 As LM33 was originally responsible for a ventilator-associated pneumonia in an ICU patient,
163 pneumonia was initiated by intranasal administration of 1×10^8 cfu of strain LM33 on
164 anesthetized mice (n=20) as previously described.³² The septicemia model, essentially used
165 to study intrinsic extraintestinal virulence of *E. coli* isolates,⁷ was carried out with 1×10^9 cfu
166 of the strain H1659 (ST131-O25b:H4), injected subcutaneously into the nape of the neck
167 (n=12 mice). We used this strain because of its virulence, previously investigated.⁶ The non-
168 lethal urinary tract infection model consists in a retrograde kidneys infection occurring after
169 an intra-urethral injection of 5×10^7 cfu of strain LM33 into the bladder (n=23 mice), as
170 previously described.³³ In every case, the organs were aseptically removed and mechanically
171 homogenized in cold PBS before counting bacterial and bacteriophage content.

172

173 Ethics

174 The animals were housed in animal facilities in accordance with French and European
175 regulations on the care and protection of laboratory animals. The protocols were approved
176 of by the veterinary staff of the Institut Pasteur and INSERM animal facilities together with
177 the National Ethics Committee regulating animal experimentation (authorization CETEA
178 #2012-0018). Food and drink were provided *ad libitum*.

179

180 Statistical analysis

181 All the statistical analyses were performed by using GraphPad Prism version 5.00 (Graph-Pad
182 Software, La Jolla, CA). The normal distribution of all the variables was checked using the
183 Kolmogorov-Smirnov test, and the results are then expressed as mean \pm SD. In case of a non-

184 Gaussian distribution, the results are expressed as median [25th, 75th percentile]. The
185 statistical tests (Student t test or Mann-Whitney test) were chosen accordingly.

186

187 **Results**

188

189 **Bacteriophage LM33_P1 targets antibiotic resistant O25b *E. coli* strains.** *E. coli* strain LM33
190 was used to isolate bacteriophage LM33_P1. The characteristics of strain LM33 are as
191 follows: an O25b:H4 serotype, a B2 phylogroup (subgroup I), a ST131 (Warwick
192 scheme)/ST43 (Pasteur Institute scheme)²² sequence-type, a *fimH* allele *H22* as well as a
193 multi-drug resistance phenotype with an extended spectrum beta-lactamase, a resistance to
194 aminoglycosides (kanamycin, tobramycin, gentamicin, netilmicin except for amikacin where
195 an intermediate phenotype is found), sulphonamides, chloramphenicol and an intermediate
196 susceptibility to nalidixic acid. The beta-lactam resistance is supported by both a plasmid
197 (pLM33) and the bacterial chromosome (**Table 1**).

198 We determined the host range of bacteriophage LM33_P1 on a panel of 283 *E. coli* strains
199 belonging to various O-types (**Fig. 1** and **Data sheet 1** provided separately for O25 and O16
200 strains). One hundred and eighty-three (64%) of these strains were not O25b and none of
201 them was infected by LM33_P1, including twelve O25a strains and six ST131-O16 strains.
202 Among the remaining one hundred O25b strains (encompassing 83 ST131, 4 ST69, 10 ST95
203 and 3 other STs), 64 (64%) were infected by LM33_P1 with a median efficiency of plaquing of
204 0.46 [0.09-1.27] (**Fig. S1**). Interestingly, LM33_P1 was found to be more efficient on STs
205 associated with high antibiotic resistance (ST131 and ST69, n=87) where 61 of these strains
206 (70%) were lysed whereas its efficacy was weak on STs associated with low antibiotic
207 resistance (ST95 and others, n=13) where only 3 of these strains (23%) were susceptible
208 (**Fig. 1**). Finally, we did not find a correlation between the susceptibility to bacteriophage
209 LM33_P1 and the *fimH* allele *H30*, which is strongly associated with fluoroquinolone
210 resistance among ST131 strains.³⁴ Indeed, considering the two more frequent *fimH* alleles in
211 our O25b strains (*H22* and *H30*, n=83), the proportion of susceptible strains to LM33_P1 was
212 72% (21 out of 29) and 66% (36 out of 54) in the strains displaying respectively the *H22* or
213 the *H30* allele (p=0.6, Fisher's exact test).

214

215 **Bacteriophage LM33_P1 is a lytic *Podoviridae* distantly related to bacteriophage T7.** The
216 genome of bacteriophage LM33_P1 (38 979 bp; GC content of 50.8%; 49 ORFs predicted,
217 accession number PRJEB12445) lacks putative ORFs with homologies to integrase or
218 recombinase.

219 A BLAST analysis of the genomic sequence revealed that the four closest related
220 bacteriophages were enterobacteria bacteriophages (**Table S1**): three coliphages called PE3-
221 1, K1F,³⁵ EcoDS1 (with 94% identity on $\geq 88\%$ of its length for all of them) and bacteriophage
222 Dev2 infecting *Cronobacter turicensis* (with 83% identity on 85% of its length).³⁶ The
223 alignment of these related bacteriophages with LM33_P1 revealed a similar spatial genome
224 organization and confirmed the high homology between them (**Fig. 2**). Strikingly, the 5'
225 extremity (the first 650 nucleotides) of the tail fiber gene is highly conserved in each
226 bacteriophage genome, while the remaining part is highly divergent. The corresponding N-
227 terminal region (IPR005604 / PF03906, InterPro / Pfam database) of this tail fiber protein is
228 involved in its connection to the tail-tube³⁷ while the C-terminal part, involved in host
229 recognition, often carries hydrolase activities such as the endosialidase of bacteriophage K1F
230 used for exopolysaccharide degradation.^{35, 38} BLAST searches on the C-terminal part of the
231 tail fiber of bacteriophage LM33_P1 revealed a homology to a domain belonging to the
232 pectin lyase superfamily (IPR011050). Tridimensional structure prediction using Phyre²
233 database³⁹ confirmed its close proximity to the endopolygalacturonase of *Erwinia carotovora*
234 that belongs to the pectin lyase superfamily (100% amino-acid predicted with a confidence
235 $>90\%$ for the tertiary structure, index of confidence for homologous protein 94.1%, Protein
236 Data Bank entry: 1BHE, **Fig. S2**).

237

238 **Bacteriophage LM33_P1 is highly efficient and rapid *in vitro*.** The adsorption of LM33_P1
239 bacteriophage on its host is fast with $\geq 90\%$ of the viral population attached to cells after 3.5
240 minutes with an adsorption constant of 1.2×10^{-8} mL/min (**Fig. S3-A**). Newly produced virions
241 are detected within the bacteria by 7 minutes post-infection (eclipse period) while host lysis
242 occurs in 9 minutes (latent period) with a burst size of 317 (95% confidence interval: 289-
243 345) (**Fig. S3-B**).

244 In liquid medium, when LM33_P1 was mixed with its host, the absorbance value of LM33
245 cells started to decline (a sign of lysis) within 15 minutes at a multiplicity of infection (MOI)
246 of 1. With much fewer bacteriophages (MOI of 10^{-6}) lysis still occurred within 60 minutes

247 **(Fig. S4).** On solid medium, LM33_P1 forms clear and large plaques, whose diameter
248 increases rapidly overtime with a visible halo around clear areas. This halo suggests the
249 presence of a diffusible enzyme that most likely carries a depolymerase activity⁴⁰ **(Fig. S5).**

250

251 **Bacteriophage LM33_P1 specifically binds to O25b LPS O-antigen.** The host range of
252 bacteriophage LM33_P1 strongly suggested that the O-chain of LPS could be involved in its
253 specificity. Using LPS competition assays we observed that purified LPS from strain LM33
254 was able to inhibit the interaction between bacteriophage LM33_P1 and strain LM33 as well
255 as other O25b strains.

256 First, we demonstrated that purified LPS reduced the number of plaque-forming units when
257 mixed with bacteriophages before application on a bacterial layer (mean reduction of 1.0
258 ± 0.23 Log₁₀ from 15 assays with five different O25b strains, **Fig. S6-A**). Together with the
259 reduction of the number of plaques, we observed a reduction of the plaque diameters,
260 suggesting that the LPS molecules prevented newly released bacteriophages from infecting
261 the surrounding hosts **(Fig. 3)**. These observations are specific of the interaction of
262 bacteriophage LM33_P1 with O25b strains since: i) an O25b LPS extract from strain LM33
263 was not able to affect the interaction of other bacteriophages targeting non O25b strains
264 and ii) an LPS extract from non O25b strains (O25a, O6 and O17) was unable to alter the
265 interaction between bacteriophage LM33_P1 and strain LM33 **(Table S2)**.

266 Second, an LPS extract from O25b strain (LM33) also reduced the infectivity of
267 bacteriophage LM33_P1 in liquid medium in a dose dependent manner **(Fig. S6-B)**, whereas
268 LPS extracts from O6 and O25a strains had no effect.

269 Third, using an O-type specific antibody to aggregate O25 strains for serotyping, we found
270 that bacteriophage LM33_P1 prevented the aggregation of strain LM33 **(Fig. S7)**.

271 Fourth, using *E. coli* O25b 81009 and its isogenic rough derivative (an LPS deficient strain
272 obtained by deleting the gene encoding for the O-antigen ligase)²⁴ we observed that
273 bacteriophage LM33_P1 infects the wild type strain 81009 while the LPS deficient strain is
274 resistant. Conversely, we confirmed that bacteriophage LM33_P1 could not adsorb on the
275 LPS defective strain.

276

277 **The adsorption of bacteriophage LM33_P1 is most likely hindered by capsule production.**

278 The production of exopolysaccharides is a well-known bacteriophage resistance mechanism

279 and might be involved in the non-adsorption of bacteriophage LM33_P1 observed in five
280 randomly chosen LM33_P1 resistant strains (81009 WT, JJ1886, S242, B-1, C-1). Since, in
281 some cases (type II capsule), the synthesis of exopolysaccharides is temperature dependent,
282 we investigated the susceptibility of LM33_P1 on all the O25b resistant strains (n=36) at
283 20°C. We observed that nine of them (25%) became susceptible at this temperature (**see**
284 **Data sheet 1**), supporting this hypothesis.

285
286 **Bacteriophage LM33_P1 efficiently infects its host *in vivo*.** As bacteriophage LM33_P1
287 exhibited impressive *in vitro* characteristics, we investigated its *in vivo* activity in three
288 different animal infection models relevant to ST131 clinical epidemiology: pneumonia,
289 septicemia and urinary tract infection (**Fig. 4-6**). Since strain LM33 was isolated from a
290 patient with pneumonia, we first attempted to trigger pneumonia in mice. Using an
291 inoculum 50 times higher than previously reported in such model³² and despite clear
292 macroscopic lung lesions, strain LM33 was not lethal, preventing us from using survival as an
293 indicator of the efficacy of the bacteriophage. We therefore evaluated LM33_P1 efficacy by
294 counting the bacteria from lung homogenates collected 17 hours following infection. Three
295 groups of mice were treated 4 hours post-infection either by control solution (PBS), or
296 intranasal (MOI 50) or intraperitoneal (MOI 500) bacteriophages. Independently of the
297 administration route, we observed a 3 Log₁₀ reduction in bacterial load when the mice
298 received bacteriophage treatments compared to control group (PBS-treated animal: 5.4x10⁷
299 cfu/g, intranasally LM33_P1-treated: 2.7x10⁴ cfu/g, intraperitoneally LM33_P1-treated:
300 3.3x10⁴ cfu/g, p <0.01, **Fig. 4**). Interestingly, the number of bacteriophages in the lung tissue
301 was similar between the intranasally and intraperitoneally-treated mice despite the fact that
302 the latter had received a 10 times higher dose (**Fig. 4**).

303 Then, we tested bacteriophage LM33_P1 in a murine model of septicemia previously
304 reported^{6, 7} using the H1659 ST131-O25b:H4 strain⁶ (strain LM33 was not lethal in this
305 model), on which LM33_P1 is as efficient as on strain LM33 (EOP = 1). Following a
306 subcutaneous inoculation of 1x10⁹ cfu, septic metastases were rapidly observed in several
307 organs (the first deaths occurred in less than 24 hours). Intraperitoneal administrations of
308 bacteriophage LM33_P1 (MOI 60, a single dose 2 hours post-infection or two doses 2 hours
309 and 12 hours post-infection) were not sufficient to prevent the death of the animals.
310 However, in the subset of the animals that died within the same time interval (between 24

311 and 30 hours), the bacteria and bacteriophage content was analyzed in the liver, the spleen
312 and the lung-heart homogenates (**Fig. 5**). In these organs, the number of bacteria was
313 reduced compared to the control group (untreated infected animals). A two doses regimen
314 appeared to be more efficient than a single one, enabling to reach a significant reduction of
315 approximately 1.4 Log₁₀ (the median bacterial count decreases from 8.5x10⁶ to 2.9x10⁵ in
316 the heart-lungs, from 7.7x10⁵ to 3.2x10⁴ in the liver and from 3.5x10⁵ to 1.4x10⁴ cfu/g in the
317 spleen). The bacteriophage counts were in the same order of magnitude in all the organs,
318 but were significantly higher when two doses had been administered (2.0x10¹⁰ versus
319 4.0x10⁹ pfu/g, p <0.01). In addition, the amount of bacteriophages was 3 to 6 Log₁₀ higher
320 than the amount of bacteria in each mouse for all the organs. All these observations
321 revealed that bacteriophage LM33_P1 was able to infect and multiply *in vivo* in strain H1659.
322 Finally, as *E. coli* is a major pathogen in UTIs, we assessed the efficacy of bacteriophage
323 LM33_P1 in a murine UTI model (**Fig. 6**). Twenty-four hours following intra-urethral injection
324 of 5.10⁷ cfu of strain LM33, the mice received a single bacteriophage treatment
325 intraperitoneally (MOI of 200). Forty-eight hours post-infection, a 2 Log₁₀ reduction of
326 bacterial load was observed in the kidneys in the treated group compared to control group
327 (1.5x10⁵ versus 8.8x10² cfu/g, p <0.001).
328 Altogether these data firmly show the ability of bacteriophage LM33_P1 to infect O25b
329 strains *in vivo*.

330

331 **Discussion**

332 One of the main advantages of bacteriophages which has often been reported is their
333 specificity to infect a few strains only within a species, having then a limited impact on the
334 patient's microbiota. Along with monoclonal antibodies (anti-O25b antibodies have been
335 proven to exert a protective effect in mouse septicemia model),⁴¹ bacteriophages are the
336 only anti-infectious tools that could reach such specificity. Using an ST131-O25b:H4 clinical
337 isolate of *E. coli* (strain LM33), we isolated a bacteriophage, LM33_P1, which was found to
338 exclusively infect O25b strains. Interestingly, O25b O-antigen is present in the archetypal
339 ST131 clonal complex but also in the ST69, another antibiotic resistant spreading clone of *E.*
340 *coli*, the "clonal group A".^{12, 42} In a therapeutic projection and taking into account the
341 pandemic lineages of extraintestinal pathogenic *E. coli*,⁴³ we observed a greater

342 susceptibility among both of these STs (70%) compared to less antibiotic-resistant O25b STs
343 such as ST95 and minor ones (23%).

344 Additionally, a majority of the strains belonging to the ST131 clonal complex display an O25b
345 O-antigen whereas a minor part, less resistant to antibiotics, display an O16 serogroup.¹⁷ The
346 specificity of bacteriophage LM33_P1 is linked to the O25b O-antigen and not to the
347 sequence type (*i.e.* none of the non-O25b ST131 strains were susceptible to bacteriophage
348 LM33_P1 while all the O25b-ST69 strains tested were susceptible). Furthermore, the
349 susceptibility of ST131-O25b:H4 strains to bacteriophage LM33_P1 is independent of the
350 *fimH* allele, a marker of the epidemiologic evolution of this clone.³⁴ Besides, bacteriophage
351 LM33_P1 was unable to infect the O25a strains, despite a highly similar O-antigen structure
352 where polysaccharides repeated units only differ by one monosaccharide (fucose *versus*
353 rhamnose), a fine discrimination that is not possible with the classical antibodies used for
354 serotyping until the recent description of O25b monoclonal antibodies.²⁴

355 Our investigations led us to estimate that the global host coverage of bacteriophage
356 LM33_P1 on O25b strains is 64%. We consider that this coverage is reliable as we first
357 avoided sampling bias by screening a large collection obtained from different sources with
358 many serotypes. Second, we assessed strain susceptibility in a rigorous way using EOP
359 determination that excludes atypical results and false positives such as those obtained with
360 lysis from without.^{44, 45} On the other hand, one limitation of our study is the origin of the
361 strains we tested: as most of them originate from European countries (76%), further studies
362 will be required to assess whether coverage of LM33_P1 is higher, equal or lower if
363 submitted to strains originating from Asian, Indian, African or American countries.

364 Finally, compared to the data available in literature, we found that LM33_P1 is the quickest
365 T7-like bacteriophage ever reported, lysing its host within 10 minutes while T7 takes 13 to 16
366 minutes.^{46, 47} Part of this success is due to its absorption constant (1.2×10^{-8} mL/min) which
367 was found to be 10 times higher than that of most bacteriophages⁴⁸⁻⁵¹ and its burst size
368 which is also in the top half of the values usually observed.⁵²

369 To prevent phage adsorption, bacteria can mask phage receptors by the production of
370 extracellular exopolysaccharides (capsules), which can also help bacteria to escape
371 recognition by immune cells.^{53, 54} We found that 25% of the strains reversed their phenotype
372 towards bacteriophage LM33_P1 from resistant to susceptible, when grown and tested at

373 20°C, a temperature known to turn off type II capsule production.⁵⁵ Therefore, the
374 bacteriophage LM33_P1 coverage increased to 80% among all the ST131-O25b:H4 strains
375 and to 73% among all the O25b strains tested. It was also previously shown that
376 bacteriophages can defeat such an exopolysaccharide shield by using tail fibers that possess
377 depolymerase activities.⁵⁶ We can reasonably assume that the discovery of new
378 bacteriophages and/or the isolation of LM33_P1 variants could provide viruses equipped
379 with such a tail fiber-associated enzyme and thus enable to improve (by restoring the O25b
380 antigen accessibility) the coverage rate of O25b strains.⁵⁷⁻⁵⁹

381 With the goal of using bacteriophages to treat human bacterial infections, the translation
382 from *in vitro* activity (forming plaques) to *in vivo* efficacy (curing a disease) is not
383 guaranteed, despite a high success rate.⁶⁰ Our investigation into the *in vivo* curative
384 potential of bacteriophage LM33_P1 revealed indeed that, in the three models tested, this
385 bacteriophage was able to infect targeted bacteria in several body compartments and via
386 different administration routes. With all the limits inherent to animal experiments, our data
387 should not be over-translated to the clinical setting. However, these results clearly show that
388 bacteriophages, including LM33_P1 as shown in this study, can quickly reduce the load of
389 their host within a complex environment including the gut of mammals.⁶¹ In a therapeutic
390 approach, such bacteriophages could be used as a selective antimicrobial agent to control
391 the passive carriage of ST131-O25b:H4 strains in human gut in order to reduce its
392 dissemination, particularly in healthcare-associated environments. Indeed, *E. coli* strains
393 residing in the digestive tract constitute a well-known reservoir for urinary tract infections
394 but probably also for ventilator-associated pneumonia.¹⁵

395 Beside the classical phage therapy approach, bacteriophage LM33_P1 or its proteins offer
396 opportunities to develop several tools. The tail fiber could be used to specifically kill O25b *E.*
397 *coli* strains using bacteriocins, as previously shown for the O104 *E. coli* strains involved in
398 enterohemorrhagic colitis.⁶² Other approaches could be considered where bacteriophages are
399 reprogrammed and could suppress antibiotic resistance genes using CRISPR-Cas system⁶³ or
400 express well-chosen beneficial enzymes to fight biofilm.⁶⁴ Deeper investigations on the
401 infectious cycle of this bacteriophage are now required to determine what molecular
402 mechanisms are responsible for its fast-killing component. Bacteriophage LM33_P1 could

403 also be used from now as a starting platform to develop highly virulent synthetic
404 bacteriophages with various host specificity.⁶⁵

405

406 **Acknowledgments**

407 We thank Gabor Nagy for providing strain 81009 and its rough derivative. We thank
408 Catherine Branger for her helpful discussion concerning antibiotic resistance genotype and
409 phenotype. We are grateful to David Gordon for providing us with the Australian strains. We
410 warmly thank Yvonne Cloarec for English language revisions.

411 We are indebted to the following physicians (microbiologists and intensivists) who collected
412 the clinical *E. coli* strains as part of the Colocoli study: M. Eveillard, A. Kouatchet, S. Lasocki,
413 P. Asfar (CHU d'Angers), T. Billard-Pomares, F. Magdoud, G. Barnaud (CHU Louis Mourier,
414 Colombes), S. Corvec, K. Lakhal (CHU de Nantes), L. Armand, M. Wolff (CHU Bichat, Paris), S.
415 Bourdon, J. Reignier, S. Martin (CHD de Vendée, La Roche sur Yon), V. Fihman, N. Deprost,
416 (CHU Henri Mondor, Créteil), J. Bador, P-E. Charles (CHU de Dijon), J. Goret, A. Boyer (CHU
417 de Bordeaux), F. Wallet, E. Jaillette, S. Nseir (CHU de Lille), L. Landraud, R. Ruimy, P-E. Danin,
418 J. Dellamonica (CHU de Nice), J. Cremniter, J-P. Frat (CHU de Poitiers), F. Jauréguy, C. Clec'h
419 (CHU Avicenne, Bobigny), D. Decré, E. Maury (CHU Saint-Antoine, Paris).

420

421 **Funding**

422 This project was supported by a joint research grant from both Institut Pasteur and
423 Assistance Publique–Hôpitaux de Paris (Programme Transversal de Recherche #417 and
424 Poste d'Accueil pour praticien hospitalier).

425

426 **Transparency declarations**

427 None to declare.

428

- 431 1. Tenailon O, Skurnik D, Picard B et al. The population genetics of commensal *Escherichia coli*.
432 *Nat Rev Microbiol* 2010; **8**: 207-17.
- 433 2. Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* ST131, an intriguing clonal
434 group. *Clin Microbiol Rev* 2014; **27**: 543-74.
- 435 3. Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic,
436 multiresistant, community-associated strain. *J Antimicrob Chemother* 2011; **66**: 1-14.
- 437 4. Russo TA, Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic
438 isolates of *Escherichia coli*: ExPEC. *J Infect Dis* 2000; **181**: 1753-4.
- 439 5. Picard B, Garcia JS, Gouriou S et al. The link between phylogeny and virulence in *Escherichia*
440 *coli* extraintestinal infection. *Infect Immun* 1999; **67**: 546-53.
- 441 6. Mora A, Dahbi G, Lopez C et al. Virulence patterns in a murine sepsis model of ST131
442 *Escherichia coli* clinical isolates belonging to serotypes O25b:H4 and O16:H5 are associated to
443 specific virotypes. *PLoS One* 2014; **9**: e87025.
- 444 7. Johnson JR, Porter SB, Zhanel G et al. Virulence of *Escherichia coli* clinical isolates in a murine
445 sepsis model in relation to sequence type ST131 status, fluoroquinolone resistance, and virulence
446 genotype. *Infect Immun* 2012; **80**: 1554-62.
- 447 8. Peirano G, Bradford PA, Kazmierczak KM et al. Global incidence of carbapenemase-producing
448 *Escherichia coli* ST131. *Emerg Infect Dis* 2014; **20**: 1928-31.
- 449 9. Coque TM, Novais A, Carattoli A et al. Dissemination of clonally related *Escherichia coli*
450 strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerg Infect Dis* 2008; **14**: 195-200.
- 451 10. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V et al. Intercontinental emergence of
452 *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J Antimicrob Chemother* 2008; **61**: 273-81.
- 453 11. Hasman H, Hammerum AM, Hansen F et al. Detection of *mcr-1* encoding plasmid-mediated
454 colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken
455 meat, Denmark 2015. *Euro Surveill* 2015; **20**: 30085.
- 456 12. Colomer-Lluch M, Mora A, Lopez C et al. Detection of quinolone-resistant *Escherichia coli*
457 isolates belonging to clonal groups O25b:H4-B2-ST131 and O25b:H4-D-ST69 in raw sewage and river
458 water in Barcelona, Spain. *J Antimicrob Chemother* 2013; **68**: 758-65.
- 459 13. Reardon S. Phage therapy gets revitalized. *Nature* 2014; **510**: 15-6.
- 460 14. Lefort A, Panhard X, Clermont O et al. Host factors and portal of entry outweigh bacterial
461 determinants to predict the severity of *Escherichia coli* bacteremia. *J Clin Microbiol* 2011; **49**: 777-83.
- 462 15. Messika J, Magdoud F, Clermont O et al. Pathophysiology of *Escherichia coli* ventilator-
463 associated pneumonia: implication of highly virulent extraintestinal pathogenic strains. *Intensive Care*
464 *Med* 2012; **38**: 2007-16.
- 465 16. Massot M, Daubie AS, Clermont O et al. Phylogenetic, virulence and antibiotic resistance
466 characteristics of commensal strain populations of *Escherichia coli* from community subjects in the
467 Paris area in 2010 and evolution over 30 years. *Microbiology* 2016; **162**: 642-50.
- 468 17. Johnson JR, Clermont O, Johnston B et al. Rapid and specific detection, molecular
469 epidemiology, and experimental virulence of the O16 subgroup within *Escherichia coli* sequence type
470 131. *J Clin Microbiol* 2014; **52**: 1358-65.
- 471 18. Clermont O, Olier M, Hoede C et al. Animal and human pathogenic *Escherichia coli* strains
472 share common genetic backgrounds. *Infect Genet Evol* 2011; **11**: 654-62.
- 473 19. Clermont O, Christenson JK, Daubie AS et al. Development of an allele-specific PCR for
474 *Escherichia coli* B2 sub-typing, a rapid and easy to perform substitute of multilocus sequence typing. *J*
475 *Microbiol Methods* 2014; **101**: 24-7.

- 476 20. Ochman H, Selander RK. Standard reference strains of Escherichia coli from natural
477 populations. *J Bacteriol* 1984; **157**: 690-3.
- 478 21. Clermont O, Christenson JK, Denamur E et al. The Clermont Escherichia coli phylo-typing
479 method revisited: improvement of specificity and detection of new phylo-groups. *Environ Microbiol*
480 *Rep* 2013; **5**: 58-65.
- 481 22. Clermont O, Gordon D, Denamur E. A guide to the various phylogenetic classification
482 schemes for Escherichia coli and the correspondence among schemes. *Microbiology* 2015; **161 (Pt 5)**:
483 980-8.
- 484 23. Clermont O, Johnson JR, Menard M et al. Determination of Escherichia coli O types by allele-
485 specific polymerase chain reaction: application to the O types involved in human septicemia. *Diagn*
486 *Microbiol Infect Dis* 2007; **57**: 129-36.
- 487 24. Szijarto V, Lukasiewicz J, Gozdziejewicz TK et al. Diagnostic potential of monoclonal antibodies
488 specific to the unique O-antigen of multidrug-resistant epidemic Escherichia coli clone ST131-
489 O25b:H4. *Clin Vaccine Immunol* 2014; **21**: 930-9.
- 490 25. Van Twest R, Kropinski AM. Bacteriophage enrichment from water and soil. *Methods Mol Biol*
491 2009; **501**: 15-21.
- 492 26. Boulanger P. Purification of bacteriophages and SDS-PAGE analysis of phage structural
493 proteins from ghost particles. *Methods Mol Biol* 2009; **502**: 227-38.
- 494 27. Sausseureau E, Vachier I, Chiron R et al. Effectiveness of bacteriophages in the sputum of
495 cystic fibrosis patients. *Clin Microbiol Infect* 2014; **20**: O983-90.
- 496 28. Davis MR, Jr., Goldberg JB. Purification and visualization of lipopolysaccharide from Gram-
497 negative bacteria by hot aqueous-phenol extraction. *J Vis Exp* 2012; **(63)**. 3916.
- 498 29. Hyman P, Abedon ST. Practical methods for determining phage growth parameters. *Methods*
499 *Mol Biol* 2009; **501**: 175-202.
- 500 30. Vallenet D, Belda E, Calteau A et al. MicroScope--an integrated microbial resource for the
501 curation and comparative analysis of genomic and metabolic data. *Nucleic Acids Res* 2013; **41**: D636-
502 47.
- 503 31. Aziz RK, Bartels D, Best AA et al. The RAST Server: rapid annotations using subsystems
504 technology. *BMC Genomics* 2008; **9**: 75.
- 505 32. Dufour N, Debarbieux L, Fromentin M et al. Treatment of Highly Virulent Extraintestinal
506 Pathogenic Escherichia coli Pneumonia With Bacteriophages. *Crit Care Med* 2015; **43**: e190-8.
- 507 33. Vimont S, Boyd A, Bleibtreu A et al. The CTX-M-15-producing Escherichia coli clone O25b: H4-
508 ST131 has high intestine colonization and urinary tract infection abilities. *PLoS One* 2012; **7**: e46547.
- 509 34. Johnson JR, Tchesnokova V, Johnston B et al. Abrupt emergence of a single dominant
510 multidrug-resistant strain of Escherichia coli. *J Infect Dis* 2013; **207**: 919-28.
- 511 35. Scholl D, Merrill C. The genome of bacteriophage K1F, a T7-like phage that has acquired the
512 ability to replicate on K1 strains of Escherichia coli. *J Bacteriol* 2005; **187**: 8499-503.
- 513 36. Kajsik M, Oslanecova L, Szemes T et al. Characterization and genome sequence of Dev2, a
514 new T7-like bacteriophage infecting Cronobacter turicensis. *Arch Virol* 2014; **159**: 3013-9.
- 515 37. Steven AC, Trus BL, Maizel JV et al. Molecular substructure of a viral receptor-recognition
516 protein. The gp17 tail-fiber of bacteriophage T7. *J Mol Biol* 1988; **200**: 351-65.
- 517 38. Casjens SR, Molineux IJ. Short noncontractile tail machines: adsorption and DNA delivery by
518 podoviruses. *Adv Exp Med Biol* 2012; **726**: 143-79.
- 519 39. Kelley LA, Mezulis S, Yates CM et al. The Phyre2 web portal for protein modeling, prediction
520 and analysis. *Nat Protoc* 2015; **10**: 845-58.
- 521 40. Adams MH, Park BH. An enzyme produced by a phage-host cell system. II. The properties of
522 the polysaccharide depolymerase. *Virology* 1956; **2**: 719-36.
- 523 41. Szijarto V, Guachalla LM, Visram ZC et al. Bactericidal monoclonal antibodies specific to the
524 lipopolysaccharide O antigen from multidrug-resistant Escherichia coli clone ST131-O25b:H4 elicit
525 protection in mice. *Antimicrob Agents Chemother* 2015; **59**: 3109-16.
- 526 42. Manges AR, Johnson JR, Foxman B et al. Widespread distribution of urinary tract infections
527 caused by a multidrug-resistant Escherichia coli clonal group. *N Engl J Med* 2001; **345**: 1007-13.

- 528 43. Riley LW. Pandemic lineages of extraintestinal pathogenic *Escherichia coli*. *Clin Microbiol*
529 *Infect* 2014; **20**: 380-90.
- 530 44. Khan Mirzaei M, Nilsson AS. Isolation of phages for phage therapy: a comparison of spot tests
531 and efficiency of plating analyses for determination of host range and efficacy. *PLoS One* 2015; **10**:
532 e0118557.
- 533 45. Abedon ST. Lysis from without. *Bacteriophage* 2011; **1**: 46-9.
- 534 46. Heineman RH, Bull JJ. Testing optimality with experimental evolution: lysis time in a
535 bacteriophage. *Evolution* 2007; **61**: 1695-709.
- 536 47. Nguyen HM, Kang C. Lysis delay and burst shrinkage of coliphage T7 by deletion of terminator
537 Tphi reversed by deletion of early genes. *J Virol* 2014; **88**: 2107-15.
- 538 48. Bayer ME. Adsorption of bacteriophages to adhesions between wall and membrane of
539 *Escherichia coli*. *J Virol* 1968; **2**: 346-56.
- 540 49. Olkkonen VM, Bamford DH. Quantitation of the adsorption and penetration stages of
541 bacteriophage phi 6 infection. *Virology* 1989; **171**: 229-38.
- 542 50. Puck TT, Garen A, Cline J. The mechanism of virus attachment to host cells. I. The role of ions
543 in the primary reaction. *J Exp Med* 1951; **93**: 65-88.
- 544 51. Storms ZJ, Smith L, Sauvageau D et al. Modeling bacteriophage attachment using adsorption
545 efficiency. *Biochemical Engineering Journal* 2012; **64**: 22-9.
- 546 52. De Paepe M, Taddei F. Viruses' life history: towards a mechanistic basis of a trade-off
547 between survival and reproduction among phages. *PLoS Biol* 2006; **4**: e193.
- 548 53. Labrie SJ, Samson JE, Moineau S. Bacteriophage resistance mechanisms. *Nat Rev Microbiol*
549 2010; **8**: 317-27.
- 550 54. Jann K, Jann B. Polysaccharide antigens of *Escherichia coli*. *Rev Infect Dis* 1987; **9 Suppl 5**:
551 S517-26.
- 552 55. Whitfield C. Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. *Annu*
553 *Rev Biochem* 2006; **75**: 39-68.
- 554 56. Pires DP, Oliveira H, Melo LD et al. Bacteriophage-encoded depolymerases: their diversity
555 and biotechnological applications. *Appl Microbiol Biotechnol* 2016; **100**: 2141-51.
- 556 57. Born Y, Fieseler L, Klumpp J et al. The tail-associated depolymerase of *Erwinia amylovora*
557 phage L1 mediates host cell adsorption and enzymatic capsule removal, which can enhance infection
558 by other phage. *Environ Microbiol* 2014; **16**: 2168-80.
- 559 58. Lin TL, Hsieh PF, Huang YT et al. Isolation of a bacteriophage and its depolymerase specific for
560 K1 capsule of *Klebsiella pneumoniae*: implication in typing and treatment. *J Infect Dis* 2014; **210**:
561 1734-44.
- 562 59. Schmerer M, Molineux IJ, Bull JJ. Synergy as a rationale for phage therapy using phage
563 cocktails. *PeerJ* 2014; **2**: e590.
- 564 60. Henry M, Lavigne R, Debarbieux L. Predicting in vivo efficacy of therapeutic bacteriophages
565 used to treat pulmonary infections. *Antimicrob Agents Chemother* 2013; **57**: 5961-8.
- 566 61. Galtier M, De Sordi L, Maura D et al. Bacteriophages to reduce gut carriage of antibiotic
567 resistant uropathogens with low impact on microbiota composition. *Environ Microbiol* 2016.
- 568 62. Scholl D, Gebhart D, Williams SR et al. Genome sequence of *E. coli* O104:H4 leads to rapid
569 development of a targeted antimicrobial agent against this emerging pathogen. *PLoS One* 2012; **7**:
570 e33637.
- 571 63. Yosef I, Manor M, Kiro R et al. Temperate and lytic bacteriophages programmed to sensitize
572 and kill antibiotic-resistant bacteria. *Proc Natl Acad Sci U S A* 2015; **112**: 7267-72.
- 573 64. Lu TK, Collins JJ. Dispersing biofilms with engineered enzymatic bacteriophage. *Proc Natl*
574 *Acad Sci U S A* 2007; **104**: 11197-202.
- 575 65. Ando H, Lemire S, Pires DP et al. Engineering Modular Viral Scaffolds for Targeted Bacterial
576 Population Editing. *Cell Systems* 2015; **1**: 187-96.
- 577 66. Zankari E, Hasman H, Cosentino S et al. Identification of acquired antimicrobial resistance
578 genes. *J Antimicrob Chemother* 2012; **67**: 2640-4.

579

580 **Table 1. Main genotypic characteristics of strain LM33 and its plasmid pLM33**

581

Strain LM33 chromosome (accession number: PRJEB9970)

General information

Genome size: 5 450 287 bp	GC content: 51.5 %	Number of genes: 5 276
Sequence type : ST131 (according to the Achtman scheme)	Serotype: O25b:H4	Phylogroup: B2 <i>fimH</i> allele: 22

Genes coding for antibiotic resistance*

Aminoglycoside resistance: *strB*, *aacA4*, *strA*, *aac(6′)-IIc*
Beta-lactam resistance: *blaDHA-7*, *blaSHV-12*, *blaTEM-1C*
Quinolone resistance: *aac(6′)Ib-cr*, *qnrB4*
MLS resistance: *ere(A)*
Sulphonamide: *sul1* ; thrimethoprim: *dfrA18*

Plasmid pLM33 (accession number: PRJEB9970)

General information

Plasmid size: 296 909 bp	GC content: 47.2%	Number of genes: 382
Incompatibility group: H		

Genes coding for antibiotic resistance*

Aminoglycoside resistance: *strA*, *strB*, *aacA4*, *aac(6′)-IIc*
Beta-lactam resistance: *blaSHV-12*, *blaTEM-1C*
Quinolone resistance: *aac(6′)Ib-cr*
MLS resistance: *ere(A)*

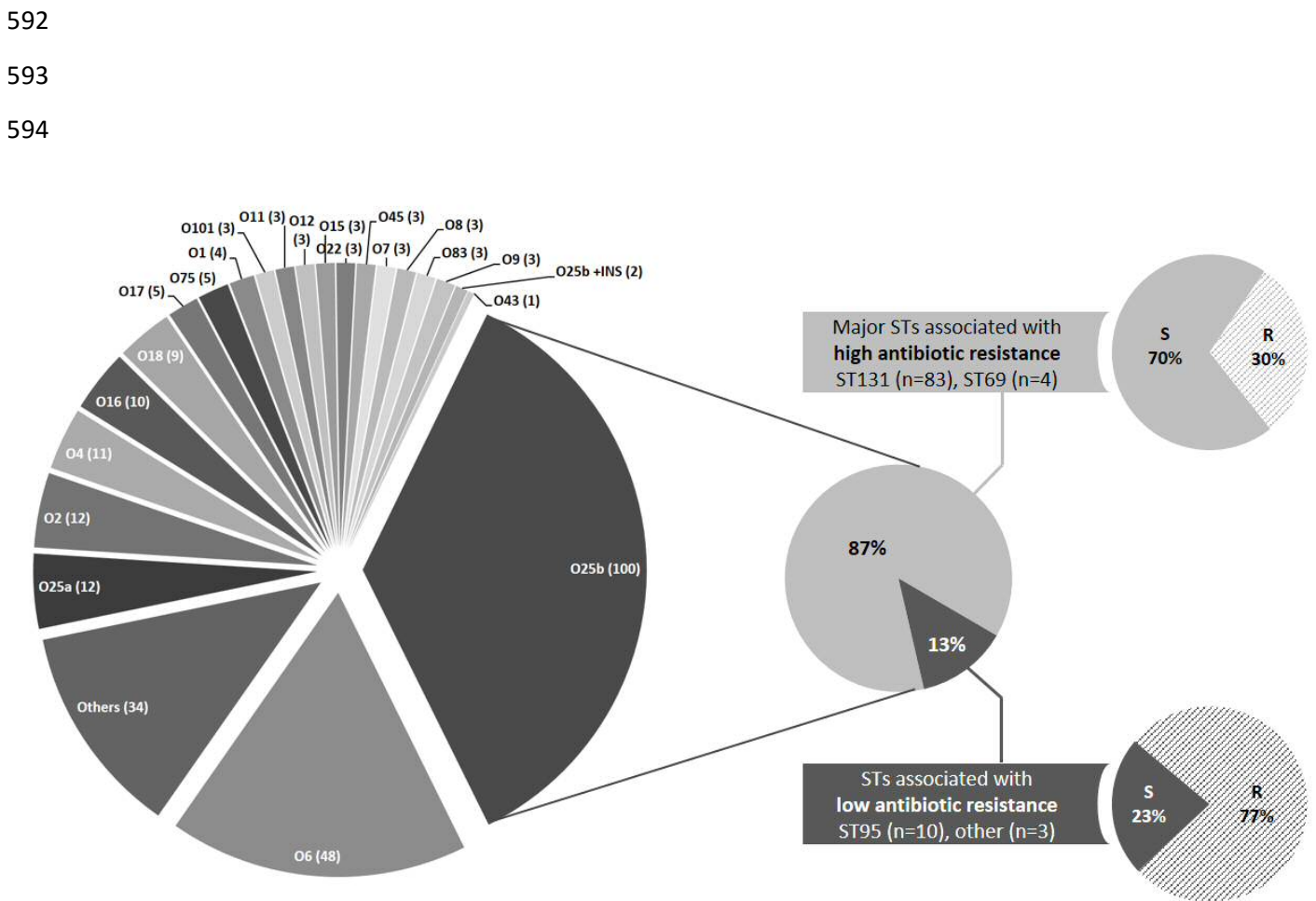
*data obtained using the center for genetic epidemiology server⁶⁶

582

583

584 **Figure 1: O-type distribution of the 283 *E. coli* strains tested for their susceptibility to**
 585 **bacteriophage LM33_P1.** The numbers in brackets represent the number of strains tested,
 586 for each O-type. None of the non-O25b strains was infected by bacteriophage LM33_P1. The
 587 proportion of O25b strains regarding bacteriophage LM33_P1 susceptibility (S, susceptible or
 588 R, resistant) and sequence type is represented on the right part.

589 O25b+INS stands for the strains possessing an O25b genotype based on the *rfb* locus
 590 sequence but with an insertion sequence within the *wbbL* gene located at the end of the
 591 operon, just upstream the *gnd* gene, and responsible for a non-O25b phenotype.



595

596

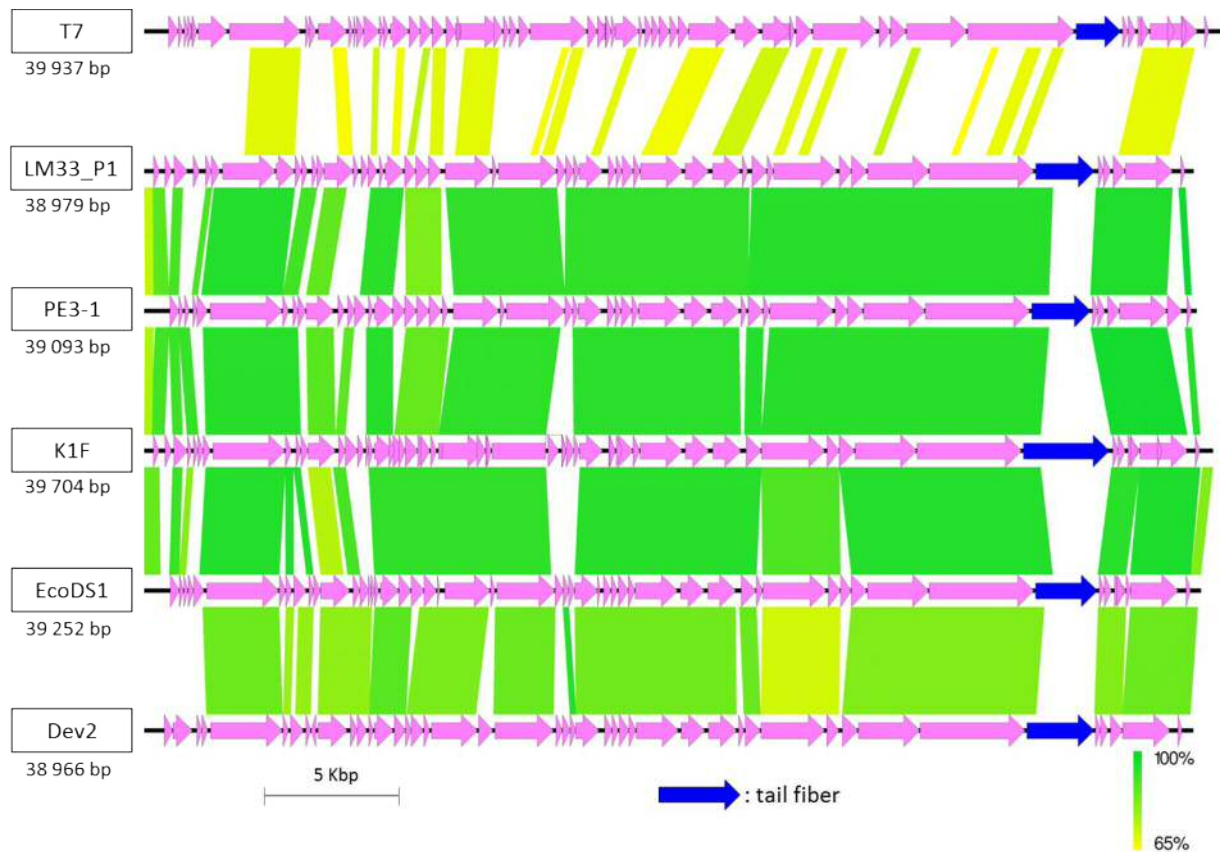
597

598

599

600 **Figure 2. Genome alignment of bacteriophage LM33_P1 with its four closest related**
601 **bacteriophages and model bacteriophage T7.** The homology in nucleotide sequence is
602 color-coded (see scale). The blue arrows correspond to the genes and indicate their
603 transcription direction. Blue arrows correspond to the gene coding for the tail fiber protein.
604 Only the homologies with an E-value $\leq 10^{-3}$ and a nucleotide length ≥ 230 are represented.

605

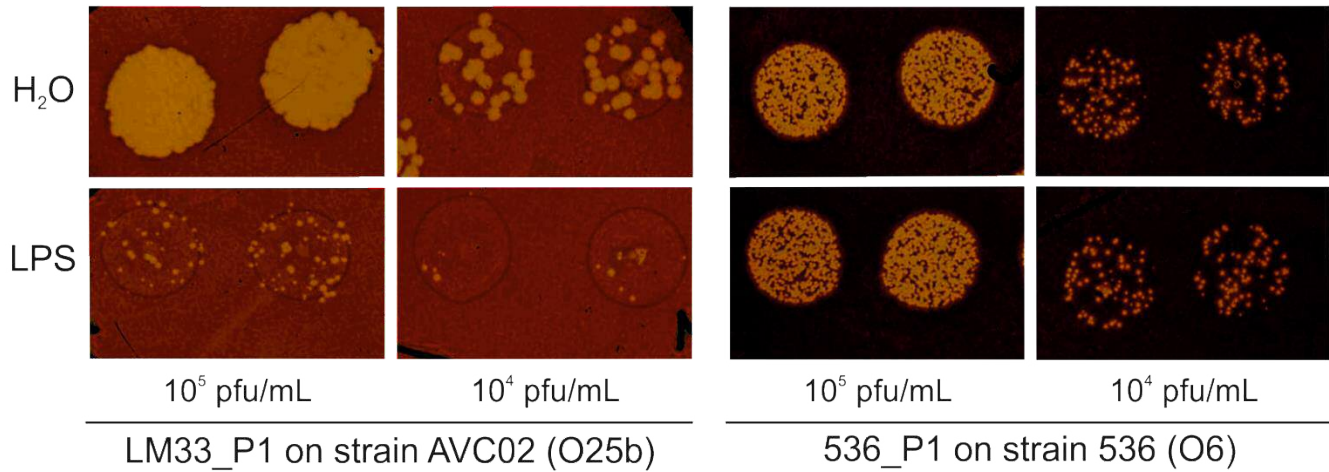


606

607

608 **Figure 3. O25b LPS extract inhibits bacteriophage LM33_P1 infection: appearance on agar**
609 **plates.** An LPS extract from strain LM33 was mixed with bacteriophage LM33_P1 (left) or
610 536_P1 (right) at two different concentrations (10^5 and 10^4 pfu/mL) and assayed on two agar
611 plates overlaid with an O25b strain (AVC02) or an O6 strain (536) as control. Enlargements of
612 these two plates are shown to facilitate the observation.

613
614

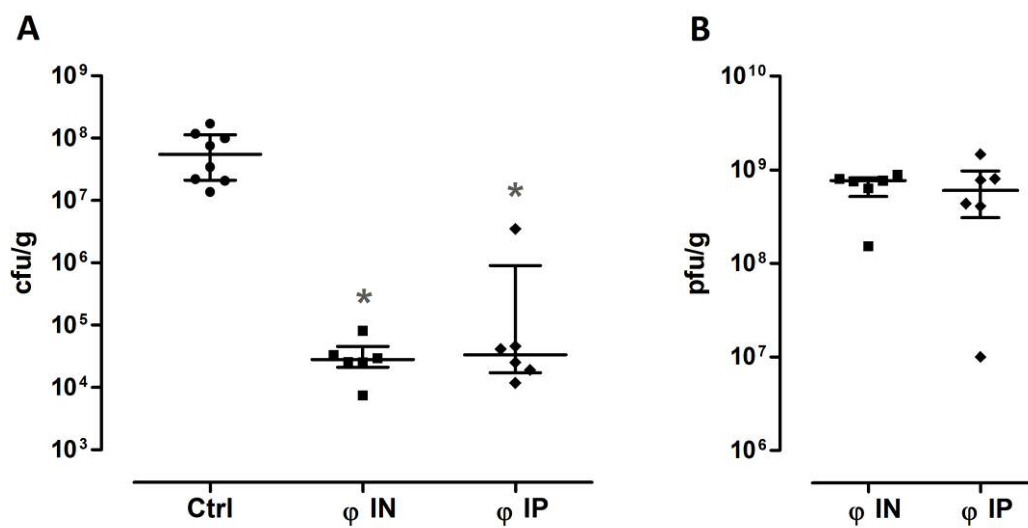


615
616
617
618
619
620
621
622
623
624
625
626
627
628
629

630 **Figure 4. Bacteriophage LM33_P1 *in vivo* activity in a lung infection model.** Bacterial (panel
631 A) and viral (panel B) counts 17 hours post-infection in lungs homogenates of mice infected
632 with 1×10^8 cfu of strain LM33. Four hours post-infection, the mice received either PBS (Ctrl,
633 $n=8$, one half intranasally and the other half intraperitoneally) or bacteriophage LM33_P1 by
634 intranasal route (ϕ IN, MOI 50, $n=6$) or by intraperitoneal route (ϕ IP, MOI 500, $n=6$). The
635 results are expressed as individual values with median and interquartile ranges (25th and 75th
636 percentiles). *: $p < 0.001$ compared to control group.

637

638



639

640

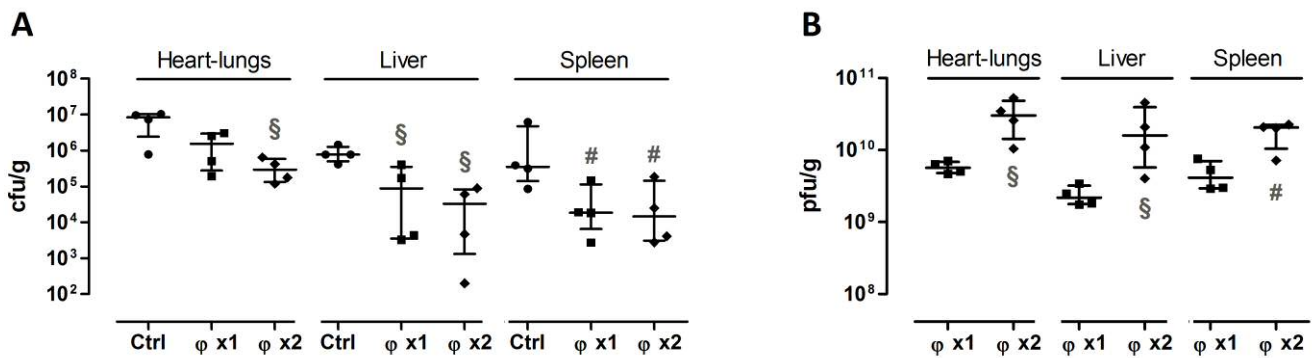
641

642

643

644 **Figure 5. Bacteriophage LM33_P1 *in vivo* activity in a septicemia model.** Bacterial (panel A)
 645 and viral (panel B) counts 20 hours post-infection in the indicated organs of mice infected
 646 with 1×10^9 cfu of strain H1659 (ST131-O25b:H4). Two hours post-infection, the mice
 647 received intraperitoneally either PBS (Ctrl) or bacteriophage LM33_P1 at a MOI of 60 (ϕ X1:
 648 one dose 2 hours post-infection, ϕ X2: two doses 2 and 12 hours post-infection). The results
 649 are expressed as individual values (4 animals per condition) with median and interquartile
 650 ranges (25th and 75th percentiles). §, #: $p < 0.05$ (§) or $p = 0.057$ (#) compared to the control
 651 group (panel A) or the single-dose treatment (panel B).

652



653

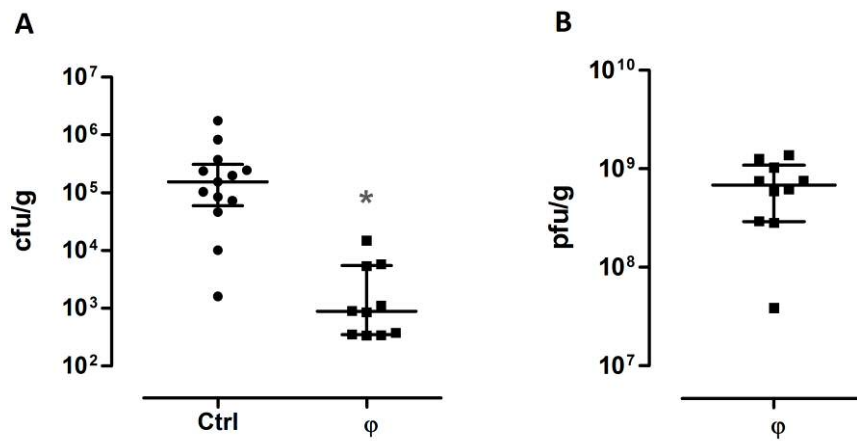
654

655

656

657 **Figure 6. Bacteriophage LM33_P1 *in vivo* activity in a urinary tract infection model.**
658 Bacterial (panel A) and viral (panel B) counts 48 hours post-infection in kidneys homogenates
659 of mice infected with 5×10^7 cfu of strain LM33. Twenty four hours post-infection, the mice
660 received intraperitoneally either PBS (Ctrl, n=13) or bacteriophage LM33_P1 (ϕ , MOI 200,
661 n=10). The results are expressed as individual values with median and interquartile ranges
662 (25^{th} and 75^{th} percentiles). *: $p < 0.001$ compared to control group.

663



664

665

666

Table S1

Genomic characteristics of bacteriophage LM33_P1, its four closest homologs and the reference bacteriophage T7, all belonging to the *Autographivirinae* subfamily of viruses.

Bacteriophage	Host	Genome size (bp)	ORFs (n)	GC %	Accession number
LM33-P1	<i>E. coli</i>	38 979	49	50.8	PRJEB12445
T7	<i>E. coli</i>	39 937	60	49.0	NC_001604.1
PE3-1	<i>E. coli</i>	39 093	48	50.4	NC_024379.1
K1F	<i>E. coli</i>	39 704	43	49.8	NC_007456.1
EcoDS1	<i>E. coli</i>	39 252	53	49.9	NC_011042.1
Dev2	<i>C. turicensis</i>	38 966	45	52.6	NC_023558.1

Table S2

Data obtained during plaque test inhibition assays with different LPS extracts and randomly chosen couples of viruses-bacteria. (+)/(-): presence/absence of an inhibitory effect of LPS extract, – : not tested.

Interaction tested		Inhibitory effect of various LPS extracts			
Bacteriophage	Bacteria (serotype)	O25b (LM33)	O6 (536)	O17 (LM02)	O25a (ECOR51)
LM33_P1	LM33 (O25b)	(+)	(-)	(-)	(-)
" "	LM34 (O25b)	(+)	(-)	(-)	(-)
" "	LM36 (O25b)	(+)	(-)	(-)	(-)
" "	AVC02 (O25b)	(+)	(-)	(-)	(-)
" "	AVC03(O25b)	(+)	(-)	(-)	(-)
536_P1 ^a	536 (O6)	(-)	(-)	–	–
423_P1 ^b	H17 (O16)	(-)	–	–	–
416_P1 ^b	LM49 (O2b)	(-)	–	–	–
LF82_P2 ^c	LF82 (O83)	(-)	–	–	–
LF82_P2 ^c	RY09 (O4)	(-)	–	–	–

^a described in reference 31 in the manuscript, ^b bacteriophages isolated using ventilator-associated pneumonia (VAP) strains (423, 416) and active on others VAP strains (H17, LM49), ^c bacteriophage isolated using an adherent-invasive *E. coli* (LF82) and active on VAP strain RY09.

Figure S1

Distribution of the efficiency of plaquing values of bacteriophage LM33_P1. Dots represent individual values for each of the 64 strains tested whereas median (0.46) and 25th-75th percentiles [0.09-1.27] are indicated by lines. Y-axis is in Log scale.

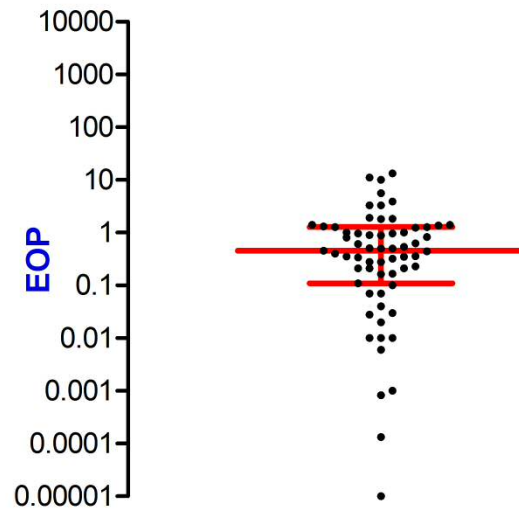


Figure S2

Predicted tertiary structure of the the C-terminal part of bacteriophage LM33_P1 tail fiber using Phyre², compared to its closest homolog (*Erwinia carotovora* endopolygalacturonase).

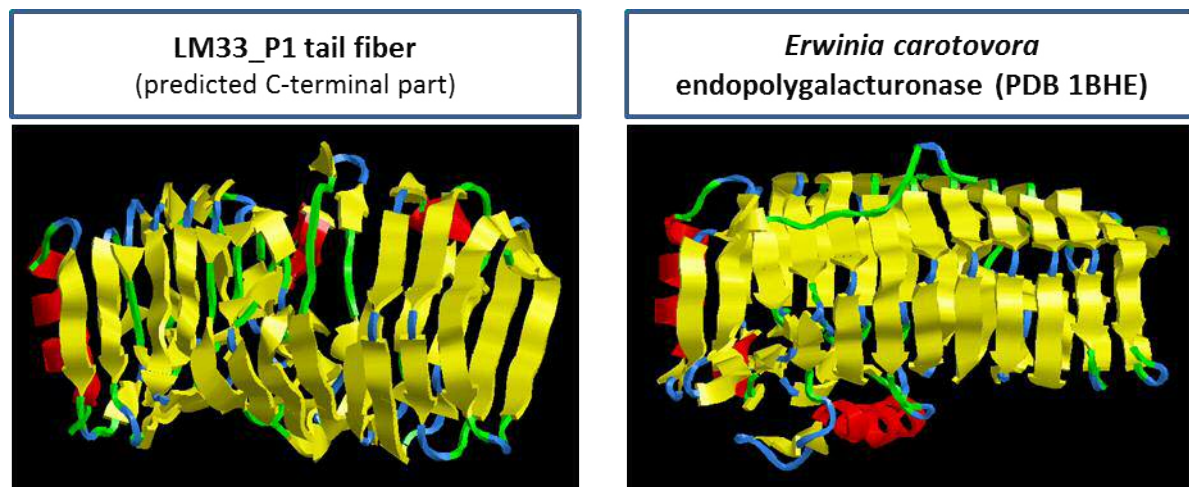


Figure S3

A. Adsorption of bacteriophage LM33_P1 on its host LM33. Strain LM33 grown in LB at 37 °C with shaking (100 rpm) was mixed with bacteriophage LM33_P1 at a MOI of 10^{-4} and aliquots were taken at the indicated time points. Dots represent the mean of 3 independent experiments with the standard deviation.

B. Bacteriophage LM33_P1 growth parameters. Bacteriophage LM33_P1 was mixed with strain LM33 (MOI 10^{-1}) at 37 °C with shaking. At the indicated time points, samples were analyzed in absence (grey lines) or in presence (black lines) of chloroform. The continuous lines represent the experimental measurements (mean of 3 independent experiments with standard error) while the dashed lines are the nonlinear regression obtained from these points.

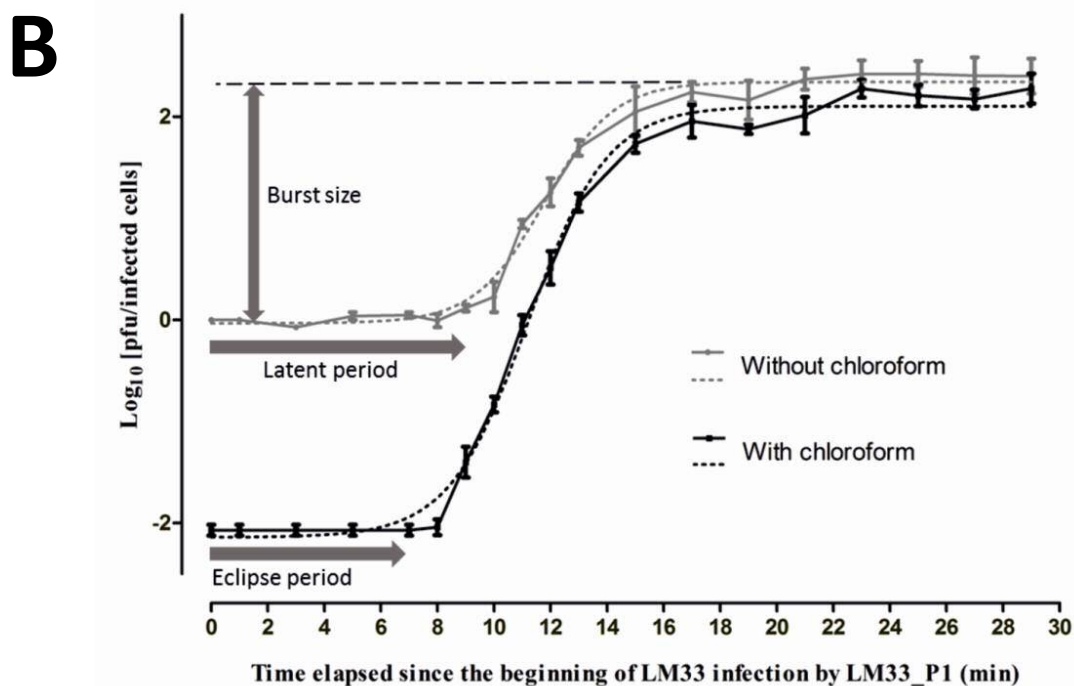
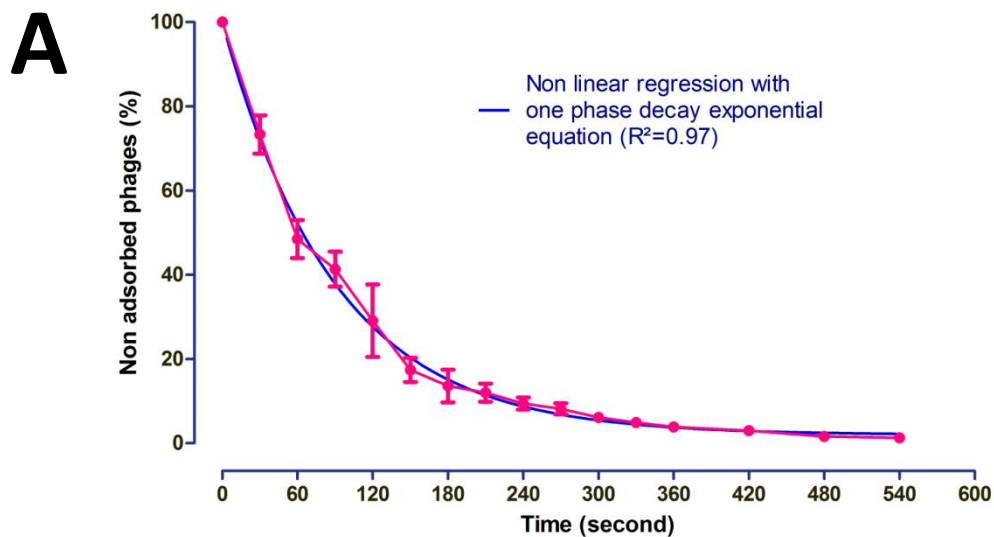


Figure S4

Lysis kinetics of strain LM33 by bacteriophage LM33_P1 at different multiplicity of infection. The **panel A** displays the first 22 hours of kinetic and the **panel B** displays a magnification of the first 2 hours. The lysis kinetics of the strain LM33 were performed as described previously (Maura et al., Environ Microbiol, 2012;14(8):1844-1854) in LB, at 37°C and with various multiplicity of infection (MOI). Optical density (600 nm) was followed over time and recorded each 15 minutes on a Glomax plate reader (Promega, Madison, USA). Strain LM33 without LM33_P1 was used as a control. All the conditions were performed in triplicates and the experiment was repeated three times independently (only one representative experiment is shown). For readability purpose, only mean of triplicates are displayed without the corresponding error bars.

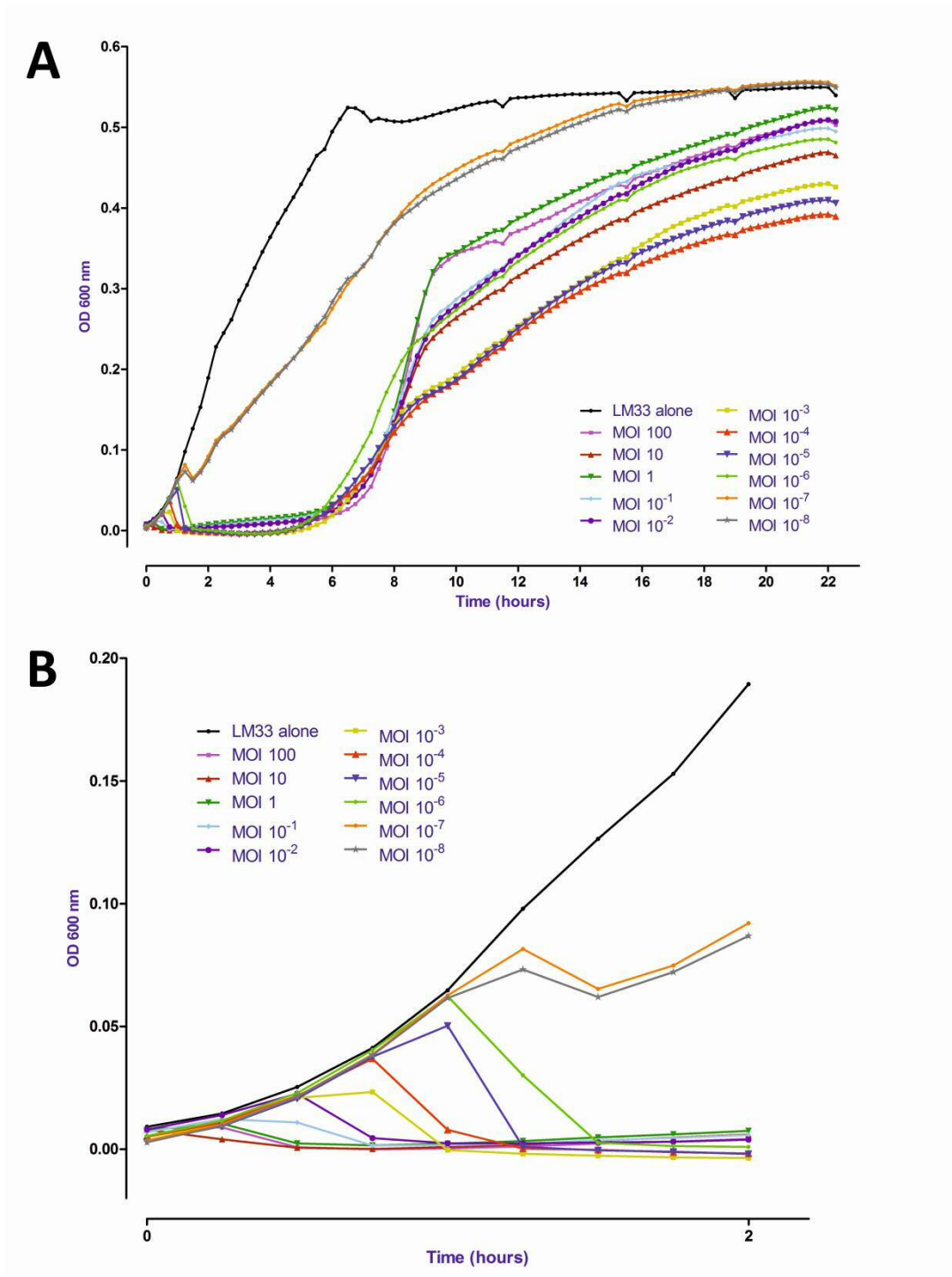
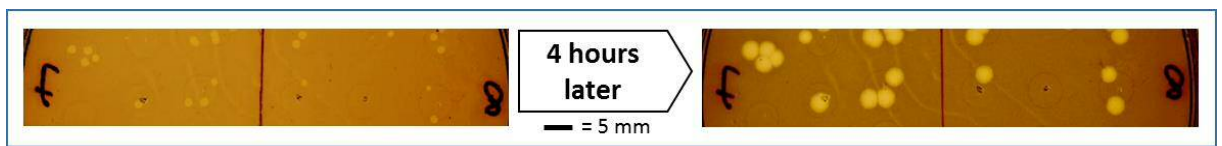


Figure S5

A. The size of the plaques made by bacteriophage LM33_P1 increases rapidly over time. LM33_P1 plaque-forming units on strain LM33 as visualized on LB-agar plate after 2 hours of incubation at 37 °C (left picture) and after 6 hours of infection (right picture).

B. A large halo surrounds plaques and area of lysis made by bacteriophage LM33_P1. Three drops of 10 μL of LM33_P1 (5×10^3 pfu/mL) have been dropped off on a bacterial host layer and incubated at 37 °C for 21 (left) and 36 hours (right). A halo is clearly visible at 36 hours, surrounding the area of lysis.

A



B



Figure S6

A: An O25b LPS extract inhibits interactions between bacteriophage LM33_P1 and O25b strains. Variations of bacteriophage titers from 15 individual plaque tests in presence or absence of O25b LPS extract are shown. For each individual assay, the same starting bacteriophage solution was titrated in triplicate, without and with LPS extract obtained from strain LM33. Each dot represents therefore the mean titer of the 3 replicates. Four O25b strains and different concentrations of LM33_P1 have been tested (see methods). **B: O25b LPS extract inhibits bacteriophage LM33_P1 activity in liquid medium.** The growth of strain LM33 in liquid broth was recorded every 15 minutes using optical density (OD_{600nm}) in absence (red curve) or in presence of bacteriophage LM33_P1 (MOI of 10⁻⁶) without (green) or with undiluted (black), 2-fold diluted (dark blue), 4-fold diluted (light blue) O25b LPS extract.

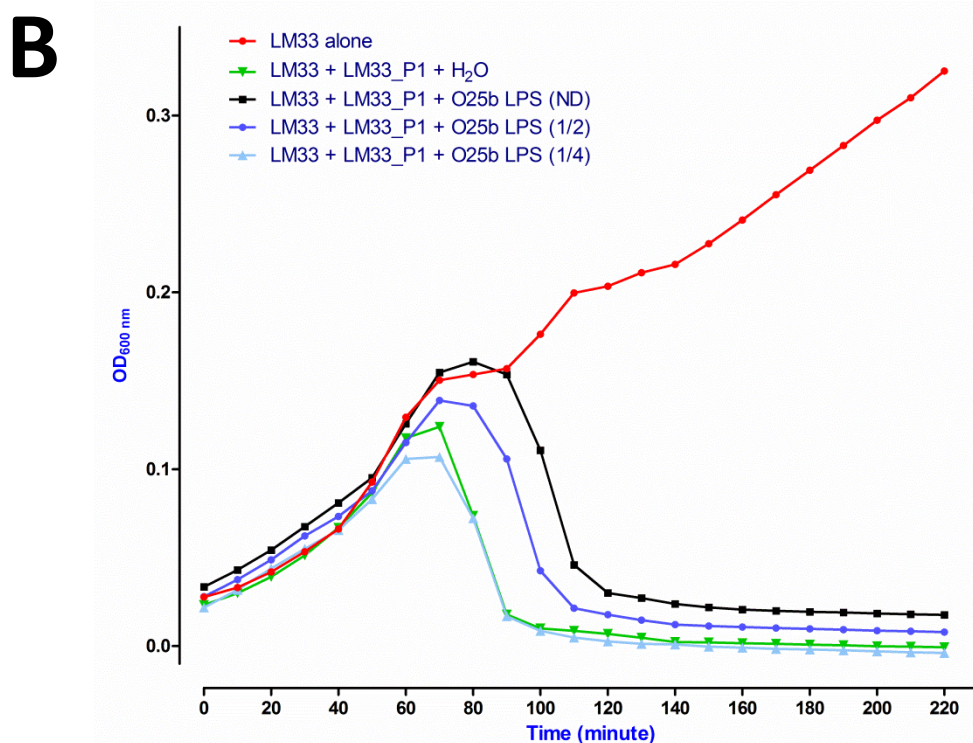
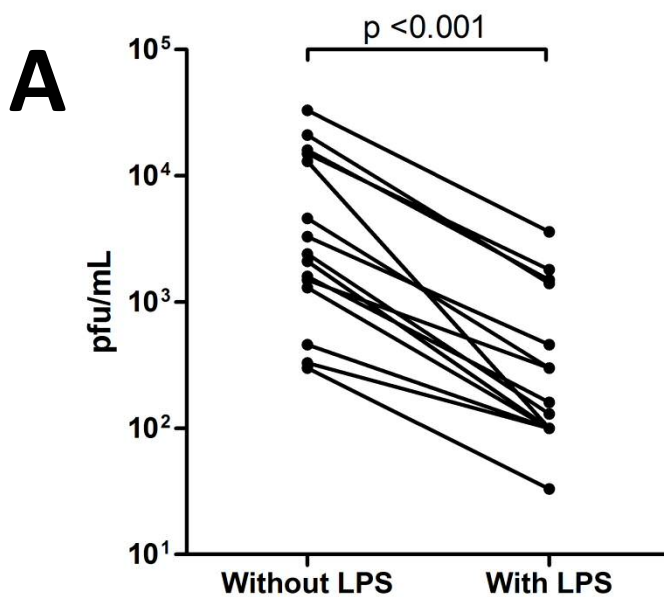
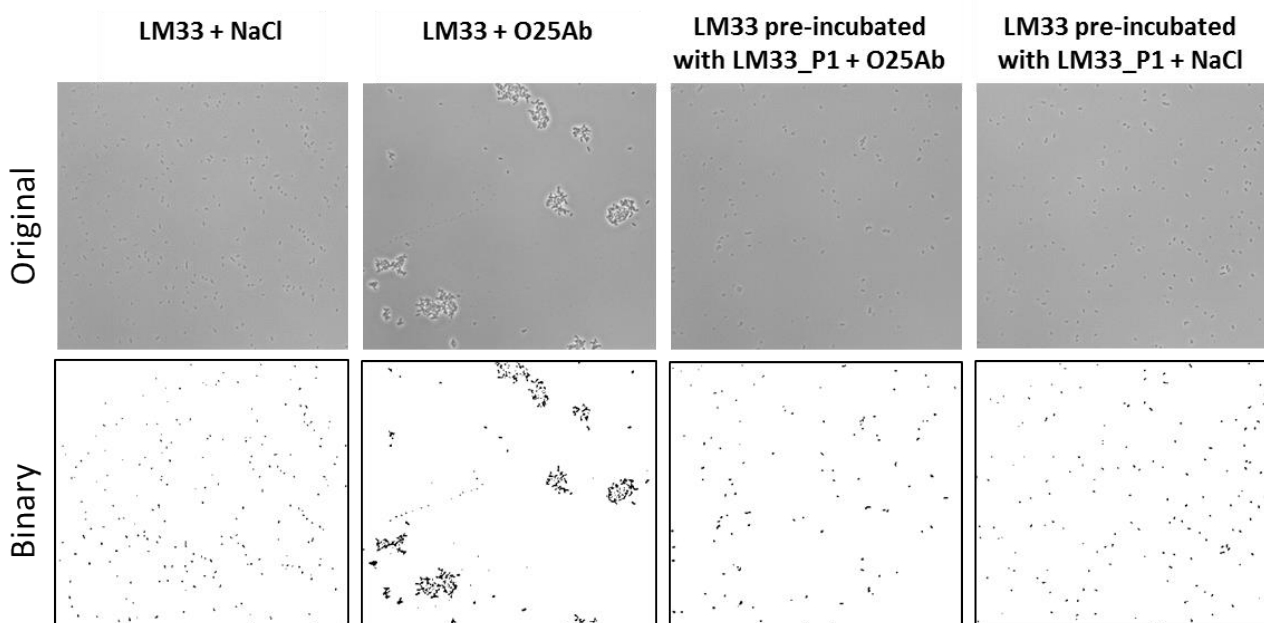


Figure S7

Bacteriophage LM33_P1 prevents O25 antibody-mediated aggregation of strain LM33. A commercially available specific O25 *E. coli* anti-serum, sold to perform O-antigen serotyping, was obtained from the Statens Serum Institut (Copenhagen, Denmark). The tests were performed using old liquid cultures of strain LM33 in stationary phase in LB (in order to slow down and delay bacterial lysis). Four conditions were tested, with the following mixes:

- I- LM33 (5 μ L) + Saline (NaCl 0.9%, 5 μ L) + TN buffer (2 μ L),
- II- LM33 (5 μ L) + O25Ab (5 μ L, in NaCl 0.9%) + TN (2 μ L),
- III- LM33 (5 μ L) pre-incubated 10 min at 20 °C with LM33_P1 (in TN, 2 μ L, MOI of 10) + O25Ab (5 μ L),
- IV- LM33 (5 μ L) pre-incubated 10 min at 20 °C with LM33_P1 (in TN, 2 μ L, MOI of 10) + Saline (5 μ L).

Following the incubation at 37 °C during 30 min, a drop (5 μ L) of each condition was put on a glass slide and covered with a cover slip for direct examination under a phase contrast microscope. The upper line shows original pictures as obtained with a 40x magnification and the lower line represents a binary black and white transformation of the above pictures generated by ImageJ software (rsb.info.nih.gov/ij/).



Procedures

Allele-specific PCR amplification for the identification of the *fimH* alleles 22, 30 and 41

The primers for the *fimH* allele-specific PCR and the length of the PCR products were as follows:

fimH22.f (5'-TATTGGCGGTGGCAGCGCC-3'), *fimH22.r* (5'-GTTTCGCTGGTAGTAGGAAA-3'), 234 bp;

fimH30.f (5'-CCGCCAATGGTACCGCTATT-3'), *fimH30.r* (5'-CAGCTTTAATCGCCACCCCA-3'), 354 bp;

fimH41.f (5'-TTTATGTAAACCTTGCGCCC-3'), *fimH41.r* (5'-AACATCACAGCCGCCAGTG-3'), 431 bp.

PCR reactions were carried out in a 20- μ l volume containing 4 μ l of 5X buffer (supplied with Taq polymerase), 10 pmol of each primer, 200 μ M each dNTP, 2 U of Taq polymerase (Promega, Charbonnières-les-Bains, France), and 3 μ l of bacterial lysate or 2 μ l of DNA. PCR was performed with an Eppendorf Mastercycler with MicroAm tubes in the following conditions: denaturation 4 min at 94° C, 30 cycles of 5 sec at 94° C and 10 sec at 65°C, and a final extension step of 5 min at 72° C.

PCR products were loaded on 2% agarose gel with SYBR® Safe DNA gel stain (Invitrogen, Cergy-Pontoise, France). Following electrophoresis, gels were photographed under UV light.

The method was validated on a panel of *E. coli* strains from which the *fimH* sequence was available, i.e. 7, 19 and 47 strains exhibiting the *fimH* allele *H22*, *H30* and *H41*, respectively, as well as 67 strains exhibiting none of these alleles (Ochman H, Selander RK. J Bacteriol. 1984 Feb;157(2):690-3 ; Clermont O, Gordon D, Denamur E. Microbiology. 2015 May;161(Pt 5):980-8 ; Johnson JR, Clermont O, Johnston B, Clabots C, Tchesnokova V, Sokurenko E, Junka AF, Maczynska B, Denamur E. J Clin Microbiol. 2014 May;52(5):1358-65.). The sensitivity and the specificity of the method were of 100%.

Bacteriophage susceptibility testing

For bacteriophage susceptibility testing, we used the double spot test technique as screening method and EOP calculation for all susceptible strains. The double spot test consisted in dropping off 10 μ L of a growing liquid culture of the bacterial strain (OD_{600nm} 0.5) on an agar plate. After drying, 1 μ L of the bacteriophage solution (LM33_P1, 10^7 pfu/mL) was added on one half of the bacterial drop. The plate was then incubated at 37°C during 4-8 hours before reading. A susceptible strain was identified by the presence of a crescent-shaped lysis area on the bacterial drop or the visualization of individualized plaques. Efficiency of plaquing (EOP) was determined for all susceptible strains by titrating the solution of LM33_P1 on both its host (LM33) and the evaluated strain. EOP was calculated as the ratio of the number of plaques formed by the bacteriophage on the non-host strain to

Procedures

the number of plaques formed on its host, using the same bacteriophage solution. Only the strains for which individualized plaques were observed were considered as susceptible strains. For strain 81009 WT and its rough derivative mutant, tests were performed at 20 °C to turn-off type II capsule expression.

Characterization of bacteriophage LM33 P1

The adsorption assay and the one-step growth experiment were performed in triplicate, using Lysogeny Broth (Difco™ Bacto-Tryptone 10 g/L, Difco™ Yeast extract Difco 5 g/L, NaCl 5 g/L), under constant shaking (100 rpm) at 37°C, as described by Hyman and Abedon (Practical methods for determining phage growth parameters, *Methods Mol Biol* 501:175-202, 2009). A correlation curve was extrapolated from raw data using nonlinear regressions (GraphPad Prism 5.0, GraphPad software, California): a dose-response model was used for the one step growth experiment ($Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$) with $Y = \log(\text{pfu}/\text{infected cell})$ and $X = \text{time}$) and an exponential model with one phase decay for adsorption experiment ($Y = (Y_0 - \text{Plateau}) * \exp(-K * X) + \text{Plateau}$ with $Y = \text{free phages}(\%)$, $X = \text{time}$). The growth parameters (eclipse and latent period, burst size) were then derived from these regressions. The adsorption constant was calculated as $-p/N$ where p is the slope of the straight line obtained after a natural logarithm transform and N the concentration of bacteria at the beginning of the adsorption assay.

Sequencing of the strain LM33 and bacteriophage LM33 P1

The sequencing of bacteriophage LM33_P1 and strain LM33 was performed using Illumina sequencing technology (Illumina Inc., San Diego, CA). The LM33_P1 DNA was extracted from a purified bacteriophage solution, using DNase and RNase pretreatments followed by a phenol-chloroform extraction as described by Pickard (Pickard DJ, 2009, Preparation of bacteriophage lysates and pure DNA. *Methods Mol Biol*). The LM33 genomic DNA was extracted using a MaxWell Tissue DNA Purification kit (Promega, Madison, WI). Genomes annotation was performed by the MicroScope platform for strain LM33 (Vallenet D, 2013, MicroScope: an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. *Nucleic Acids Res*) and with the RAST server for bacteriophage LM33_P1 (Aziz RK, 2008, The RAST Server: rapid annotations using subsystems technology. *BMC Genomics*), followed by manual curation.

Procedures

Murine experimental infections models

The animals were housed in authorized animal facilities in accordance with French and European regulations on the care and protection of laboratory animals. The protocols were approved of by the veterinary staff of the Institut Pasteur and INSERM animal facilities, as well as the National Ethics Committee regulating animal experimentation. The animals were housed in a SPF animal facility rated biosafety level 3 (Institut Pasteur) or 2 (INSERM). Food and drink were provided *ad libitum*. The mice were housed in separate ventilated cages corresponding to each group (control or phage-treated) and were visited at least twice daily to monitor health status. A daily weighing was performed and the mice were euthanized if weight loss greater than 20%, a limit that had never been reached here. The control group was always handled first (before the phage-treated group) to avoid phage contamination of the control group. The period of time between the infection and the treatment (phage or mock) was recorded and was identical between each group.

For each infection model, the results provided are the sum of two or three independent experiments.

The bacteriophage solutions administered in the animal experiments were obtained from purified stock (as described in Material and Methods) and were submitted to an additional purification procedure to reduce endotoxin level using an affinity chromatography-based endotoxin removal kit (EndoTrap blue, Hyglos, Germany).

Pneumonia was initiated by intranasal administration of 1×10^8 cfu of strain LM33 on 20 anesthetized eight-week-old 25 g BALB/cJrj male mice (Janvier, Le Genest Saint Isle, France). The mice were treated using bacteriophage LM33_P1 four hours post-infection (n=12), either by using the intranasal route (multiplicity of infection of 50, *i.e.* a ratio of viruses to bacteria equal to 50) or the intraperitoneal route (MOI of 500). Control mice (n=8) received accordingly an intranasal or intraperitoneal identical volume of PBS (phosphate-buffered saline). The lungs were collected 17 hours post-infection on euthanized animals. An intraperitoneal administration of 1.25 μ g (50 μ g/kg) of buprenorphine (Buprecare; AST Farma, Oudewater, The Netherlands) was systematically performed on all the mice at 8 hours post infection to limit the pain and the dyspnea.

The septicemia model, as previously described, is essentially used to study the intrinsic extraintestinal virulence of *E. coli* isolates. Twelve four-week-old 17 g OF1 female mice (Janvier, Le Genest Saint Isle, France) were injected subcutaneously into the nape of the neck with 1×10^9 cfu of strain H1659 (ST131-O25b:H4). Because of the high inoculum used, we tested both a single and a double dose of bacteriophages: the single dose (MOI 60) was administered by an intraperitoneal injection 2 hours post-infection (n=4) while the double dose consisted in an injection (MOI 60) administered 2 and 12 hours post-infection (n=4). Control mice (n=4) received an identical volume of PBS. Organs targeted by septic metastasis

Procedures

(heart-lung, spleen and liver) were collected on the animals that died between 24 to 30 hours post-infection.

The urinary tract infection model consists in a retrograde kidneys infection occurring after an intra-urethral injection of 5×10^7 cfu of strain LM33 in the bladder (23 mice). Twenty-four hours after the infection, 8-week-old 17 g CBA/j female mice (Charles River, Chatillon-sur-Chalaronne, France) were treated intraperitoneally with LM33_P1 (MOI of 200, n=10) while control mice (n=13) received an identical volume of PBS. The kidneys were collected 48 hours post-infection.

The euthanasia of animals was performed by asphyxiation with rising concentration of CO₂. In every case, the organs were mechanically homogenized in cold PBS using a gentleMACS Octo Dissociator (Milteny Biotec, Bergisch Gladbach, Germany) before being serially diluted and spread on Drigalski agar plates containing appropriate antibiotic to numerate colony, in triplicate. The bacteriophage count was performed in triplicate on supernatant after centrifugation of the homogenates according to routine methods.