

1 **Phage-plasmids spread antibiotic resistance genes through infection and lysogenic**
2 **conversion**

3

4 Authors:

5 Eugen Pfeifer^{1*}, Rémy A. Bonnin², Eduardo P.C. Rocha^{1*}

6

7 ¹ Institut Pasteur, Université Paris Cité, CNRS UMR3525, Microbial Evolutionary Genomics, Paris

8 75015, France

9 ² Team "Resist" UMR1184 "Immunology of Viral, Auto-Immune, Hematological and Bacterial diseases

10 (IMVA-HB)," INSERM, Université Paris-Saclay, CEA, LabEx LERMIT, Faculty of Medicine, Le Kremlin-

11 Bicêtre, France. Associated French National Reference Center for Antibiotic Resistance:

12 Carbapenemase-Producing *Enterobacteriaceae*, Le Kremlin-Bicêtre, France.

13

14 *corresponding authors: eugen.pfeifer@pasteur.fr, erocha@pasteur.fr

15

16 Running title:

17 **AMR genes spread by phage-plasmids**

18

19 Abstract

20 Antibiotic resistance is rapidly spreading by horizontal transfer of resistance genes in mobile
21 genetic elements. While plasmids are key drivers of this process, very few integrative phages
22 encode antibiotic resistance genes. Here, we find that phage-plasmids, elements that are
23 both phages and plasmids, often carry antibiotic resistance genes. We found 60 phage-
24 plasmids with 184 antibiotic resistance genes, including broad-spectrum-cephalosporins,
25 carbapenems, aminoglycosides, fluoroquinolones and colistin. These genes are in a few
26 hotspots, seem to have been co-translocated with transposable elements, and are often in
27 class I integrons, which had not been previously found in phages. We tried to induce six
28 phage-plasmids with resistance genes (including four with resistance integrons) and
29 succeeded in five cases. Other phage-plasmids and integrative prophages were co-induced in
30 these experiments. As a proof of principle, we focused on a P1-like element encoding an
31 extended spectrum β -lactamase, *bla*_{CTX-M-55}. After induction, we confirmed that it's capable
32 to infect and convert four other *E. coli* strains. Its re-induction led to further conversion of a
33 sensitive strain, confirming it's a fully functional phage. This study shows that phage-plasmids
34 carry a large diversity of clinically relevant antibiotic resistance genes that they transfer across
35 bacteria. As plasmids, these elements seem very plastic and capable of acquiring genes from
36 other plasmids. As phages, they may provide novel paths of transfer for resistance genes,
37 because they can infect bacteria distant in time and space from the original host. As a matter
38 of alarm, they may also eventually mediate transfer to other types of phages.

39 Importance

40 Dissemination of antimicrobial resistances is a major threat to global health. Here, we show
41 that a group of temperate bacterial viruses (=phages), termed phage-plasmids, commonly
42 encode different and multiple types of resistance genes of high clinical importance, often in
43 integrons. This is unexpected since phages typically do not carry resistance genes and, hence,
44 do not confer their hosts with resistance upon infection and genome integration. Our
45 experiments with phage-plasmids isolated from clinical settings confirmed they infect
46 sensitive strains, rendering them antibiotic resistant. The spread of antibiotic resistance genes
47 by phage-plasmids is worrisome because it dispenses cell-to-cell contact, necessary for the
48 canonical plasmid transfer (=conjugation). Furthermore, their integrons are now genetic
49 platforms for the acquisition of novel resistance genes.

50 Introduction

51 Antimicrobial resistances (AMR) are fast disseminating among human-associated bacteria
52 and have been classified as major challenges to Global Health (1). Enterobacterales are
53 identified as the most critical group (2) against which new drugs need to be developed.
54 Resistance is the result of one of multiple mechanisms: limiting drug uptake; target
55 modification; active drug efflux and drug inactivation. The latter includes extended spectrum
56 β -lactamases (e.g. ESBLs) that allow Enterobacterales to become resistant against most β -
57 lactams (such as penicillins or broad-spectrum cephalosporins). Although ESBLs do not
58 provide directly resistance to carbapenems (last-resort antibiotics within β -lactams), the wide
59 and improper use of carbapenems, especially as a first-line treatment, has promoted the
60 emergence of carbapenem-resistant Enterobacterales (CRE) strains that are commonly found
61 to be resistant to others antibiotic classes (3). While low-level resistance to β -lactams can be
62 provided by many mechanisms such as qualitative or quantitative modifications of porins,
63 high resistance is usually associated with the acquisition of genes encoding ESBLs or
64 carbapenemases by horizontal gene transfer (4). The most important and clinically relevant
65 carbapenemases identified in Enterobacterales belong to class A (KPC-like enzyme), class B
66 (NDM-, VIM- and IMP-like enzyme) and class D (OXA-48-like enzyme) type β -lactamases (5).
67 Plasmids are key drivers of the transmission of antibiotic resistance genes (ARGs) between
68 bacteria, usually by conjugation (6, 7). Transfer is also facilitated by the presence of mobile
69 genetic elements (MGEs) translocating genetic information between replicons (8). Notably,
70 ARGs are often flanked by transposable elements that facilitate their translocation between
71 plasmids or between plasmids and the chromosome (9). Integrons can also facilitate the
72 translocation of ARG cassettes (10). Mobile integrons are usually associated with plasmids
73 and/or transposons and consist of one integrase (here of the type IntI1) and a small array of
74 gene cassettes flanked by recombination sites. Integrons can acquire new gene cassettes from
75 other integrons and shuffle the existing ones (11). A large fraction of the cassettes of mobile
76 integrons consists of ARGs (10). The co-transfer of multiple ARGs in an integron facilitates the
77 emergence of multi-drug resistance strains.

78 Temperate bacteriophages (phages) can mobilize genes by different types of transduction
79 processes (generalized, specialized and lateral) (12) or introduce new genes by lysogenic
80 conversion (13). Generalized transduction relies on erroneous packaging of non-phage DNA

81 by specific types of phages and tends to occur at low frequencies (14), whereas lateral and
82 specialized transduction require proximity between the transferring genes and the phage
83 (12). All these processes have been shown to result in the transfer of ARGs in the lab, but
84 there is extensive controversy on the extent and pertinence of this process in natural
85 environments (15–19). In contrast, lysogeny is common in nature (20–22). In this case, the
86 phage remains mostly silent in the cell (as prophage), but accessory genes can be expressed
87 and change the host phenotype. Many toxins with key impact on the virulence of bacterial
88 pathogens are present and expressed from prophages (13). However, very few phages encode
89 *bona fide* ARGs (16). To the best of our knowledge, no natural phage with ARGs has been
90 shown to be fully functional – i.e., to lyse the original host cell, infect another cell and then
91 repeat the cycle to infect a third cell – and provide antibiotic resistance by lysogenic
92 conversion.

93 While most prophages integrate the chromosome, some remain in cells as phage-plasmids
94 (P-Ps). These are temperate phages that transfer horizontally (infect) as viruses but remain
95 and replicate within cells as plasmids. In a previous work, we found P-Ps to be numerous,
96 widespread and organized in different groups (23). A few of these groups are frequent in
97 enterobacteria and other important nosocomial pathogens, *e.g.* P1-like P-Ps are very frequent
98 in *Escherichia coli*, SSU5-like and N15-like elements in *Klebsiella pneumoniae*, and AB-like P-
99 Ps in *Acinetobacter baumannii*. P-Ps tend to be larger than prophages integrated in the
100 chromosome. The P-Ps have loci that are very plastic and contain genes typical of plasmids
101 and other more conserved loci encoding phage-related genes (23). Some of the P-Ps, notably
102 the P1-like, can also be efficient transducers (24). The double nature of P-Ps, being a plasmid
103 and a phage, led us to think that they might contribute more, especially by lysogenic
104 conversion, to the spread of ARGs than the other phages. Furthermore, a few reports have
105 identified elements resembling P-Ps carrying ARGs. For example, P1-like elements were
106 identified encoding an *mcr-1* gene conferring resistance to colistin in *K. pneumoniae*, and
107 ESBLs in *Salmonella* spp. and *E. coli* but induction and transmission could not be confirmed
108 (25–27). Recently, a P1-like element with several predicted ARGs could lysogenize one
109 commensal *E. coli* strain and provide resistance to streptomycin (28). This shows that P-Ps can
110 carry and transfer ARGs, although the viability of the full phage lifecycle (infection and re-
111 infection) was not yet confirmed.

112 Here, we test the hypothesis that P-Ps are more likely to encode ARGs than the other phages
113 because they share characteristics of plasmids such as presence of transposable elements and
114 regions of high genetic plasticity. For this, we searched a large number of P-Ps, plasmids and
115 phages from reference databases for *bona fide* ARGs. We found many ARGs and their
116 acquisition seems to have been driven by transposable elements and integrons. To test if the
117 P-Ps can be induced we scanned a collection of carbapenem-resistant strains for putative P-
118 Ps. The tested cases showed almost systematic induction of P-Ps. Among those induced, we
119 then tested if P-Ps were able to convert a panel of sensitive strains into bacteria resistant to
120 broad-spectrum cephalosporins.

121 Methods

122 Genomic data

123 We used the completely assembled genomes of 8399 bacterial strains, including their 21550
124 plasmids, and the completely assembled genomes of 3725 phages. All genome data was
125 retrieved from the non-redundant NCBI RefSeq database (29) (March, 2021).

126

127 Similarity between mobile genetic elements

128 The weighted gene repertoire relatedness (wGRR) assesses the similarity of gene repertoires
129 between pairs of mobile genetic elements, by taking into account their number of bi-
130 directional best hits (BBH) and their sequence identity. It is computed as described previously
131 (23) for all genomes/contigs of phages, plasmids and P-Ps. Briefly, MMseqs2 (v. 13-45111)
132 (30) was used to conduct an all-vs-all gene comparisons between the elements. BBHs
133 between two genomes were extracted if they met the following criteria: evalue $<10^{-4}$ and
134 sequence identity $>35\%$ covering at least 50% of both gene sequences. wGRR was computed
135 as:

$$wGRR(A, B) = \frac{\sum_i^P id(A_i, B_i)}{\min(\#A, \#B)}$$

136

137 A_i and B_i are the i th BBH pair of P total pairs. The gene number of the smaller genome is
138 $\min(\#A, \#B)$, and the sequence identity between the BBH pair is $id(A_i, B_i)$. The sum of the
139 sequence identities (of the BBHs) normalized to the gene number of the smaller genome is
140 defined as the wGRR between the two genomes.

141

142 Identification, and classification of phage-plasmids (P-Ps)

143 P-P genomes were identified as described previously (23). Briefly, we searched for genes
144 encoding phage-like functions in plasmids of intermediate size (>10 kb and <300 kb) by using
145 carefully-selected pVOG (31), PFAM (32) and TIGRFAM (33) HMM protein profiles. The
146 detection used HMMER v 3.3.2 (34). A positive hit was assigned if the alignment covered at
147 least 50% of the protein profile with a domain i-Evalue $<10^{-3}$. The distributions of hits in the
148 plasmids were given to previously trained random forest models that provided the list of
149 putative P-Ps. dsDNA Phages (larger than 10 kb) were screened for plasmid functions using
150 protein profiles specific for plasmid replication and partition systems (35). Phages with hits

151 for plasmid functions were extracted and were compared with plasmids and P-Ps (23). Novel
152 elements having wGRR ≥ 0.4 with elements present in the list of previously identified P-Ps
153 were added to the list of putative P-Ps. This resulted in 1416 putative P-Ps, including 740
154 previously identified.

155 The classification of novel P-Ps is based on the similarity to previously identified P-P groups.
156 P-Ps that were not identified in our previous study (23), typically because they correspond to
157 more recent genome sequences, were assigned to defined P-Ps groups when they have wGRR
158 ≥ 0.5 and at least half of their genes homologous to a previously classed P-P. When there are
159 multiple hits, the P-P was classed according to the classification of the element with the
160 highest wGRR.

161

162 Identification of antibiotic resistance genes (ARGs), IS elements and integrons

163 We searched genomes for ARGs using as a reference the databases CARD (36), ResFinder (37),
164 and ARG-ANNOT (38). We searched for sequence similarity between the genes of a MGE
165 (phage, plasmid, P-P) and these databases using blastp (v.2.12.0+) (39) (to compare with
166 protein sequences of CARD and ARG-ANNOT) and blastx (v.2.12.0+) (39) (for nucleotide
167 sequences of ResFinder). We collected all hits in all databases respecting the following
168 constraints: evalue $< 10^{-5}$, sequence identity $\geq 99\%$ and alignment covering sequences by
169 $\geq 99\%$. The results were compared with the output of AMRFinderPlus (3.10.18) (tool from
170 NCBI for ARG detection (40)) (supplementary figure S2). IS elements were identified using
171 ISEScan (v. 1.7.2.3, default parameters) (41). Integrons were identified using IntegronFinder
172 (v. 2.0rc6, default parameters) (42).

173

174 Pangenome graphs

175 To compute pangenomes of P-P groups (including newly assigned members), we followed the
176 same workflow as described previously (23). We computed the pangenome with PPanGGolin
177 (v. 1.1.136, default parameters) (43). Genes (including ARGs) were grouped into gene families,
178 if they had an identity of at least 80% covering 80% of the sequence. We made the
179 visualization of the pangenome graphs with Gephi (<https://gephi.org/>) and igraph
180 (<https://igraph.org/r/>) in the R environment.

181

182 ARG-encoding P-Ps in carbapenem-resistant *Enterobacteriaceae*

183 Draft genomes of carbapenem-resistant Enterobacterales (CRE) received from the French
184 National Reference center were screened for ARG-containing P-Ps. For this, we predicted the
185 genes using prodigal (v2.6.3, with default parameters) (44) and compared each contig with
186 known P-Ps using the wGRR. We selected contigs with wGRR \geq 0.4 for further study. These
187 contigs were annotated in terms of ARGs using the same method as that used for the P-Ps
188 (see section on the identification of ARGs).

189 Strains with contigs that were regarded as parts of putative P-Ps and encoding ARGs, were
190 then re-sequenced using long reads. Cells were cultivated in 4 ml LB-Miller medium (w/
191 Carbenicillin 50 $\mu\text{g/ml}$ at 37 °C, 250 RPM) for ~16h, pelleted and their DNA were isolated with
192 a modified version of the guanidium thiocyanate method (prior to DNA precipitation samples
193 were treated with RNase A at 37 °C for 30 min) (45). DNA library preparation (SMRTBell Library
194 10 kb insert size) and sequencing was done with the Biomics sequencing platform of the
195 Institut Pasteur (C2RT) (Paris, France) with the technology of Pacific Biosciences. The obtained
196 reads were assembled by flye (v.2.7.1-b1590) (46) with default parameters (see
197 supplementary figure S8).

198

199 Growth experiments with Mitomycin C (MMC)

200 The CRE strains with ARG-encoding P-Ps were first cultivated in 4 ml LB-Miller medium w/
201 Carbenicillin 50 $\mu\text{g/ml}$ (37 °C, ca. 16h). The stationary cultures were diluted 1:100 and
202 cultivated in a 96-well plate (200 μl per well) in LB-Miller medium w/ Carbenicillin 50 $\mu\text{g/ml}$
203 for 1h. Subsequently, Mitomycin C (MMC) (Sigma-Aldrich, St. Louis, United States) was added
204 in final concentrations of 5 $\mu\text{g/ml}$, 1 $\mu\text{g/ml}$ and w/o MMC. The growth was monitored by
205 following the absorbance at OD₆₀₀ measured with a TECAN Genios™ plate reader (Männedorf,
206 Switzerland) (supplementary figure S9).

207

208 Polyethylene glycol (PEG) precipitation of phage virions

209 CRE strains w/ P-Ps were cultivated as described in the MMC growth experiment. 4 ml-
210 cultures were started in LB-Miller w/ Carbenicillin 50 $\mu\text{g/ml}$ by using 1:100 dilutions of the
211 overnight cultured strains and 5 $\mu\text{g/ml}$ MMC was added after 1h. 4h after the MMC addition,
212 samples (2ml) were taken for PEG precipitation, pelleted and the supernatant was filtered

213 (0.22 μm) (=phage lysate). To these phage lysates, 5xPEG/NaCl (PEG-8000 20%, NaCl 2.5 M)
214 solution was added in a 1:5 ratio, inverted several times and chilled on ice for 1h.
215 Subsequently, virions were pelleted at 3 min and 13 000 g and the supernatant carefully
216 discarded. The pellets were resolved in TBS buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl) in
217 1/10 of the initial phage lysate volume and incubated for another hour on ice. The PEG
218 precipitated samples were further used for phage DNA extraction or infection experiments.

219

220 Extraction and sequencing of DNA located in virions

221 Virion DNA was extracted as described by Jakočiūnė and Moodley (47) after PEG precipitation
222 and starting from step 3.2. Residual bacterial DNA was removed by treating samples with
223 DNase I and RNase at 37 °C. The phage protein capsid was digested with Proteinase K and the
224 DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) was used to purify the DNA. Quantity
225 and quality of purified DNA was checked by a Qubit™ fluorometer and a NanoDrop™
226 spectrometer. Library preparation (Illumina® TruSeq™ DNA PCR-Free), sequencing and quality
227 checks were done by the Biomics sequencing platform of the Institut Pasteur (C2RT) (Paris,
228 France) by short-reads (paired-end, 250 bp length) on a MiSeq system (Illumina®, San Diego,
229 U.S.).

230

231 Sequence data processing

232 We took DNA obtained after the MMC induction experiment and tried to assemble the P-Ps.
233 However, given the presence of repeats in these elements, they were not fully assembled. To
234 obtain the complete sequences, we put together the long reads from the genome sequencing
235 (obtained before) and the short reads from the MMC induction experiment (see section
236 above). These were then co-assembled using Unicycler (v. 0.4.8) (48) with default parameters.
237 The hybrid assembly resulted in 4-15 linear and/or circular contigs per strain representing the
238 sequence of induced P-Ps, prophages and other DNA found in virions after MMC treatment
239 (supplementary figure S10). We evaluated the assemblies by checking if the P-P contigs were
240 closed (fully assembled) or if they weren't, by comparing them with known P-Ps
241 (supplementary figure S11). Subsequently, we mapped the reads (obtained after the MMC
242 induction) on these assemblies to assess how they cover them using bowtie2 (v. 2.4.4) (49)
243 with default parameters. To extract the coverage, we converted the output SAM-files to

244 sorted BAM-file using SAMtools (v. 1.13) (50) and obtained the coverage with BEDTools
245 (v2.30.0) (51). In addition, we computed the (background) coverage caused by undigested
246 gDNA. For this, we took the short reads (from the MMC induction experiment) that did not
247 map on the hybrid assembled contigs (= DNA outside of virions) and aligned them on the
248 contigs acquired from the genome sequencing experiment. The mean coverage was
249 computed by dividing the absolute read coverage per contig (genome) by the size of the
250 contig (genome).

251

252 Generation of antibiotic resistant phage-plasmid lysogens

253 PEG precipitated phage lysates were prepared and stored at 4 °C. Potential host strains were
254 cultivated the day before in 4 ml LB-Miller medium for approx. 16h at 37 °C and 250 RPM. The
255 stationary cultures were diluted 1:100 in LB-Miller medium and grown until an OD₆₀₀ of 0.5
256 to 1. Subsequently, 50 µl of the phage lysate was added to 50 µl host culture w/ 2mM CaCl₂
257 and incubated under non-shaking conditions at 37 °C for 1h. After incubation, the cell/phage-
258 lysate mixture was plated on agar plates with the required antibiotic concentration (to screen
259 for lysogens). Antimicrobial susceptibility tests were performed as described (52) and
260 interpreted according to the EUCAST guidelines. Colonies were tested by PCR for the presence
261 of P-P genomes (amplifying two regions; for region 1 with 522 bp PP-R1A 5'-
262 CTACCAGACCGCCTTTCTCAAAC-3', PP-1B: 5'-TTGCCGAACTAGAGAATAAATACGG-3' and for
263 region 2 with 423 bp PP-R2A 5'-TTAACCTTTGTCGGCGTCGG-3', PP-R2B 5'-
264 ATGTCATTCTTTTCTACATTAACAGC-3') and finally confirmed by genome re-sequencing.
265 Genomic DNA was isolated as described in the section on ARG-encoding P-Ps in CRE strains.
266 Library preparation (Illumina® TruSeq™ DNA PCR-Free) and sequencing was done on the C2RT
267 Biomics platform of the Institut Pasteur (using short reads, paired-end, 250 bp) on an Illumina
268 MiSeq system. The resulting reads were mapped on the P-Ps and on the host genomes (*E. coli*
269 55989: NC_011748, *E. coli* CIP 105917: NZ_CP041623, *E. coli* CIP 53.126: NZ_CP022959, *E. coli*
270 CIP 76.24: NZ_CP009072). Coverage was extracted as described in the section on processing
271 sequencing data.

272

273 Data processing, storage and availability

274 If not otherwise stated, all analysis and illustrations were done in the R environment
275 (<https://www.r-project.org/>) with Rstudio (v. 1.4.1106). All reads were uploaded to the
276 European Nucleotide Archive (ENA) (<https://www.ebi.ac.uk/ena>). Short and long reads from
277 the MMC induction, the genome sequencing experiment, the verification of P-P acquisition
278 as well as the P-P nucleotide sequences gained by the hybrid assemblies are accessible under
279 the following ENA study number PRJEB52357. Details on accession numbers and experiments
280 are listed in supplementary table S5.

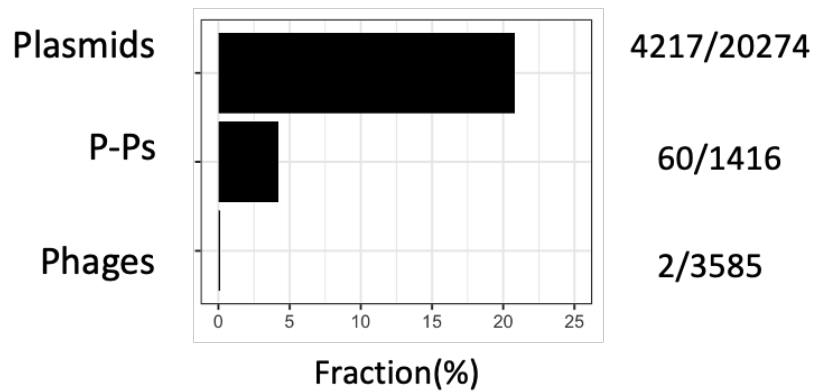
281 Results

282 Antibiotic resistance genes are common in phage-plasmids but rare in other phages

283 To assess quantitatively the distribution of ARGs in plasmids, phages, and P-Ps, we searched for
284 these genes in the complete bacterial and phage genomes of the RefSeq database. For this,
285 we first updated our database of P-Ps using a previously described detection method (in (23))
286 (supplementary figure S1A). The novel P-Ps were classed in groups using their similarities to
287 previously classed elements as measured by the weighted gene repertoire relatedness
288 (wGRR) (see methods and supplementary figure S1). This led to an almost doubling of the
289 database of P-Ps to a total of 1416 P-Ps. These elements represent 5.6% of the 25,275 phages
290 and plasmids.

291 We searched for genes in phages, plasmids and P-Ps with very high sequence similarity (at
292 least 99% identity and 99% coverage) to verified ARGs from three reference databases (ARG-
293 ANNOT, ResFinder and CARD). In agreement with previous studies (8), ARGs were frequently
294 found in plasmids (20.8%) and almost never found in phages (2 out of 3585 genomes, <1 ‰)
295 (see figure 1). A total of 4.2% of the P-Ps carried ARGs, a frequency that is intermediate
296 between that of phages (ca. 76.0 times more) and plasmids (4.9 times less). To further validate
297 the annotation of ARGs, we compared the results of the three databases with the analysis of
298 our data using the NCBI AMRFinderPlus software (40). We found similar ARGs in P-Ps and
299 phages, and an increase in the number of plasmids with ARG of about 13.5% (supplementary
300 figure S2). In P-Ps, the ARGs encode a variety of enzymes e.g. β -lactamases, dihydrofolate
301 reductases, and aminoglycosides-modifying enzymes. We also identified a few genes
302 encoding efflux pumps (supplementary table S1). Overall, our analysis shows that P-Ps encode
303 ARGs much more often than the remaining phages. In some cases, they encode resistance
304 genes to last-line antibiotics, like the *mcr-1* against colistin, various *blaKPC* (type 2, 3, 4 and
305 33) and *blaNDM-1* genes against carbapenems (supplementary table S1).

306



307

308 **Figure 1: Number of mobile genetic elements encoding ARGs.** The values after the bars indicate the number of
309 elements encoding ARGs over the total number of elements considered in the analysis.

310

311 Resistance genes are in specific types and loci of phage-plasmids

312 Most of the P-Ps carrying ARGs (47 of the 60 P-Ps) were found in genomes of just four species:

313 *Acinetobacter baumannii* (n=8), *Escherichia coli* (n=20), *Klebsiella pneumoniae* (n=14) and

314 *Salmonella* (*spp.* and *enterica*) (n=5). This is not overly surprising; our previous study showed

315 these species had many P-Ps (23), many genomes of these species are available in the

316 database, and these are all pathogenic bacteria known to develop antibiotic resistance (53).

317 The majority of these P-Ps were assigned to well-defined P-P groups (23). P1-like P-Ps

318 represent a third of the elements with ARGs (21 cases) of which all are in the P1-subgroup 1.

319 We also detected 12 SSU5-related P-Ps and 8 AB-like P-Ps with ARGs (supplementary table

320 S1). Interestingly, we could not detect any ARGs in P-Ps of the N15 group, the pMT1 group

321 and the P1 subgroup 2. The results for N15 are particularly intriguing, because these elements

322 are very abundant in nosocomial species, like *E. coli* and *K. pneumoniae* (23).

323 We analyzed the genomic locations of ARGs in P-Ps to shed light on how these genes were

324 acquired and how these events may have affected the genetic organization of P-Ps. For this,

325 we computed the pangenomes of the P-P groups, selected gene families present in high or

326 intermediate frequency in the pangenomes to build a graph of the genetic organization of the

327 elements, and placed the ARGs in relation to this backbone (figure 2). ARGs were never found

328 in the persistent genome of the P-Ps, in light with the hypothesis that they were recently

329 horizontally acquired and that they are not essential. Some P-Ps harbor one ARG, but the

330 majority (n=39) has multiple genes, with up to 13 ARGs detected in a single putative P-P

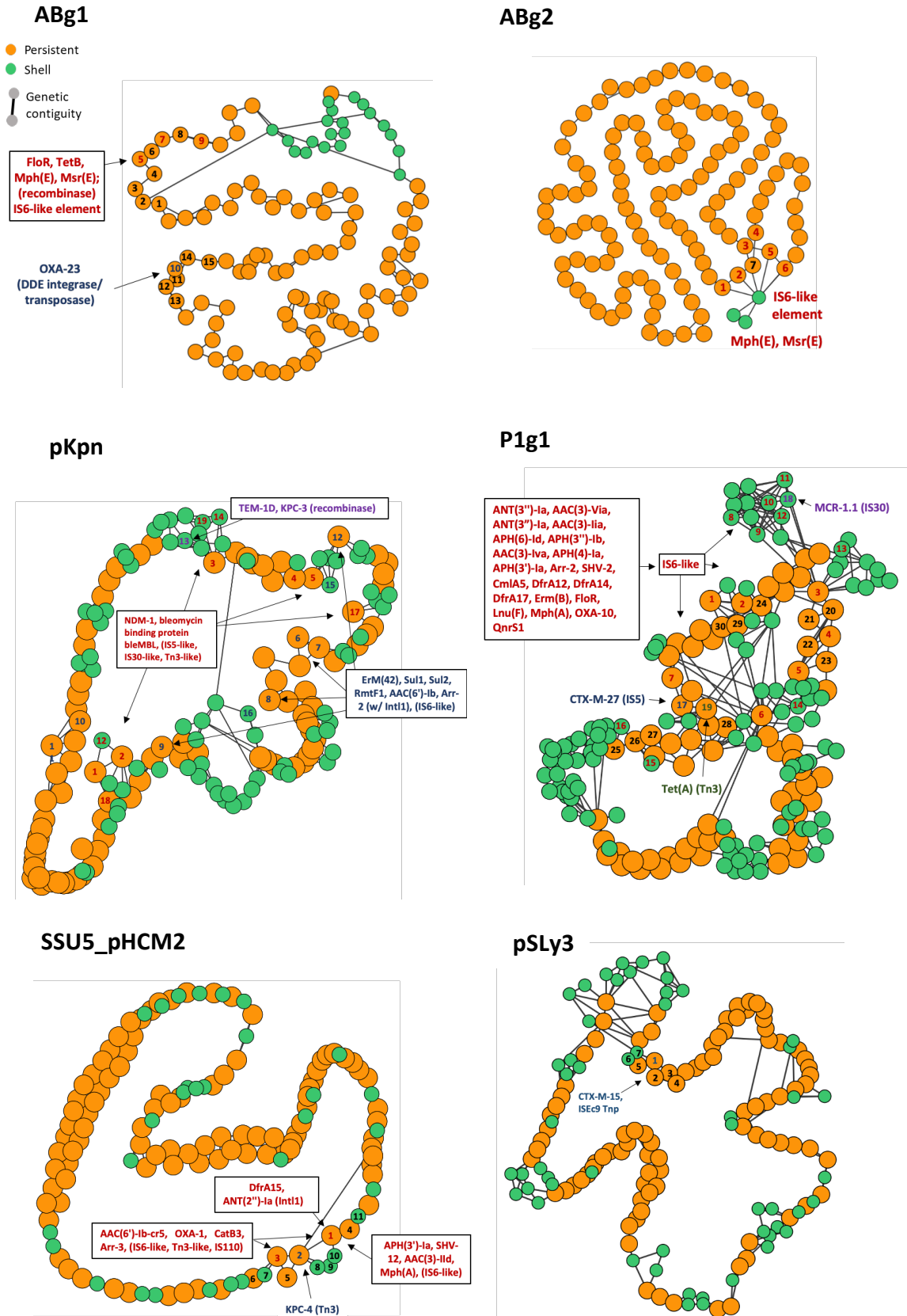
331 (pASP-135, NZ_CP016381) from the *Aeromonas hydrophila* strain AHNIH1 (supplementary

332 table S1). This fits previous suggestions that *Aeromonas* spp have a key role in the genetic

333 transfer of ARGs (54). One of the ARGs is the *bla*_{KPC-2} conferring resistance to carbapenems
334 and is of great concern since it could act as a reservoir for this gene. Genes that commonly
335 promote recombination and genomic plasticity, such as transposases and recombinases, were
336 systematically identified in close proximity to the ARGs (supplementary figure S3-S7).
337 Transposases of the IS6-like family were particularly frequently found next to the ARGs,
338 especially those of the type IS26 (figure 2). This family of ISs has been previously involved in
339 the spread of clinically relevant ARGs, commonly causes plasmid co-integration, and its
340 insertion results in hybrid promoters that can influence the expression of neighboring genes
341 (55). Notably, most ARG families (21/24) of the P1 subgroup 1 are close to an IS6-like
342 transposase (figure 2), suggesting that this transposable element drives the ARG acquisition
343 in these P-Ps. In addition, in the AB, pKpn, and SSU5_pHCM2 groups some ARGs are found
344 next to IS5-like, IS30-like, Tn3-like transposases, and several other types of recombinases. In
345 the pSLy3-like group, we found no IS6-like transposases next to ARGs, but we did find an *ISEc9*
346 transposase (figure 2, supplementary figure S7).

347 Interestingly, we found that ARGs tend to be present in a small number of loci in the genomes
348 of P-Ps. Within the P1-like and the pKpn-like genomes, the IS6-like transposable elements are
349 inserted into a few distinct positions, whereas in genomes of SSU5-like and AB-like P-Ps all
350 the insertions are concentrated in just one locus (figure 2). This is in line with our previous
351 finding that P1 and pKpn genomes are more plastic than the average P-Ps (more complex,
352 larger shell and cloud genomes, high number of plastic regions (23)). The conserved genes
353 flanking the regions with ARGs often encode regulators, enzymes associated with DNA repair,
354 or unknown functions. Few of them flank key highly conserved phage functions.

355 Overall, this analysis revealed diverse classes of ARGs in different groups of P-Ps (figure 2,
356 supplementary figure S3-S7) that seem to have been acquired by the action of transposable
357 elements.



358
359

360 **Figure 2: Genetic environments of ARGs in P-Ps' pangenome graphs. Nodes are gene families. Genes (including**
 361 **ARGs) were groups into gene families (default parameters of PPanGGolin (43)) if they had a similarity of >80%**
 362 **identity covering at least 80% of the sequence. Orange = persistent, green = shell. Edges are shown for adjacent**

363 genes within the gene families (genetic contiguity). Gene families with colored numbers (red, blue, violet, green)
364 are direct neighbors of ARG containing genetic elements (transposon, IS, integron, recombinase (separated by
365 colors)). Black numbers are given for proximal gene families. **ABg1**: 1-3,6,8-9:hypothetical, 4:nucleoside,
366 5:ATPase AAA, 7:3'-5' exonuclease pyrophospho-hydrolase. **ABg2**: 1,3:hypothetical, 2,7:ribonucleoside-
367 diphosphate red. 4:toprim domain protein, 5:ATPase AAA, 7:3'-5' exonuclease pyrophosphohydrolase.
368 **SSU5_pHCM2**: 1:PhoH, 2-5, 7-11:hypothetical, 6:DNA ligase. **P1**: 1:SSB, 2-5,15,17,19-20,23-24,28: hypothetical,
369 6:cell division inhibitor (lcd-like), 7-11:tail fiber, 12:recombinase, 13-14:tail fiber assembly, 16:ResMod subM,
370 18:Ref family, 21:bleomycin hydrolase, 22:transglycosylase, 25:DNA repair, 26:Phd/ YefM (T-A), 27:doc (T-A),
371 29:lysozyme, 30:head processing. **pKpn**: 1:transcriptional regulator, 2,7,9,12-15,17-19:hypothetical, 3:phoH,
372 4:porphyrin biosynthesis protein, 5-6: AAA family ATPase, 8: ribonucl.-diphosphate reductase subunit 10-11:tail
373 fiber domain-containing protein, 16:HsdR. **pSLyr3**: 1:DUF3927 family, 2:tellurite/colicin resistance, 3-
374 7:hypothetical

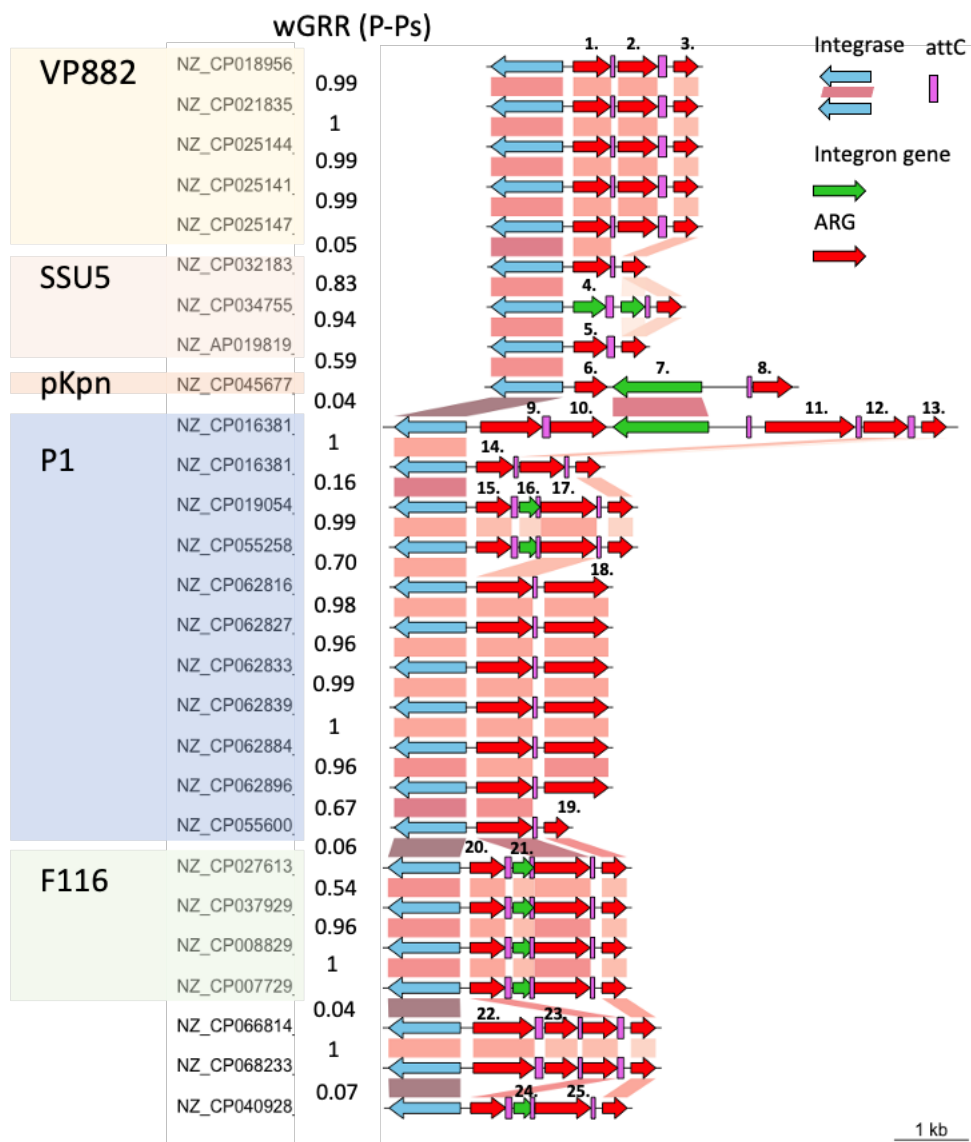
375

376 Integrons carrying ARGs are frequent in phage-plasmids

377 Class 1 integrons are not mobile by themselves, but plasmids often carry such integrons with
378 ARGs (resistance integrons) (56). A recent analysis identified more than 1400 complete
379 integrons in plasmids on the genome dataset used in our study (42). In contrast, no integron
380 carried by a phage was reported so far. Accordingly, we searched for integrons in 3585 phages
381 lacking evidence of being P-Ps and found no single integron in these elements. Since P-Ps have
382 characteristics intermediate between plasmids and phages, we screened them for integrons.
383 We found 27 integrons in P-Ps. Integrons were especially abundant in P1-like elements (n=11)
384 (figure 3). Although, the SSU5 supergroup has the most members (n=268), just four P-Ps were
385 predicted to have integrons in this set. Just in one P-P (NZ_CP016381), isolated from an
386 *A. hydrophila* strain, two dissimilar integrons were detected. Furthermore, the *A. hydrophila*
387 P-P has some similarity to P1 (wGRR = 0.07, 19 homologous genes), but not enough to class it
388 as P1-like. Nine P-Ps with integrons were found in VP882-like and F116-like P-Ps.

389 These integrons have between two and five cassettes. Remarkably, nearly all genes within the
390 cassettes were predicted to be ARGs (figure 3). As usual, *qacEdelta1* conferring resistance to
391 antiseptics, was detected in most integrons (20/27) being part of the 3' conserved segment.
392 We found 15 co-occurrences of this cassette with that of *aadA2* (aminoglycoside
393 nucleotidyltransferase) and 10 with those with *dfrA12/dfrA15* genes (trimethoprim
394 resistance). A large diversity of other resistance genes was identified in integrons including
395 aminoglycosides modifying enzymes (*ant(2'')-Ia*, *aac(6')-33*, *aac(3)-Via*), rifampicin resistance
396 gene (*arr-2*, *arr-6*), chloramphenicol resistance (*cmlA6*, *catB11*) and different β -lactamases
397 including the minor ESBL *bla_{GES-1}* and the penicillinase *bla_{CARB-2}* (figure 3). Hence, integrons in
398 P-Ps encode a diverse panel of ARGs.

399 We compared the gene repertoire relatedness (wGRR) between integron-encoding P-Ps and
 400 the similarity between the integrons themselves. The gene cassette arrays tend to be very
 401 similar when they are in the same type of P-P and very distinct across unrelated P-Ps.
 402 However, in a few cases (pointed out by black arrows in figure 3), dissimilar P-Ps have similar
 403 integron cassettes (>90% identity and 90% coverage) suggesting an epidemic spread of genes
 404 providing a selective advantage (resistance to aminoglycosides and antiseptics). In all cases,
 405 the integrons of P-Ps had very similar IntI1 type integrases. These results suggest that, like it
 406 is the case for plasmids, type I integrons act as reservoirs for multiple ARGs in P-Ps.
 407



408

409

410 **Figure 3: Integrons encoded in P-Ps.** A. Genomic organization of integrons found in P-Ps, arranged by P-P groups

411 and co-aligned by the class I IntI1 integrase. The P-P type is highlighted by differently colors. Gene-to-gene

412 assignment is based on a blastp comparison when the alignment is at least 90% identical and covers at least 90%

413 of the sequences. Blue gene arrows indicate the integrase gene, red arrows show AMR genes (99% identity, 99%
414 coverage to reference sequences), and green arrows represent the rest of the genes within the integrons.
415 Numbers indicated above non-homologous cassettes represent different types of ARGs: [1,14]:*ant(2'')*-Ia,
416 [2]:*aac(6')*-33, [3,13,19]:*qacED1*, [4]:*aac(3)*-Ib, [5]:*dfrA15*, [6]:*arr-2*, [7]:group II intron reverse
417 transcriptase/maturase, [8]:*aac(6')*-Ib, [9]:*bla_{CARB-2}*, [10,17,25]:*aadA2*, [11]:*cmlA6*, [12]:*catB11*, [15,20]:*dfrA12*,
418 [16,21,24]:DUF1010 protein, [18]:*aac(3)*-VIa, [22]:*bla_{GES-1}*, [23]:*arr-6*.

419

420 Phage-plasmids with resistance genes are induced by mitomycin C

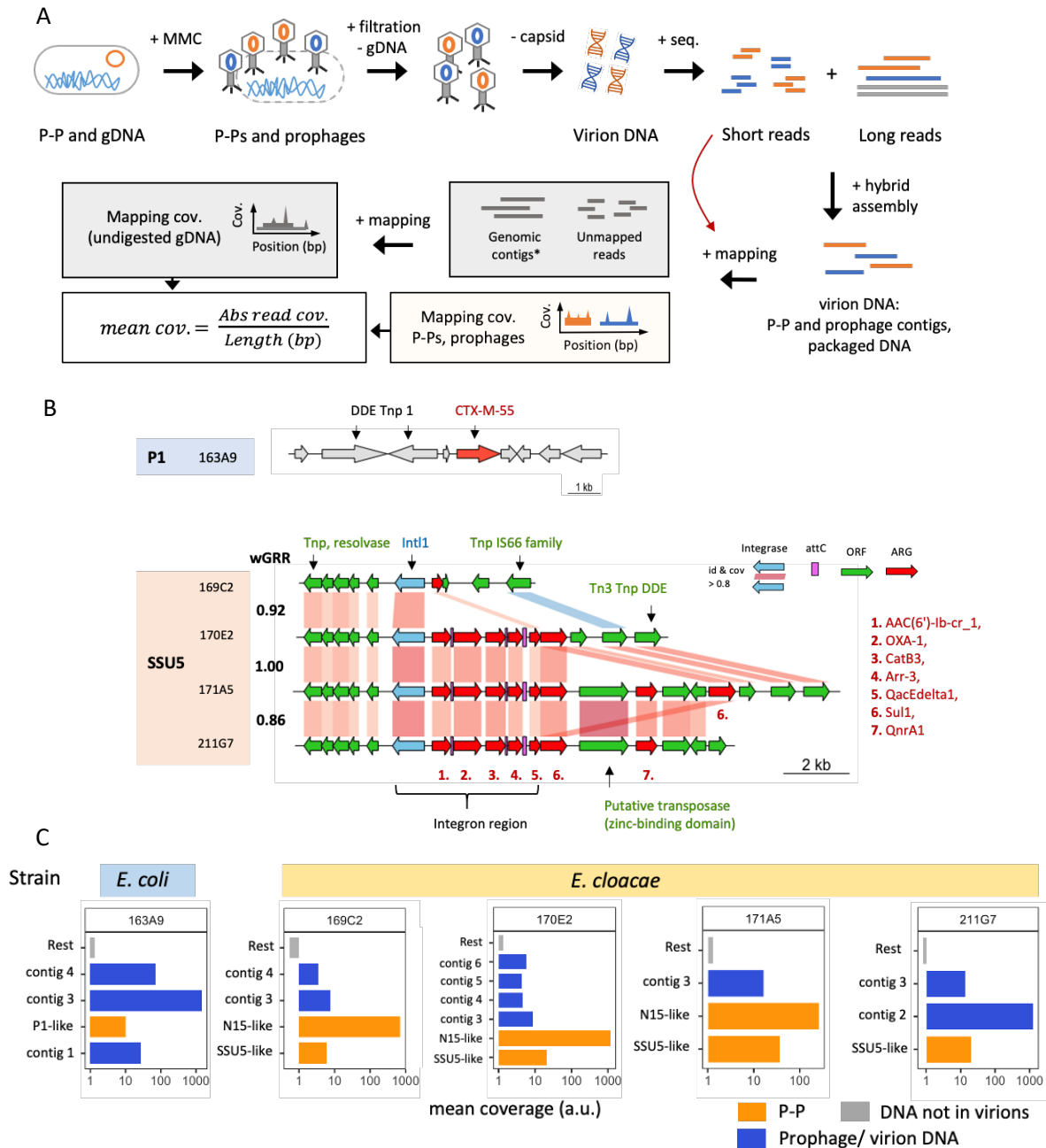
421 The recent acquisition of ARGs in P-Ps might make them inactive phages, as previously
422 observed in a plasmid resembling a P1-like element (25), either because the insertion
423 inactivates relevant functions or because natural selection of the bacterium could select for
424 inactive elements. To test if some ARG-encoding P-Ps are functional phages, we screened a
425 collection of draft genomes of CRE strains for ARG-encoding P-Ps. We identified six strains
426 (two *E. coli* and four *E. cloacae*) that we sequenced using long reads (supplementary figure
427 S8). These genomes had six P-Ps with ARGs: one P1-like (of 163A9) in *E. coli*, and five SSU5-
428 like P-Ps, one from *E. coli* (of 166F4) and four from *E. cloacae* (169C2, 170E2, 171A5, 211G7)
429 (supplementary table S2). We then tested if these P-Ps are induced by DNA damage by
430 exposing the bacterial cells to mitomycin C (MMC). 3-4h after MMC addition, a drop of cell-
431 density indicated the phage-dependent cell lysis (caused by SOS response and consecutive
432 prophage and/or P-P induction) (supplementary figure S9). Phage particles were purified,
433 their DNA extracted and sequenced by short reads (after digestion of chromosomal DNA
434 (gDNA), see methods). We then conducted hybrid assemblies by combining the short reads
435 from the MMC experiment with the long reads from the genome sequencing (figure 4A).

436 For all P-Ps, except of strain 166F4, the co-assembly resulted in closed circular contigs or in
437 larger assemblies with good homology to known P-Ps (supplementary figure S10-S11), that
438 confirmed the structure of the replicons. This opened the possibility of studying the genetic
439 context of the ARGs in these P-Ps. The P1-like P-P of the *E. coli* strain 163A9 contains two DDE
440 transposases next to the β -lactamase encoded by a *bla_{CTX-M-55}* gene (figure 4B). The four SSU5-
441 like P-Ps from *E. cloacae* are very similar (wGRRs from 0.86 to 1, figure 4B, supplementary
442 figure S11) and their ARGs are in the same loci, in a complex region including various
443 transposases and a type 1 integron with a very similar integrase (identity and coverage >80%)
444 (figure 4B). Three P-Ps contain integron regions with five ARGs, whereas the one from the
445 strain 169C2 has a very similar integrase gene but lacks gene cassettes (figure 4B). In addition,
446 downstream of the integron region a few (one to three) more ARGs are in a locus with
447 multiple transposases. The number of ARGs of the SSU5-like P-Ps ranges from one (169C2) to

448 eight (171A5) and they are predicted to encode resistance against several antibiotics including
449 penicillins (*bla*_{OXA-1}), fluoroquinolones (*qnrA1*), aminoglycosides (*aac(6′)-Ib*) or sulfonamides
450 (*sul1*) (figure 4B, supplementary table S2).

451 To verify that P-P DNA is found in the virions, we mapped the short reads from the MMC
452 experiment on the co-assemblies and retrieved the average mapping coverage (figure 4A).
453 Unmapped reads were extracted and used to obtain the relative frequency of non-digested
454 chromosomal DNA (=background signal) by mapping them on the genome contigs (see
455 methods) (figure 4A). We considered that highly covered P-Ps and prophages, relative to the
456 background chromosome, were induced and packaged into virions. Five of the six tested
457 strains were indeed induced and produced viral particles with the ARG-encoding P-Ps (figure
458 4C). The DNA of these elements was present at diverse frequencies, possibly a result of
459 different burst sizes (which might be further affected by the presence of other prophages
460 (57)). For one of the six strains (*E. coli* 166F4), we did not obtain any reads mapping the P-P
461 contig suggesting that this SSU5-like P-P is inactive or not inducible by MMC under our
462 experimental conditions. Interestingly, in the genomes of three strains (*E. cloacae* 169C2,
463 170E2 and 171A5) we found two different types of P-Ps: the SSU5-like encoding the ARG and
464 another P-P lacking ARGs that is related to the N15 group. Noteworthy, we could also assign
465 some of the sequences retrieved from the viral particles to chromosomal loci with prophages
466 in the CRE strains, which shows that they were also induced by MMC (figure 4C,
467 supplementary table S3). The coverage of P-Ps and integrative prophages in the analysis of
468 the viral particles is typically at least one order of magnitude higher than the average coverage
469 of the background, showing that the result is not due to random contamination by bacterial
470 DNA. Hence, most of the analysed P-Ps, with or without ARGs, are inducible and are packaged
471 into virions.

472



473

474 **Figure 4: Induction of P-Ps and prophages in CRE strains.** **A.** CRE strains w/ ARG-encoding P-Ps were induced by 5 µg/ml
 475 MMC. 4h after induction, phage particles were purified and chromosomal DNA (=gDNA) removed by DNase I digestion. The
 476 phage capsid was degraded by proteinase K, virion DNA was purified and sequenced. The obtained short reads were co-
 477 assembled with long-reads from the genomic sequencing experiment (see methods). The assemblies were compared to P-P
 478 and phage genomes, subsequently assigned and the read mapping coverage was computed (by mapping the short reads
 479 from the MMC experiment on them). The reads that did not map the assemblies were used to compute the background
 480 coverage caused by undigested gDNA (by mapping on genomic contigs obtained by the long-read assembly). **B.** In the
 481 genome of the P1-like P-P of the *E. coli* 163A9 the CTX-M-55 gene is found next to two DDE transposases. The ARGs encoded
 482 in the SSU5-like P-Ps from the *E. cloacae* strains 169C2, 170E2, 171A5 and 211G7 are in a complex region containing
 483 transposases and integrons. Homology assignments between P-Ps were done when sequence similarity was of at least 80%
 484 identity and covered 80% of the sequence of the gene (retrieved from an all-vs-all blastp comparison). The similarity between
 485 P-Ps is indicated by the weighted gene repertoire relatedness (wGRR). **C.** Average read coverage was obtained and calculated
 486 as described in A. Shown are all contigs larger than 10kb. The coverage (a.u. = arbitrary unit) is plotted on a logarithmic x axis
 487 for P-P contigs (orange), contigs assigned to prophages or virion loaded DNA (blue) and average background coverage (rest
 488 coverage) obtained after mapping the remaining reads on genomic contigs (grey) for each tested CRE strain.

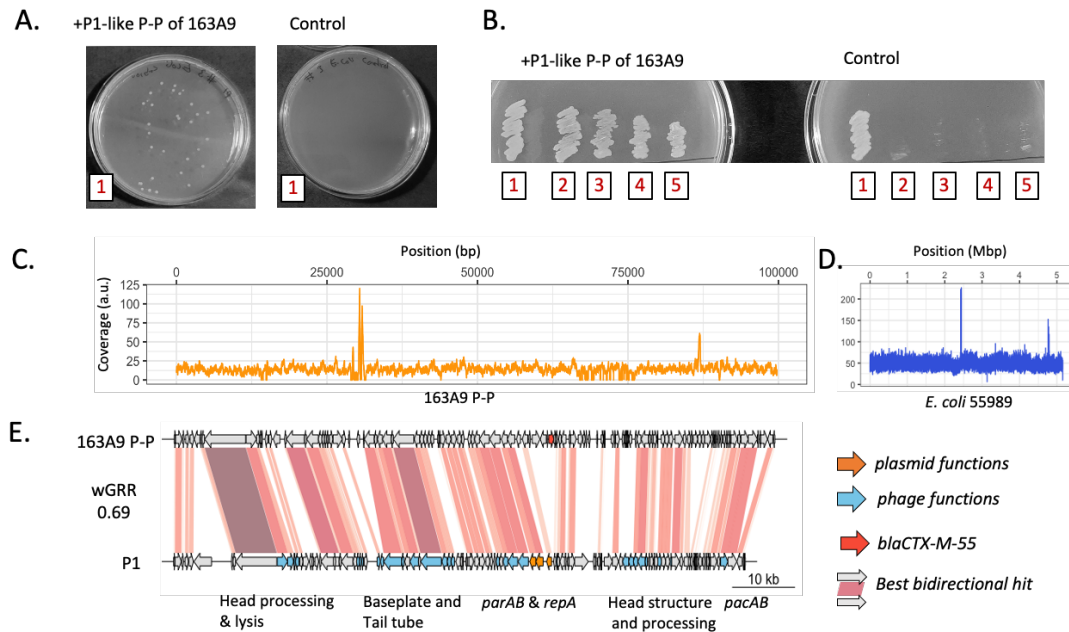
489

490 The MMC induction experiments confirmed that P-Ps with ARGs are inducible and packaged
491 into virions. Hence, these P-Ps confer resistance to antibiotics to the bacterium and function
492 as real phages. Here, we test whether they are also capable of infecting, lysogenizing, and
493 converting other host strains into becoming antibiotic resistant. Phages tend to have
494 narrower host ranges than conjugative plasmids, possibly because of their requirement for
495 specific receptors at the cell envelope (58), the existence of numerous bacterial defenses
496 against phages (59), and the presence of other prophages (60). This results in phage-bacteria
497 interaction matrices that tend to reveal a very low frequency of productive infections for
498 temperate phages (61, 62). Hence, the first challenge was to identify permissive hosts
499 different from the strain carrying the P-P.

500 The four SSU5-like PPs are very similar and presumably have similar host ranges. Since the
501 requirements of host range of these phages in this host are poorly understood, we tested 18
502 different *E. cloacae* strains retrieved from the Pasteur and the German collections (DSMZ)
503 (supplementary table S4). However, no lysogenic conversion was observed by the SSU5-like
504 P-P.

505 We chose a diverse panel of 12 *E. coli* and one *S. enterica* (CIP 82.29T) strains from the Pasteur
506 collection to infect with the P1-like P-P (supplementary table S4). The *S. enterica* strain was
507 of particular interest to test the host range of the P-P. We then conducted infection
508 experiments, where we purified the phage particles, incubated them with the potential host
509 strains and screened for resistant lysogens by plating the mixture on antibiotic-containing
510 plates (here carbenicillin, see Methods). For the *S. enterica* strain we did not obtain lysogens.
511 However, we found four different *E. coli* recipient strains (55989, CIP 105917, CIP 53.126, CIP
512 76.24), all from phylogroup B, that were initially sensitive but became resistant to carbenicillin
513 after the infection with the P1-like P-P of strain 163A9 (figure 5AB). The sequencing of the
514 genomes of the recipient strains confirmed the acquisition of the ARG and the rest of the P-P
515 (figure 5CDE, supplementary figure S12). Moreover, susceptibility tests with various β -lactam
516 antibiotics (broad-spectrum penicillins, cephalosporins of different generations,
517 carbapenems) confirmed the presence of the CTX-M-55 ESBL in the lysogenized strains. All
518 four strains show resistance to three broad spectrum penicillins (Ticarcillin, Piperacillin,
519 Amoxicillin) and a 3rd generation cephalosporin (Cefotaxime) (supplementary figure S13).
520 Finally, we tested if the P-P is fully functional, by testing if it can be induced in the new host
521 and used to infect another cell. We exposed the *E. coli* 55989 strain with the P1-like P-P to

522 MMC, purified the particles and used them to infect the original antibiotic-sensitive *E. coli*
 523 55989 strain. This revealed the acquisition of the P-P and the lysogenic conversion
 524 (supplementary figure S14), thereby confirming that it is a fully functional phage. This
 525 demonstrates that natural P1-like P-Ps can transfer ARGs as phages and result in lysogenic
 526 conversion of other strains.
 527



528

529 **Figure 5: Lysogenic conversion of different *E. coli* (55989 [2], CIP 105917 [3], CIP 53.126 [4], CIP 76.24 [5]) by**
 530 **the P1-like P-P of strain 163A9 [1]. A + B.** After the infection and plating experiment four tested *E. coli* strains
 531 acquired resistance to carbenicillin. Examples of colonies of strain 55989 with the P1-like P-P of 163A9 are shown
 532 on LB agar plates with carbenicillin 100 ug/ml (A). The original host of the P1-like P-P and all four lysogens are
 533 resistant to ampicillin 100 ug/ml (B). **C & D.** Their genomes were isolated and sequenced by short reads
 534 (supplementary figure 12). Shown is the read coverage for the P-P genome (C) and the chromosomes of the host
 535 strain *E. coli* 55989 (D). **E.** Genome comparison of the P-P from 163A9 and P1 and are co-aligned to the first gene
 536 of P1. The alignment is matched with the read coverage plot in C. The function of P1 genes were retrieved from
 537 Łobocka et al (63).

538 Discussion

539 ARGs and integrons are commonly found in plasmids (8), but very rarely in phages (16). P-Ps
540 are temperate phages and plasmids. Here, we show they are more much likely to encode
541 ARGs than the other phages. We also show that they frequently encode integrons with ARGs,
542 which had never been observed in functional phages. We demonstrated that one of the P-P
543 was a fully functional phage that could be induced, produced lysogens resistance to broad-
544 spectrum cephalosporins, and this could be shown for two full cycles of induction, infection
545 and conversion. Nevertheless, there are some limitations to our study. Some P-Ps may be
546 inactive, a trait common among integrative prophages (64). Alternatively, some P-Ps may not
547 be inducible by MMC. Still, we tested six strains that had nine putative P-Ps and all but one
548 could be induced to produce viral particles containing phage DNA. This suggests that many P-
549 Ps are still functional. We could not obtain new lysogens for a group of closely related SSU5-
550 like P-Ps by screening for antibiotic resistance. To identify ARGs, we applied very strict criteria
551 (99% identity over 99% sequence coverage), however their expression may depend on the
552 genetic background. The lack of lysogens may also be caused by bacterial resistance to phage
553 infection. Previous works have shown that interaction matrices of bacteria with temperate
554 phages tend to be very sparse (61), i.e. most combinations do not result in productive
555 infection. This is because many bacteria have anti-phage systems, lack appropriate cell
556 receptors, or have phages repressing infecting P-Ps (60). In addition, the replication initiators
557 of P-Ps may be incompatible with those of resident plasmids, preventing their establishment
558 in lysogens. Further work will be needed to explore the host range of P-Ps carrying ARGs. In
559 contrast, we found multiple *E. coli* strains susceptible to the P1-like phage of strain 163A9.
560 These differences may be associated with P-Ps host range, which is known to be unusually
561 broad in P1 (65, 66).

562 A few previous reports identified ARGs in P-P-like elements among enterobacteria, even
563 though evidence of induction is often lacking (25, 26, 67). In our study, we show that this is a
564 general trend of P1-like elements but also of different other types of P-Ps. Such cases can be
565 found in other bacterial clades of important nosocomial pathogens, e.g., in *Acinetobacter*.
566 Overall, they are much more likely to carry ARGs than the other phages. We show that they
567 carry a wide diversity of resistances. Most worrisome, many clinically relevant ARGs are found
568 in P-Ps, including the carbapenemase genes *bla*_{KPC-3} and *bla*_{NDM-1}. The *bla*_{KPC}-like genes are

569 involved in the diffusion of carbapenem resistance in Italy, Israel and USA, whereas the
570 *bla*_{NDM}-like gene is disseminated worldwide (68). Among the last-resort antibiotic, colistin was
571 reintroduced in the armamentarium to fight against carbapenem-resistant Gram-negative
572 rods despite its nephrotoxicity. Plasmid-mediated colistin resistance has been recently
573 described (69). This ARG, named *mcr-1*, was also identified in P-Ps. Beyond these two
574 important resistance mechanisms, the *rmtF* gene is of further importance and also carried by
575 P-Ps. This gene encodes a 16S RNA methyltransferase conferring resistance to almost all
576 aminoglycosides used for treatment (70). Thus, P-Ps are involved in the diffusion of resistance
577 to the main antibiotic families including β -lactams (e.g. *bla*_{KPC-3}), aminoglycosides,
578 fluoroquinolones (e.g. *qnrA1*) and polymyxins.

579 The presence of ARGs genes in P-Ps raises concerns that are common to the resistances found
580 in other plasmids, notably that they can spread these genes across bacterial populations.
581 However, the fact that P-Ps are also phages raises additional concerns. First, and unlike
582 conjugative plasmids, phages transfer their DNA in viral particles and do not require direct
583 contact between cells for the transfer. Hence, they can transfer between bacteria present in
584 different time and places. Second, the lytic cycle of phages amplifies their genomes hundreds
585 of times (e.g. 400 for P1 (71)) for packaging in the viral particles, which may result in bursts of
586 transfer of ARGs. The process of phage replication in the cell could also lead to over-
587 expression of ARGs and liberate enzymes that detoxify the environment for the remaining
588 bacteria, a process akin to the production of the Shiga toxin from the prophages encoding it
589 (72). However, it must be stated that in our experiences the only drug that induced P-Ps was
590 MMC. Finally, P-Ps are more likely to recombine with other phages than the remaining
591 plasmids, because they share numerous homologous genes (23). This may pose a threat of
592 ARG transfers to other phages.

593 Plasmids are known to contain many transposable elements and integrons that facilitate the
594 translocation of ARGs within and between replicons (9). In contrast, phages typically have
595 very few if any such elements (73). Here, we show that P-Ps have numerous transposable
596 elements associated with ARGs and integrons. Hence, P-Ps can take advantage of genetic
597 elements typical of plasmids, transposable elements and integrons, to acquire ARGs that can
598 then be spread horizontally by viral particles. Integrons are reservoirs of ARGs and, especially
599 in clinical settings, promote the spread of multi drug resistances (74). Their identification in
600 P-Ps is worrisome, because the integron ability to incorporate novel cassettes from other

601 integrons implies that upon acquisition of an integron the repertoire of ARGs of the P-Ps can
602 evolve faster to incorporate novel types of resistance.

603 This raises the question of why P-Ps have so many more ARGs than the other phages. About
604 half of the sequenced phages are virulent (75). They are not expected to carry ARGs because
605 they do not produce lysogens, although this cannot be completely excluded, since they may
606 produce pseudo-lysogens (76). The causes for the different frequency of ARGs in P-Ps and
607 other temperate phages are less obvious. It could be argued that the frequency of ARGs in P-
608 Ps was caused by their abundance in bacterial pathogens. However, most sequenced phages
609 in the database are also from a few genera including many enterobacteria, and this does not
610 seem sufficient to explain the difference in the number of ARGs present in the two types of
611 elements. For example, the naturally-occurring phages of *E. coli* used in this study, which were
612 not P-Ps, are completely devoid of identifiable ARGs. We propose that differences between
613 P-Ps and integrative, temperate phages result from a combination of factors. First, P-Ps tend
614 to be larger than the other phages (23). This is particularly relevant for phages, because they
615 can only package an excess of a few percent of their genome size. A sudden larger increase in
616 genome size precludes packaging of the genome and thus blocks phage transfer. Hence, a
617 genome of 49 kb, like phage lambda, can only accommodate very small insertions. In contrast,
618 P1-like elements are on average 96 kb (23), and can tolerate larger changes. Since the
619 integration of ARGs in P-Ps involves the transposition of the gene and flanking transposable
620 elements and/or integrons, these insertions may be too large for most integrative phages.
621 Accordingly, we found ARGs in the largest P-Ps, like P1-like and SSU5-like, but not in the much
622 smaller N15-like elements (average 55 kb, (23)). Differences between P-Ps and other phages
623 could also be caused by the regions of homology to plasmids of the former. This might
624 facilitate genetic exchanges between plasmids and P-Ps-

625 Independently of the reasons leading to an over-representation of ARGs in P-Ps relative to
626 the other phages, the subsequent evolution of the loci containing them in P-Ps may result in
627 streamlined compact loci that could be easier to transfer to other phages. Indeed, integrative
628 temperate phages already encode many virulence factors, and we cannot find a reason why,
629 given enough time, they will not eventually acquire ARGs. This would be a most worrisome
630 outcome of the recent evolutionary process of acquisition of ARGs by human-associated
631 bacteria, since, as stated above, phages are extremely abundant, spread very fast in the
632 environment, and can infect bacteria in different geographical locations and time.

633

634 [Acknowledgements](#)

635

636 INCEPTION project (PIA/ANR-16-CONV-0005), Equipe FRM (Fondation pour la Recherche
637 Médicale): EQU201903007835, Laboratoire d'Excellence IBEID Integrative Biology of
638 Emerging Infectious Diseases [ANR-10-LABX-62-IBEID], SALMOPROPHAGE ANR-16-CE16-
639 0029. Sequencing was done at the Biomics Platform, C2RT, Institut Pasteur, Paris, France,
640 supported by France Génomique (ANR-10-INBS-09) and IBISA. We thank especially Marc
641 Monot, Goerges Haustant, Laure Lemée, Valérie Briolat, Laurence Ma, Elodie Turc, Amandine
642 Buffet and Matthieu Haudiquet for providing support, advice and services in DNA isolation,
643 library preparation and sequencing. This work used the computational and storage services
644 (TARS & MAESTRO cluster) provided by the IT department at Institut Pasteur, Paris.

645

646 [References](#)

647

648 1. Hernando-Amado S, Coque TM, Baquero F, Martínez JL. 2019. Defining and combating
649 antibiotic resistance from One Health and Global Health perspectives. *Nat Microbiol*
650 4:1432–1442.

651 2. Spera AM, Esposito S, Pagliano P. 2019. Emerging antibiotic resistance:
652 carbapenemase-producing enterobacteria. Bad new bugs, still no new drugs. *Infez Med*
653 27:357–364.

654 3. Lin Q, Wang Y, Yu J, Li S, Zhang Y, Wang H, Lai X, Liu D, Mao L, Luo Y, Tang G, Chen Z,
655 Sun Z. 2021. Bacterial characteristics of carbapenem-resistant *Enterobacteriaceae*
656 (CRE) colonized strains and their correlation with subsequent infection. *BMC Infect Dis*
657 21:638.

658 4. Worthington RJ, Melander C. 2013. Overcoming Resistance to β -Lactam Antibiotics. *J*
659 *Org Chem* 78:4207–4213.

660 5. Bonnin RA, Jousset AB, Emeraud C, Oueslati S, Dortet L, Naas T. 2020. Genetic
661 Diversity, Biochemical Properties, and Detection Methods of Minor Carbapenemases in
662 Enterobacterales. *Front Med (Lausanne)* 7:616490.

663 6. Che Y, Yang Y, Xu X, Břinda K, Polz MF, Hanage WP, Zhang T. 2021. Conjugative
664 plasmids interact with insertion sequences to shape the horizontal transfer of
665 antimicrobial resistance genes. *PNAS* 118:e2008731118.

666 7. Carattoli A. 2013. Plasmids and the spread of resistance. *Int J Med Microbiol* 303:298–
667 304.

668 8. Partridge SR, Kwong SM, Firth N, Jensen SO. 2018. Mobile Genetic Elements Associated
669 with Antimicrobial Resistance. *Clin Microbiol Rev* 31:e00088-17.

670 9. Yao Y, Maddamsetti R, Weiss A, Ha Y, Wang T, Wang S, You L. 2022. Intra- and
671 interpopulation transposition of mobile genetic elements driven by antibiotic selection.
672 5. *Nat Ecol Evol* 6:555–564.

- 673 10. Deng Y, Bao X, Ji L, Chen L, Liu J, Miao J, Chen D, Bian H, Li Y, Yu G. 2015. Resistance
674 integrons: class 1, 2 and 3 integrons. *Ann Clin Microbiol Antimicrob* 14:45.
- 675 11. Mazel D. 2006. Integrons: agents of bacterial evolution. *Nat Rev Microbiol* 4:608–620.
- 676 12. Chiang YN, Penadés JR, Chen J. 2019. Genetic transduction by phages and
677 chromosomal islands: The new and noncanonical. *PLOS Pathog* 15:e1007878.
- 678 13. Brüssow H, Canchaya C, Hardt W-D. 2004. Phages and the Evolution of Bacterial
679 Pathogens: from Genomic Rearrangements to Lysogenic Conversion. *Microbiol Mol
680 Biol Rev* 68:560–602.
- 681 14. Volkova VV, Lu Z, Besser T, Gröhn YT. 2014. Modeling the Infection Dynamics of
682 Bacteriophages in Enteric *Escherichia coli*: Estimating the Contribution of Transduction
683 to Antimicrobial Gene Spread. *Appl Environ Microbiol* 80:4350–4362.
- 684 15. Borodovich T, Shkoporov AN, Ross RP, Hill C. 2022. Phage-mediated horizontal gene
685 transfer and its implications for the human gut microbiome. *Gastroenterol Rep*
686 10:goac012.
- 687 16. Enault F, Briet A, Bouteille L, Roux S, Sullivan MB, Petit M-A. 2017. Phages rarely
688 encode antibiotic resistance genes: a cautionary tale for virome analyses. *ISME J*
689 11:237–247.
- 690 17. Calero-Cáceres W, Ye M, Balcázar JL. 2019. Bacteriophages as Environmental
691 Reservoirs of Antibiotic Resistance. *Trends Microbiol* 27:570–577.
- 692 18. Colavecchio A, Cadieux B, Lo A, Goodridge LD. 2017. Bacteriophages Contribute to the
693 Spread of Antibiotic Resistance Genes among Foodborne Pathogens of the
694 *Enterobacteriaceae* Family – A Review. *Front Microbiol* 1108.
- 695 19. Brown-Jaque M, Calero-Cáceres W, Muniesa M. 2015. Transfer of antibiotic-resistance
696 genes via phage-related mobile elements. *Plasmid* 79:1–7.
- 697 20. Howard-Varona C, Hargreaves KR, Abedon ST, Sullivan MB. 2017. Lysogeny in nature:
698 Mechanisms, impact and ecology of temperate phages. *ISME Journal* 11:1511–1520.

- 699 21. Kim M-S, Bae J-W. 2018. Lysogeny is prevalent and widely distributed in the murine gut
700 microbiota. *ISME J* 12:1127–1141.
- 701 22. Tuttle MJ, Buchan A. 2020. Lysogeny in the oceans: Lessons from cultivated model
702 systems and a reanalysis of its prevalence. *Environ Microbiol* 22:4919–4933.
- 703 23. Pfeifer E, Moura de Sousa JA, Touchon M, Rocha EPC. 2021. Bacteria have numerous
704 distinctive groups of phage-plasmids with conserved phage and variable plasmid gene
705 repertoires. *Nucleic Acids Res* 49:2655–2673.
- 706 24. Sternberg N. 1990. Bacteriophage P1 cloning system for the isolation, amplification,
707 and recovery of DNA fragments as large as 100 kilobase pairs. *PNAS* 87(1):103–107.
- 708 25. Zhou W, Liu L, Feng Y, Zong Z. 2018. A P7 Phage-Like Plasmid Carrying *mcr-1* in an ST15
709 *Klebsiella pneumoniae* Clinical Isolate. *Front Microbiol* 9:11.
- 710 26. Yang L, Li W, Jiang G-Z, Zhang W-H, Ding H-Z, Liu Y-H, Zeng Z-L, Jiang H-X. 2017.
711 Characterization of a P1-like bacteriophage carrying CTX-M-27 in *Salmonella spp.*
712 resistant to third generation cephalosporins isolated from pork in China. *Sci Rep*
713 7:40710.
- 714 27. Billard-Pomares T, Fouteau S, Jacquet ME, Roche D, Barbe V, Castellanos M, Bouet JY,
715 Cruveiller S, Médigue C, Blanco J, Clermont O, Denamur E, Branger C. 2014.
716 Characterization of a P1-Like Bacteriophage Carrying an SHV-2 Extended-Spectrum β -
717 Lactamase from an *Escherichia coli* Strain. *Antimicrob Agents Chemother* 58:6550–
718 6557.
- 719 28. Venturini C, Zingali T, Wyrsh ER, Bowring B, Iredell J, Partridge SR, Djordjevic SP. 2019.
720 Diversity of P1 phage-like elements in multidrug resistant *Escherichia coli*. *Sci Rep*
721 9:18861.
- 722 29. Pruitt KD, Tatusova T, Maglott DR. 2007. NCBI reference sequences (RefSeq): a curated
723 non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids*
724 *Res* 35:D61–D65.

- 725 30. Steinegger M, Söding J. 2017. MMseqs2 enables sensitive protein sequence searching
726 for the analysis of massive data sets. *Nat Biotechnol* 35:1026–1028.
- 727 31. Graziotin AL, Koonin EV, Kristensen DM. 2017. Prokaryotic Virus Orthologous Groups
728 (pVOGs): a resource for comparative genomics and protein family annotation. *Nucleic
729 Acids Res* 45:D491–D498.
- 730 32. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, Tosatto
731 SCE, Paladin L, Raj S, Richardson LJ, Finn RD, Bateman A. 2021. Pfam: The protein
732 families database in 2021. *Nucleic Acids Res* 49:D412–D419.
- 733 33. Haft DH, Loftus BJ, Richardson DL, Yang F, Eisen JA, Paulsen IT, White O. 2001.
734 TIGRFAMs: a protein family resource for the functional identification of proteins.
735 *Nucleic Acids Res* 29:41–43.
- 736 34. Eddy SR. 2011. Accelerated Profile HMM Searches. *PLoS Comput Biol* 7:e1002195.
- 737 35. Cury J, Touchon M, Rocha EPC. 2017. Integrative and conjugative elements and their
738 hosts: composition, distribution and organization. *Nucleic Acids Res* 45:8943–8956.
- 739 36. Alcock BP, Raphenya AR, Lau TTY, Tsang KK, Bouchard M, Edalatmand A, Huynh W,
740 Nguyen A-LV, Cheng AA, Liu S, Min SY, Miroshnichenko A, Tran H-K, Werfalli RE, Nasir
741 JA, Oloni M, Speicher DJ, Florescu A, Singh B, Faltyn M, Hernandez-Koutoucheva A,
742 Sharma AN, Bordeleau E, Pawlowski AC, Zubyk HL, Dooley D, Griffiths E, Maguire F,
743 Winsor GL, Beiko RG, Brinkman FSL, Hsiao WWL, Domselaar GV, McArthur AG. 2020.
744 CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic
745 resistance database. *Nucleic Acids Res* 48:D517–D525.
- 746 37. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe
747 RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G,
748 Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H,
749 Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wiczorek K,
750 Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. 2020.
751 ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother*
752 75:3491–3500.

- 753 38. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, Rolain J-
754 M. 2014. ARG-ANNOT, a New Bioinformatic Tool To Discover Antibiotic Resistance
755 Genes in Bacterial Genomes. *Antimicrob Agents Chemother* 58:212–220.
- 756 39. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009.
757 BLAST+: architecture and applications. *BMC Bioinform* 10:421.
- 758 40. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH, Hoffmann
759 M, Pettengill JB, Prasad AB, Tillman GE, Tyson GH, Klimke W. 2021. AMRFinderPlus and
760 the Reference Gene Catalog facilitate examination of the genomic links among
761 antimicrobial resistance, stress response, and virulence. *Sci Rep* 11:12728.
- 762 41. Xie Z, Tang H. 2017. ISEScan: automated identification of insertion sequence elements
763 in prokaryotic genomes. *Bioinformatics* 33:3340–3347.
- 764 42. Néron B, Littner E, Haudiquet M, Perrin A, Cury J, Rocha E. 2022. IntegronFinder 2.0:
765 Identification and Analysis of Integrons across Bacteria, with a Focus on Antibiotic
766 Resistance in *Klebsiella*. *Microorganisms* 10:700.
- 767 43. Gautreau G, Bazin A, Gachet M, Planel R, Burlot L, Dubois M, Perrin A, Médigue C,
768 Calteau A, Cruveiller S, Matias C, Ambroise C, Rocha EPC, Vallenet D. 2020.
769 PPanGGOLiN: Depicting microbial diversity via a partitioned pangenome graph. *PLoS*
770 *Comput Biol* 16:e1007732.
- 771 44. Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal:
772 prokaryotic gene recognition and translation initiation site identification. *BMC*
773 *Bioinform* 11:119.
- 774 45. Pitcher D g., Saunders N a., Owen R j. 1989. Rapid extraction of bacterial genomic DNA
775 with guanidium thiocyanate. *Lett Appl Microbiol* 8:151–156.
- 776 46. Kolmogorov M, Yuan J, Lin Y, Pevzner PA. 2019. Assembly of long, error-prone reads
777 using repeat graphs. *Nat Biotechnol* 37:540–546.
- 778 47. Jakočiūnė D, Moodley A. 2018. A Rapid Bacteriophage DNA Extraction Method.
779 *Methods Protoc* 1:E27.

- 780 48. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: Resolving bacterial genome
781 assemblies from short and long sequencing reads. *PLoS Comput Biol* 13:e1005595.
- 782 49. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. *Nat*
783 *Methods* 9:357–359.
- 784 50. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin
785 R. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–
786 2079.
- 787 51. Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic
788 features. *Bioinformatics* 26:841–842.
- 789 52. Bonnin RA, Emeraud C, Jousset AB, Naas T, Dortet L. 2022. Comparison of disk
790 diffusion, MIC test strip and broth microdilution methods for cefiderocol susceptibility
791 testing on carbapenem-resistant enterobacterales. *Clin Microbiol Infect* S1198-
792 743X(22)00221-X.
- 793 53. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, Han C, Bisignano C,
794 Rao P, Wool E, Johnson SC, Browne AJ, Chipeta MG, Fell F, Hackett S, Haines-
795 Woodhouse G, Hamadani BHK, Kumaran EAP, McManigal B, Agarwal R, Akech S,
796 Albertson S, Amuasi J, Andrews J, Aravkin A, Ashley E, Bailey F, Baker S, Basnyat B,
797 Bekker A, Bender R, Bethou A, Bielicki J, Boonkasidecha S, Bukosia J, Carvalheiro C,
798 Castañeda-Orjuela C, Chansamouth V, Chaurasia S, Chiurchiù S, Chowdhury F, Cook AJ,
799 Cooper B, Cressey TR, Criollo-Mora E, Cunningham M, Darboe S, Day NPJ, Luca MD,
800 Dokova K, Dramowski A, Dunachie SJ, Eckmanns T, Eibach D, Emami A, Feasey N,
801 Fisher-Pearson N, Forrest K, Garrett D, Gastmeier P, Giref AZ, Greer RC, Gupta V, Haller
802 S, Haselbeck A, Hay SI, Holm M, Hopkins S, Iregbu KC, Jacobs J, Jarovsky D, Javanmardi
803 F, Khorana M, Kissoon N, Kobeissi E, Kostyanev T, Krapp F, Krumkamp R, Kumar A, Kyu
804 HH, Lim C, Limmathurotsakul D, Loftus MJ, Lunn M, Ma J, Mturi N, Munera-Huertas T,
805 Musicha P, Mussi-Pinhata MM, Nakamura T, Nanavati R, Nangia S, Newton P, Ngoun C,
806 Novotney A, Nwakanma D, Obiero CW, Olivas-Martinez A, Olliaro P, Ooko E, Ortiz-
807 Brizuela E, Peleg AY, Perrone C, Plakkal N, Ponce-de-Leon A, Raad M, Ramdin T, Riddell
808 A, Roberts T, Robotham JV, Roca A, Rudd KE, Russell N, Schnall J, Scott JAG,

- 809 Shivamallappa M, Sifuentes-Osornio J, Steenkeste N, Stewardson AJ, Stoeva T, Tasak N,
810 Thaiprakong A, Thwaites G, Turner C, Turner P, Doorn HR van, Velaphi S, Vongpradith
811 A, Vu H, Walsh T, Waner S, Wangrangsimakul T, Wozniak T, Zheng P, Sartorius B, Lopez
812 AD, Stergachis A, Moore C, Dolecek C, Naghavi M. 2022. Global burden of bacterial
813 antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 399:629–655.
- 814 54. Ploy M-C, Berendonk TU. 2022. Editorial overview: AMR in the environment — too
815 complex for surveillance? *Curr Opin* 65:xi–xiii.
- 816 55. Varani A, He S, Siguier P, Ross K, Chandler M. 2021. The IS6 family, a clinically
817 important group of insertion sequences including IS26. *Mob DNA* 12:11.
- 818 56. Gillings MR. 2014. Integrons: Past, Present, and Future. *Microbiol Mol Biol Rev* 78:257–
819 277.
- 820 57. Refardt D. 2011. Within-host competition determines reproductive success of
821 temperate bacteriophages. *ISME J* 5:1451–1460.
- 822 58. Dowah ASA, Clokie MRJ. 2018. Review of the nature, diversity and structure of
823 bacteriophage receptor binding proteins that target Gram-positive bacteria. *Biophys*
824 *Rev* 10:535–542.
- 825 59. Bernheim A, Sorek R. 2020. The pan-immune system of bacteria: antiviral defence as a
826 community resource. 2. *Nat Rev Microbiol* 18:113–119.
- 827 60. Bondy-Denomy J, Qian J, Westra ER, Buckling A, Guttman DS, Davidson AR, Maxwell KL.
828 2016. Prophages mediate defense against phage infection through diverse
829 mechanisms. *ISME J* 10:2854–2866.
- 830 61. de Sousa JAM, Buffet A, Haudiquet M, Rocha EPC, Rendueles O. 2020. Modular
831 prophage interactions driven by capsule serotype select for capsule loss under phage
832 predation. *ISME J* 14:2980–2996.
- 833 62. Piel D, Bruto M, Labreuche Y, Blanquart F, Chenivesse S, Lépante S, James A, Barcia-
834 Cruz R, Dubert J, Petton B, Lieberman E, Wegner KM, Hussain FA, Kauffman KM, Polz
835 MF, Bikard D, Gandon S, Roux FL. 2021. Genetic determinism of phage-bacteria

- 836 coevolution in natural populations. bioRxiv
837 <https://doi.org/10.1101/2021.05.05.442762>.
- 838 63. Łobocka MB, Rose DJ, Plunkett G, Rusin M, Samojedny A, Lehnerr H, Yarmolinsky MB,
839 Blattner FR. 2004. Genome of bacteriophage P1. *J Bacteriol* 186:7032–7068.
- 840 64. Bobay L-M, Touchon M, Rocha EPC. 2014. Pervasive domestication of defective
841 prophages by bacteria. *PNAS* 111:12127–12132.
- 842 65. Murooka Y, Harada T. 1979. Expansion of the host range of coliphage P1 and gene
843 transfer from enteric bacteria to other gram-negative bacteria. *Appl Environ Microbiol*
844 38:754–757.
- 845 66. Iida S, Meyer J, Kennedy KE, Arber W. 1982. A site-specific, conservative recombination
846 system carried by bacteriophage P1. Mapping the recombinase gene *cin* and the cross-
847 over sites *cix* for the inversion of the C segment. *EMBO J* 1:1445–1453.
- 848 67. Colavecchio A, Jeukens J, Freschi L, Edmond Rheault J-G, Kukavica-Ibrulj I, Levesque RC,
849 LeJeune J, Goodridge L. 2017. Complete Genome Sequences of Two Phage-Like
850 Plasmids Carrying the CTX-M-15 Extended-Spectrum β -Lactamase Gene. 19. *Genome*
851 *Announc* 5:e00102-17.
- 852 68. Nordmann P, Dortet L, Poirel L. 2012. Carbapenem resistance in *Enterobacteriaceae*:
853 here is the storm! *Trends Mol Med* 18:263–272.
- 854 69. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu
855 L-F, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu J-H, Shen J. 2016. Emergence
856 of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human
857 beings in China: a microbiological and molecular biological study. *Lancet Infect Dis*
858 16:161–168.
- 859 70. Galimand M, Courvalin P, Lambert T. 2012. RmtF, a new member of the
860 aminoglycoside resistance 16S rRNA N7 G1405 methyltransferase family. *Antimicrob*
861 *Agents Chemother* 56:3960–3962.

- 862 71. Paepe MD, Taddei F. 2006. Viruses' Life History: Towards a Mechanistic Basis of a
863 Trade-Off between Survival and Reproduction among Phages. *PLoS Biol* 4:e193.
- 864 72. Zhang X, McDaniel AD, Wolf LE, Keusch GT, Waldor MK, Acheson DWK. 2000.
865 Quinolone Antibiotics Induce Shiga Toxin-Encoding Bacteriophages, Toxin Production,
866 and Death in Mice. *J Infect Dis* 181:664–670.
- 867 73. Leclercq S, Cordaux R. 2011. Do phages efficiently shuttle transposable elements
868 among prokaryotes? *Evol* 65:3327–3331.
- 869 74. Stalder T, Barraud O, Casellas M, Dagot C, Ploy M-C. 2012. Integron Involvement in
870 Environmental Spread of Antibiotic Resistance. *Front Microbiol* 3.
- 871 75. Moura de Sousa JA, Pfeifer E, Touchon M, Rocha EPC. 2021. Causes and Consequences
872 of Bacteriophage Diversification via Genetic Exchanges across Lifestyles and Bacterial
873 Taxa. *Mol Biol Evol* 38:2497–2512.
- 874 76. Latino L, Midoux C, Hauck Y, Vergnaud G, Pourcel C 2016. Pseudolysogeny and
875 sequential mutations build multiresistance to virulent bacteriophages in *Pseudomonas*
876 *aeruginosa*. *Microbiology* 162:748–763.

877 [Table legends](#)

878 Table legends

879 Supplementary table 1:

880 Antibiotic resistance genes detected in phage-plasmids.

881

882 Supplementary table 2:

883 Phage-plasmids with ARG in CRE strains.

884

885 Supplementary table 3:

886 VirSorter2 and wGRR analysis of co-assemblies retrieved after MMC-induction experiment
887 (including average read coverage).

888

889 Supplementary table 4:

890 Bacterial strains that were used for infection experiments with ARG encoding P-Ps.

891

892 Supplementary table 5:

893 Accession numbers on the reads and assemblies from the genome sequencing, MMC
894 induction (hybrid assembly included) and re-sequencing of lysogens.