

# A 4.5-Year Within-Patient Evolution of a Colistin-Resistant *Klebsiella pneumoniae* Carbapenemase-Producing *K. pneumoniae* Sequence Type 258

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2 **A 4.5 years within-patient evolution of a colistin resistant *Klebsiella***  
3 ***pneumoniae* Carbapenemase-producing *K. pneumoniae* Sequence Type 258**

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31 Running title: *In vivo* evolution of KPC-*K. pneumoniae* (39 characters)

32

33 Keywords: KPC, carrier, within-host evolution, NGS, phylogeny, host pathogen interaction

34

35 **40-word summary:**

36 KPC-producing *Klebsiella pneumoniae* (KPC-Kp) have emerged as a major nosocomial

37 pathogen. This study emphasizes long-term *in-vivo* genome evolution along with the selection

38 of pathoadaptive mutations in a KPC-Kp strain that might have contributed to its long-term

39 carriage, virulence and dissemination.

40

## ABSTRACT

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**Background.** KPC-producing *Klebsiella pneumoniae* (KPC-Kp) have emerged globally over the last decade as a major nosocomial pathogen that threatens patient's care. These highly resistant bacteria are mostly associated with a single Kp clonal group (CG) CG258, but the reasons for its host and hospital adaptation remain largely unknown.

**Methods.** We analyzed the *in vivo* evolution of a colistin-resistant KPC-Kp-CG258 strain that contaminated a patient following an endoscopy, and was responsible for a fatal bacteraemia 4.5 years later. Whole Genome Sequencing (WGS) was performed on 17 KPC-Kp isolates from this patient, single-nucleotide polymorphisms (SNPs) were analyzed and their implication in antimicrobial resistance and bacterial host adaptation investigated.

**Results.** The patient KPC-Kp strain diversified over 4.5 years at a rate of 7.5 substitutions per genome per year resulting in broad phenotypic modifications. After two years of carriage, all isolates restored susceptibility to colistin. Higher expression of the fimbriae conferred the ability to produce more biofilm and the isolate responsible for a bacteraemia grew in human serum. The convergent mutations occurring in specific pathways, such as the respiratory chain and the cell envelope revealed a complex long-term adaptation of KPC-Kp.

**Conclusion.** Broad genomic and phenotypic diversification and the parallel selection of pathoadaptive mutations might contribute to long-term carriage and virulence of KPC-Kp-CG258 strains and to the dissemination of this clone.

62           The increasing incidence of extended spectrum  $\beta$ -lactamase (ESBL) and  
63 carbapenemase-producing strains of *K. pneumoniae* (Kp) in health care facilities is a cause of  
64 global concern. In several countries including Greece, Italy, Israel and the United States, the  
65 rate of infections with *K. pneumoniae* resistant to all antibiotics commercially available is  
66 worryingly growing [1–5]. Most of these isolates are resistant to all  $\beta$ -lactams due to the  
67 production of a class A carbapenemase named KPC and can also be resistant to other  
68 antibiotics of last resort like tigecyclin [6] and colistin [7]. The worldwide dissemination of  
69 KPC-Kp is mainly associated with the spread of a single clonal group designated CG258  
70 [1,8,9].The evolutionary success of CG258 in combination with KPC is not yet fully  
71 understood. Several factors might have an impact on the global dissemination of a clone, such  
72 as increased pathogenicity, high transmissibility, and/or increased duration of colonization  
73 [9]. Analysis of KPC-Kp ST258 outbreaks indicated that this clone can be easily transmitted  
74 between patients for several months following the identification of the initial index case [8].  
75 However, little is known about the outcome of KPC-Kp fecal carriers and the duration of  
76 carriage, which are determining transmission factors. Some studies indicate that patients can  
77 exhibit spontaneous decolonization within 6 months after KPC-Kp acquisition [10]. In  
78 contrast, reports of carriage longer than one year has been also observed [8,10,11].

79           Here we analyzed the evolution of a KPC-Kp ST258 strain that colonized a patient  
80 over 4.5 years. This strain underwent considerable genetic and phenotypic diversification to  
81 adapt to antibiotic treatments and to promote virulence. These results highlight the capacity of  
82 adaption of a KPC-Kp ST258 strain that may contribute to its propensity to sustainably  
83 colonize patients and persist in healthcare institutions.

84

## 85 **MATERIALS AND METHODS**

### 86 **Accession numbers**

87 Kp BIC-1 sequences have been submitted to Genbank under the accessions numbers:  
88 CP022573 (chromosome), CP022574 (pBIC1-a), CP022575 (pBIC-1b), CP022576 (pBIC-  
89 1c).

90

### 91 **Bacterial strains**

92 Strain Kp BIC-1 transmitted to the patient was recovered from a contaminated endoscope  
93 [12,13]. Strains S1 to S17 were isolated from different body sites and are summarized in  
94 Table 1. Kp CIP 53153, YC2004 (KPC-Kp ST258) [14] and KN633 (KPC-Kp ST338) [14]  
95 are isolates susceptible to colistin used as control in qRT-PCR experiments. Kp LM21 was  
96 used as positive control in biofilm formation [15]. Electrocompetent *Escherichia coli* TOP10  
97 (ThermoFisher, Villebon Sur Yvette, France) was used as a recipient in electroporation  
98 experiments.

99

### 100 **Antimicrobial susceptibility testing**

101 Minimal inhibitory concentrations (MICs) for carbapenems (imipenem, ertapenem and  
102 meropenem), aminoglycosides (gentamicin, amikacin, tobramycin and netilmicin) and  
103 tigecyclin were determined using Etest® strips (bioMérieux) on Mueller-Hinton agar plates  
104 (Bio-Rad). The MICs for colistin were determined by broth culture microdilution in triplicate.  
105 Results were interpreted according to the EUCAST guidelines ([www.eucast.org](http://www.eucast.org)).

106

### 107 **Whole-genome sequencing (WGS) and bioinformatics analysis**

108 The genome of Kp BIC-1 was sequenced using PacBio RS technology (GATC Biotech AG,  
109 Mulhouse, France). Illumina WGS was performed on Kp isolates BIC-1 and S1 to 17. DNA  
110 libraries were prepared using the Nextera XT v3 kit (Illumina, Essex, UK) and run on a  
111 MiSeq system to generate paired-end 75- or 150-bp reads. Kp BIC-1 genome was used as

112 reference for detecting mutations using BRESEQ [16]. Acquisition of genetic material was  
113 assessed by analyzing unmapped reads following their *de novo* assembly by using Velvet  
114 [17]. Phylogeny of the 18 Kp isolates was constructed by considering the 85 identified SNPs  
115 among their genomes by Maximum-Parsimony (MP) using MEGA [18], refined manually and  
116 rooted by considering the direction of each SNP as described previously [19]. Evolutionary  
117 rate of the patient strain was determined based on the linear association between the root-to-  
118 tip number of mutations and year of strain isolation. The core genome phylogeny of the 18 Kp  
119 isolates together with ST258 published sequences and with the ST11 strain HS11286 as an  
120 outgroup was performed using the Parsnp program from the Harvest suite [20].

121

#### 122 **Quantification of biofilm formation**

123 Biofilm formation capacity was measured in microtiter plate as previously described [21].  
124 Briefly  $2 \cdot 10^7$  CFU/mL of an overnight culture were inoculated into 180  $\mu$ l of medium  
125 M63B1–0.4% Glucose in a 96-well PVC microtiter plate (Falcon). After 18h of incubation at  
126 37°C, culture medium was removed, bacteria were fixed by adding 100  $\mu$ l of Boin's solution  
127 (Sigma-Aldrich, Saint Quentin Fallavier, France) and rinsed once with distilled water.  
128 Bacterial cells were stained for 10 min at room temperature by adding 50  $\mu$ l of 0.5% (wt/vol)  
129 aqueous solution of crystal violet in each well and washed twice with distilled water and dried  
130 at room temperature. The bound dye was released from stained cells using 80/20  
131 ethanol/acetone solution and measured by absorbance at 595 nm.

132

#### 133 **Serum resistance assay**

134 The serum resistance of isolates S1, S11, S13, S15, S17 and Kp CIP53153 as reference were  
135 determined according to the method of Siu *et al* [22]. Briefly, bacterial suspensions were  
136 collected from mid-log phase cultures (approximately  $5 \cdot 10^7$  CFU/ml) and mixed at a 1:3

137 (vol/vol) ratios with pooled human serum (Sterile and filtered human male AB plasma from  
138 Sigma-Aldrich) and incubated at 37°C with agitation. Colony counts were determined at T0  
139 and after 30, 60, 120 and 180 min of incubation. Serum susceptibility was assessed by  
140 plotting the average survival percentage against the incubation time. Each strain was tested  
141 four times.

142

### 143 **Statistical analysis data**

144 Statistical analysis was performed on  $\geq 3$  independent experiments using GraphPad Prism v6  
145 software. Data were compared two-by-two using unpaired Student's t test with  
146 Welch' correction. p values  $<0.05$  were considered statistically significant.

147

## 148 **RESULTS**

### 149 **Clinical case**

150 In 2009, a nosocomial outbreak of KPC-Kp isolates mediated by a contaminated endoscope  
151 occurred in a French tertiary care hospital [12]. Seventeen patients that underwent  
152 gastroscopy were contaminated with an endoscope-borne KPC-Kp strain (Kp BIC-1). One of  
153 them suffered from recurrent cholangitis due to repetitive lithiasis (Figure 1A). In September  
154 2009, he underwent sphincteroscopy with the contaminated endoscope. Until April 2014, 17  
155 KPC-Kp isolates were collected from this patient (S1 to S17) (Table 1). In 2013, a prostatic  
156 adenocarcinoma and a bladder cancer were diagnosed. In 2014, infectious complications  
157 occurred a week after a cystoprostatectomy, with peritonitis secondary to a breach in the  
158 Bricker anastomosis. Sepsis secondary to a polymicrobial infection with intestinal bacteria  
159 including KPC-Kp was followed by multivisceral failure and death.

160

### 161 **Genetic diversification of a single KPC-Kp strain**



162 To demonstrate whether the patient was colonized by a single KPC-Kp strain, we performed  
163 Illumina WGS of the 17 isolates and determined the complete genome sequence of Kp BIC-1  
164 strain by combining PacBio and Illumina sequencing data. Kp BIC-1 carries a circular  
165 chromosome of 5,394,314 bp containing 5,189 predicted coding sequences and three  
166 plasmids: pBIC-1a (a 170,415 bp IncFIIK2 family, pBIC-1b (a 43,380 bp IncX3 plasmid) and  
167 pBIC-1c (a 13,841 bp ColE1 plasmid). Multiple antimicrobial, heavy-metal and arsenic  
168 resistance markers were carried by the chromosome and the three plasmids (Supplementary  
169 Table 1). Phylogenetic analysis with representative KPC-Kp ST258 isolates showed that this  
170 strain belongs to clade I of ST258 (Supplementary Figure 1) [2,23,24]. Single nucleotide  
171 polymorphisms (SNPs) based phylogeny of the 18 isolates showed that they represent a  
172 monophyletic clade (Figure 1B). Therefore, all the patients' isolates derived from Kp BIC-1.

173 Within-host colonization of this strain over 4.5 years led to the accumulation of  
174 mutations (deletions, tandem duplications and SNPs) in up to 39, 24 and 22 loci for the last  
175 three isolates S15, S16 and S17, respectively (Figure 1B). Assembly of unmapped reads  
176 revealed no gene acquisition and a remarkably stable plasmid content over 4.5 years of  
177 carriage. A total of 98 genetic events occurred (Supplementary Table 2), with most of them in  
178 the chromosome (n=91). From this longitudinal study, we estimated that the average  
179 evolutionary rate of this KPC strain was of 7.5 SNPs per year per genome (Figure 1C).

180 Most significantly, the functions affected showed convergence towards specific  
181 pathways (Supplementary Table 2): (i) the respiratory chain: with three independent  
182 mutations in complex 1, heme biosynthesis and in the *sucC* gene, (ii) anaerobic respiration  
183 with mutations in nitrate reductase genes, in the promoter region of the *fnr* gene encoding a  
184 regulator of anaerobic metabolism and in the *narL* regulatory gene, (iii) the metabolism of  
185 different carbon sources and (iv) the cell envelope with mutations in porin genes (two  
186 independent mutations in the ferric enterobactin receptor precursor, *pfeA* and an *ompF*-like

187 gene), capsular polysaccharides (the glycosyl transferase *epsJ*, the colanic acid biosynthesis  
188 gene *wcaJ* and the sensor histidine kinase *rcsC*) and the *rfaH* antiterminator.

189

### 190 **Restoring colistin susceptibility**

191 The patient received during these 4.5 years numerous antimicrobial treatments,  
192 including carbapenems and gentamicin (Figure 1A). Accordingly, we observed changes in the  
193 antibiotic susceptibility during colonization (Table 1). Kp BIC-1 isolated from the endoscope  
194 and the first twelve isolates from the patient (S1 to S12) were resistant to colistin with MICs  $\geq$   
195 64 mg/L (Table 1). This high level of resistance resulted from the insertion of IS*Kpn25*  
196 between nucleotides 141 and 142 of the *mgrB* gene. MgrB plays a prominent role in colistin  
197 resistance in Kp by repressing the PhoP-PhoQ regulatory pathway [25,26]. PhoP indirectly  
198 activates the PmrA-PmrB two component system, which results in the overexpression of the  
199 *arnBCADTEF* operon leading to addition of the cationic groups (4-amino-4-deoxy-L-  
200 arabinose, L-Ara4N) on the phosphate groups of lipid A. This modification results in a  
201 decrease of the electronegative charge of the bacterial surface and resistance to cationic  
202 peptides [27]. After two years of carriage without any colistin treatment, all isolates (S13 to  
203 S17) were susceptible to colistin (Table 1). We identified two mutations in two partners of the  
204 colistin resistance pathway that may explain this phenotypic reversion. In four isolates (S13,  
205 S14, S16 and S17) we identified a mutation in *arnC*, leading to a non-conservative amino-  
206 acid change C161Y. ArnC is a lipid-A glycosyltransferase encoded by the *arnBCADTEF*  
207 operon. Isolate S15 is mutated in *phoP* (I201N). To investigate the effect of this SNP in PhoP,  
208 we quantified the expression of *arnC*, *phoP* and *phoQ* genes (indirect and direct targets of  
209 PhoP) by qRT-PCR on strain S1 resistant to colistin, S15 (PhoP I201N), S17 (ArnC C161Y)  
210 and on three colistin susceptible Kp strains used as references (CIP 53153, YC2004, KN533).  
211 Compared to the reference strains, isolates S1 and S17 showed a significantly higher

212 expression of *arnC*, confirming the effect of the *mgrB* disruption (Supplementary Figure 2).  
213 In the S15 isolate mutated in PhoP, the expression of *arnC*, *phoP* and *phoQ* was restored at  
214 the level of the reference colistin susceptible strains (Supplementary Figure 2). Regarding the  
215 S17 isolate, no change in the expression of *arnC*, *phoP* and *phoQ* was observed as compared  
216 to S1. We therefore hypothesized that the mutation in *arnC* led to a decrease of the  
217 glycosyltransferase activity of the modified ArnC protein. To confirm the role of the SNPs in  
218 *phoP* and *arnC* in colistin susceptibility reversion, we complemented the phenotype of S15  
219 and S17 respectively by cloning the unmutated gene into a modified pUC19 vector  
220 (Supplementary methods). Both complemented isolates S15-pUC19-*phoP*(WT) and S17-  
221 pUC19-*arnC*(WT) were resistant to colistin with MICs  $\geq 32$   $\mu\text{g/ml}$  (Table 2) .

222

### 223 **Variation of aminoglycoside susceptibility**

224 Kp-BIC-1 and the first six isolates were susceptible to gentamicin whereas six later  
225 isolates (S7 to S11 and S15) shifted to gentamicin resistance and an increased susceptibility to  
226 amikacin (Table 1 and Supplementary Table 3). SNPs analysis revealed the presence of a  
227 point mutation in *aac*(6')-Ib gene carried by pBIC-1c leading to a L119S amino-acid change  
228 of the AAC(6')-Ib enzyme in all the gentamicin resistant isolates. Casin *et al.* showed that this  
229 substitution led similarly to an 8-fold decrease in amikacin MICs and to an 8-fold increase in  
230 gentamicin MICs [28]. By cloning the two *aac*(6')-Ib variants in *E. coli*, the involvement of  
231 this SNP for the gentamicin resistance switch was demonstrated (Supplementary Table 3).

232

### 233 **Strain adaptation to promote virulence**

234 Specific adaptation that could promote bacterial virulence during long-term carriage  
235 was investigated. A feature of KPC-Kp ST258 isolates is their ability to cause bacteraemia  
236 especially in immunocompromised patients [29]. Among the 17 isolates, S15 was responsible

237 for a bloodstream infection. To assess if some polymorphisms in S15 might have contributed  
238 to its ability to grow in blood, we performed serum resistance assay on five isolates from the  
239 patient and on the non-ST258 Kp CIP 53153 strain as reference. Isolates S1, S11, S13, S17  
240 and CIP 53153 were highly sensitive to serum but isolate S15 was not only resistant to human  
241 serum but also grew rapidly (Figure 2). An SNP responsible for a nonsense mutation was  
242 present in the *gmlC* gene only in this isolate. The *gml* operon is present in the vast majority of  
243 KPC-Kp ST258 strains and is known to confer improved survival in the presence of human  
244 serum [30]. However, cloning experiment of a wild-type copy of *gmlC* in S15 could not  
245 restore susceptibility to human serum (Supplementary methods). The reasons of S15 peculiar  
246 phenotype might reside in one or several of the other 35 unique mutations.

247 A second common feature of KPC-Kp ST258 is their low capacity to produce biofilm  
248 [31]. However mutations in type 1 fimbriae operon potentially implicated in biofilm  
249 production [32] were detected in several isolates. Indeed, whereas the Kp-BIC-1 strain and 16  
250 isolates were poor biofilm producers, the isolate S7 produced significantly more biofilm  
251 (Figure 3A). We have detected three differences in the genome of S7 compared to Kp-BIC-1  
252 (Supplementary Table 2) including an inversion of the *fim* element in the promoter region  
253 switching from off to on the expression of the type 1 fimbriae operon (Figure 1C) and a non-  
254 synonymous SNP (P180T) in the *fimH* gene encoding the fimbriae adhesin.

255 Isolates S6, S8, S9, S10, S15 and S17 possess also an insertion of an adenosine in the  
256 *fim* element (Figure 3B) but no significant difference in biofilm formation (Figure 3A). To  
257 investigate the effect of these genomic modifications on the expression of the type 1 fimbriae,  
258 we performed qRT-PCR on isolates Kp BIC-1, S6 and S7 (Supplementary Materials).  
259 Compared to Kp BIC-1, we observed 11- and 71-fold increase in the expression of the *fimH*  
260 gene in S6 and in S7 respectively (Figure 3C). Although the 11-fold increase in *fimH*

261 expression has no detectable effect on biofilm production under laboratory conditions, it  
262 might increase adhesion to host cells and contribute to virulence or colonization [33].

263

## 264 **DISCUSSION**

265 Here, we deciphered by WGS the within patient evolution of a single strain of KPC-  
266 Kp over 4.5 years. The study begins with a rare but yet dramatic case of nosocomial  
267 transmission of carbapenemase-producing Enterobacteriaceae due to a contaminated  
268 endoscope, and ends prematurely with the patient's death secondary to a sepsis with the same  
269 KPC-Kp. The evolutionary rate was calculated at 7.5 SNPs per year per genome but this value  
270 needs to be considered with caution since the patient received several courses of antimicrobial  
271 treatments and thus, the selective pressure was not constant. This value is between the rate of  
272 3.8 SNPs/year observed in a study of MDR Kp in Ireland and UK [34] and a rate of ten  
273 mutations per genome per year determined in KPC-Kp from a single institution [35].

274 We therefore analyzed the phenotypic diversification related to antimicrobial pressure.  
275 Regarding aminoglycosides susceptibility, we reported a *in vivo* mutation in the *aac(6')*-Ib  
276 gene that conferred resistance to gentamicin. It appeared subsequent to a ten days treatment  
277 with imipenem and gentamicin, and was stable over-time. This highlights the risk of rapid  
278 change in the specificity of AAC(6')-Ib and its possible implication in treatment failures.  
279 Strikingly, the contaminating strain was colistin resistant but after two years of carriage, all  
280 isolates became susceptible to colistin. The *in vivo* reversion from a resistant to a susceptible  
281 phenotype has been reported in *Acinetobacter baumannii* and in *Pseudomonas aeruginosa* but  
282 not yet in Kp [27]. Colistin are currently one of the last resort treatment for severe human  
283 infections caused by MDR bacteria, particularly carbapenemase-producing  
284 Enterobacteriaceae [7]. Even though plasmid-mediated colistin resistance *mcr* genes have  
285 recently been described worldwide [36,37], high level colistin resistance in

286 Enterobacteriaceae in human is still mainly due to non-transferable modifications of the lipid  
287 A resulting from chromosomal mutations [27]. After two years of intestinal carriage without  
288 colistin pressure, we identified two compensatory mutations in the clinical isolates,  
289 highlighting the potential impact of lipid A modifications on bacterial *in vivo* fitness, similarly  
290 to what was observed for colistin-resistant *A. baumannii* both *in vitro* and *in vivo* [38].  
291 To further explore the long-term adaptation of this clone, mutations that could impact  
292 bacterial virulence were searched for. An inversion in the promoter region of the *fim* locus  
293 was found in isolate S7 capable to produce significantly more biofilm than others. Search for  
294 the orientation of the *fim* element in KPC-Kp CG258 genome sequences available on public  
295 repositories revealed that out of more than 500 strains, only three were oriented to allow  
296 expression of the *fim* operon. Therefore, expression of the type 1 fimbriae seems extremely  
297 rare in KPC-Kp ST258 and might have been selected under long-term colonization.  
298 Furthermore, isolate S15 showed a unique evolutionary trajectory in the patient, as it did not  
299 share any common SNPs with the previous nor the two following isolates and had 39 genomic  
300 differences with BIC-1 (Figure 1B and Supplementary Table 2). This isolate is therefore a  
301 proof of the genomic diversity that occurred *in vivo* during persistent carriage leading to a  
302 broad range of adaptive phenotypes contributing to invasive infection. S15 strain isolated  
303 almost three years after infection acquired the ability to grow in serum, to resist to gentamicin  
304 and reversed the colistin resistance phenotype. The diversity of isolates revealed a complex  
305 within host evolution involving possibly adaptation to specific niches in the gut and leading to  
306 a diverse clonal population only partially characterized by the conservation of a single isolate  
307 at each sampling.

308 In summary, our work highlights the importance of leveraging the power of WGS to  
309 better understand how clinically-important clones are capable to adapt and cause disease in  
310 the human host. Currently, no specific clinical guidelines recommend the typing of more than

311 one isolate per patient, but our findings reveal the need to closely consider within-host  
312 variability in future studies of *K. pneumoniae* evolution and transmission.

313

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### 321 **Conflict of interest**

322 The authors declare no competing financial interests

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435

436

437 **Figures Legends**

438

439 **Figure 1. Clinical and microbiological history of a patient colonized with a unique strain**  
440 **of KPC-Kp ST258 for 4,5 years** **A.** 17 isolates were collected at different moments of  
441 patient's medical life. Kp BIC-1 designates the isolate collected from the endoscope. Known  
442 antimicrobial treatments are indicated in light grey boxes. **B.** Phylogenetic analysis of the 18  
443 isolates. Numbers indicate the number of SNPs in the corresponding branch **C.** Molecular  
444 clock rate based on the linear association between the root-to-tip number of mutations and  
445 year of strain isolation. SNPs: Single Nucleotide Polymorphisms.

446

447 **Figure 2. Serum bactericidal assay.**

448 The average of survival (in %) of each strain in human serum is plotted against the incubation  
449 time (n=4, means  $\pm$  SEM).<sup>o</sup> Kp53153 is a non-KPC non-ST258 strain. All strains were  
450 compared to the strain S17 (Student *t* test : \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  ).

451

452 **Figure 3. Biofilm formation.**

453 **A.** Biofilm formation quantification using crystal violet staining. Absorbance values (n=4,  
454 means  $\pm$  SEM) are indicated. The dotted line corresponds to the mean absorbance of KPC-Kp  
455 strains S1, S2, S3, S4, S5, S6, S8, S9, S10, S11, S12, S13, S14, S15, S16 and S17. Only the  
456 absorbance of the S7 strain (purple) is significantly increased compared to the other KPC-Kp  
457 strains (Student *t* test : \*\*\*\*,  $p < 0.0001$ ). LM21 was used as a control positive strain [37]. In  
458 C-, no bacteria were added in the well. **B.** Schematic representation of the *fim* locus in the 18  
459 isolates. The white and grey arrows indicate Right and Left Inverted Repeats respectively. **C.**  
460 Analysis of *fimH* expression using qRT-PCR (n=3, means of fold change expression  $\pm$  SEM)  
461 (Student *t* test : \*\*\*\*,  $p < 0.0001$ ).

462

463 **Table 1. Antimicrobial susceptibility testing of the 18 isolates.**

Name	Date	Origin	Susceptibility testing (mg/L)*					
			ERT <sup>a</sup>	IMI <sup>a</sup>	MER <sup>a</sup>	COL <sup>b</sup>	TGC <sup>a</sup>	GM <sup>a</sup>
Kp BIC-1	2009-09-25	endoscope	6	1.5	<b>4</b>	32	<b>1.5</b>	1.5
S1	2009-09-29	stool	12	1.5	<b>4</b>	128	<b>2</b>	1.5
S2	2009-10-12	stool	12	1.5	<b>4</b>	64	<b>1.5</b>	1.5
S3	2009-10-13	unknown	16	1.5	<b>6</b>	64	<b>2</b>	2
S4	2009-10-14	unknown	12	1.5	<b>4</b>	64	<b>1.5</b>	1.5
S5	2009-10-16	bile	12	1.5	<b>4</b>	32	<b>1.5</b>	1.5
S6	2009-11-03	stool	12	1.5	<b>4</b>	32	<b>2</b>	2
S7	2009-11-03	stool	8	1	<b>4</b>	32	<b>2</b>	12
S8	2009-11-11	urine	12	1	<b>4</b>	32	<b>2</b>	12
S9	2009-11-16	stool	12	1	<b>4</b>	64	<b>2</b>	12
S10	2009-11-21	urine	12	1	<b>4</b>	64	<b>1.5</b>	12
S11	2009-11-23	unknown	12	1	<b>4</b>	64	<b>1.5</b>	8
S12	2010-01-15	stool	16	1	<b>4</b>	64	<b>1.5</b>	2
S13	2011-07-29	stool	16	1	<b>4</b>	0.5	<b>2</b>	1.5
S14	2011-07-29	bile	24	2	<b>8</b>	0.5	<b>2</b>	1.5
S15	2012-08-23	blood culture	8	0.75	<b>3</b>	0.25	<b>1.5</b>	12
S16	2013-06-04	stool	24	1.5	<b>6</b>	0.5	<b>2</b>	1.5
S17	2014-04-29	BAL	12	1.5	<b>6</b>	0.5	<b>2</b>	1.5

464

465 ERT Ertapenem; IMI Imipenem; MEM Meropenem; COL Colistin; TGC Tigecyclin; GM

466 Gentamicin; BAL Bronchoalveolar lavage; <sup>a</sup> Performed with E-test; <sup>b</sup> Performed with broth

467 microdilution. \* Resistant MIC values are highlighted in grey, intermediate MIC values are

468 written in bold.

469

470

471 **Table 2. Effect of the SNP in *phoP* and *arnC* on colistin susceptibility**  
 472

Strain	Susceptibility testing <sup>1</sup> Colistin
Kp BIC-1	32
S15*	0,25
S15*-pUC19	0,25
S15*-pUC19- <i>phoP</i> (WT)	64
S17#	0,5
S17#-pUC19	0,5
S17#-pUC19- <i>arnC</i> (WT)	32

473  
 474 <sup>1</sup>Determined by broth microdilution. Resistant MIC values are highlighted in grey.  
 475 \* with the mutation PhoP I201N  
 476 # with the mutation ArnC C161Y  
 477

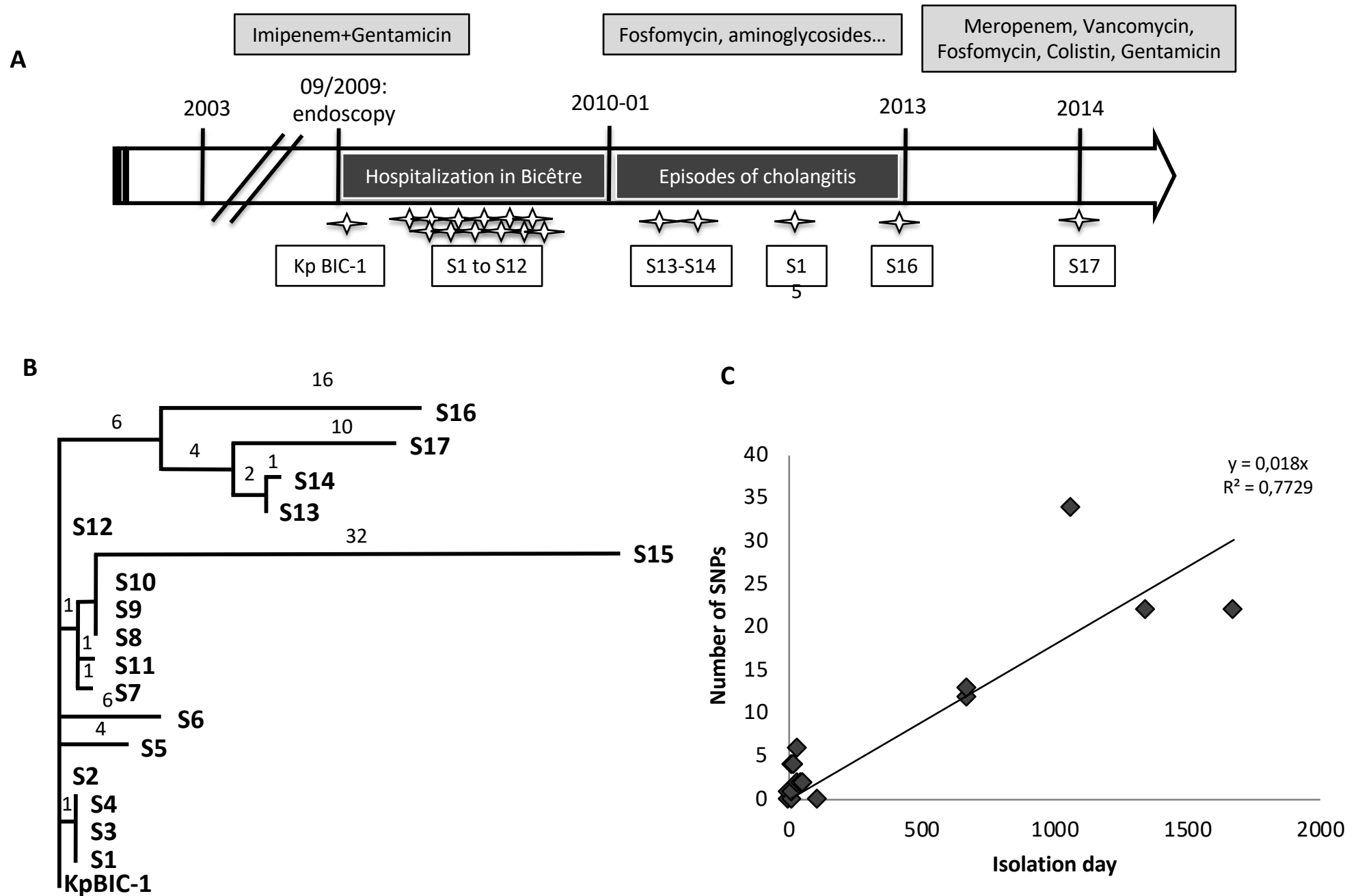


Fig.1



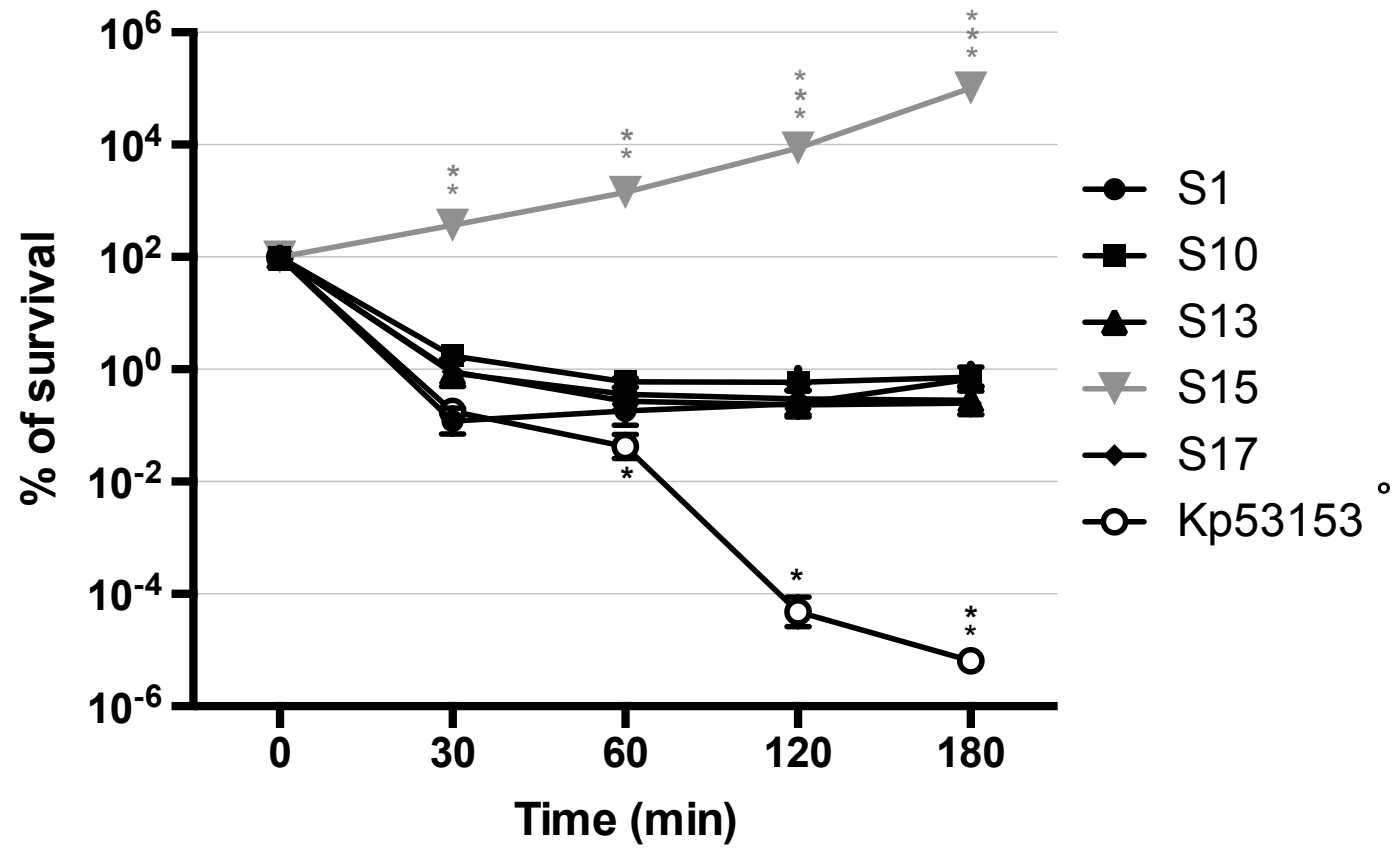


Fig.2

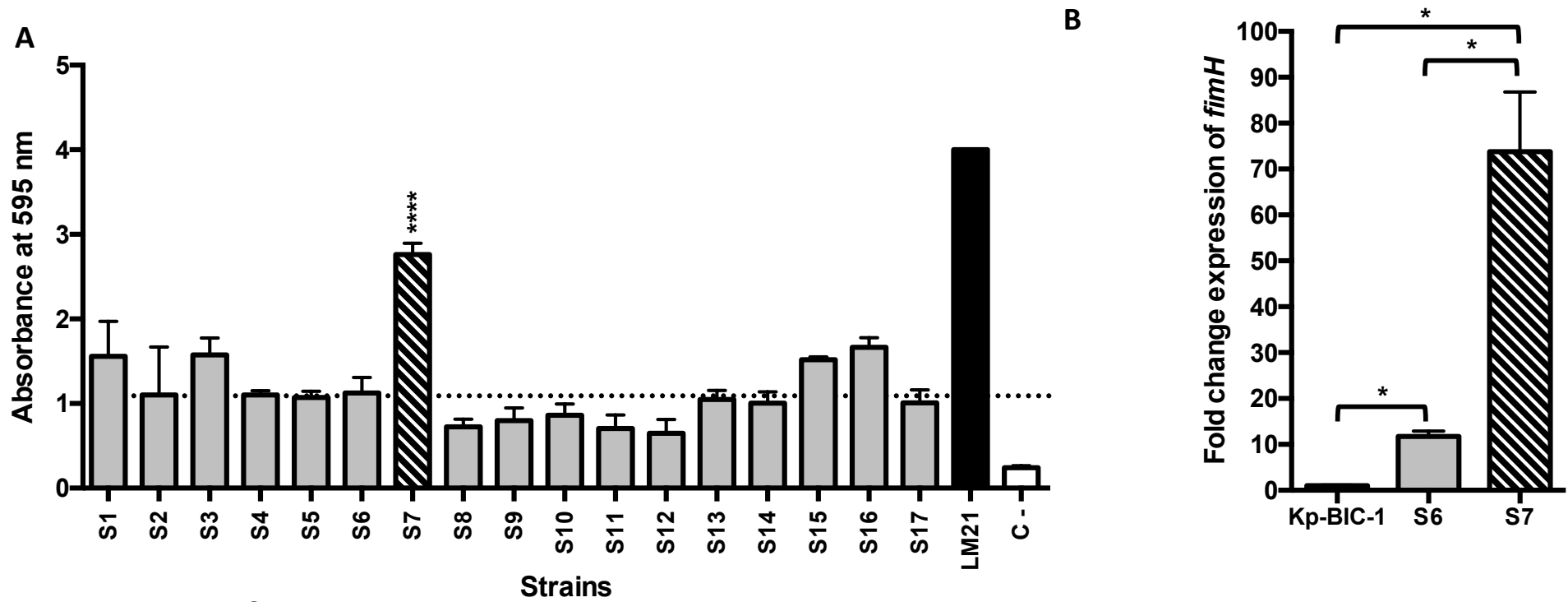


Fig.3