

1 Dissemination routes of the carbapenem resistance plasmid pOXA-48 in a 2 hospital setting

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28 **Introductory paragraph**

29 Infections caused by carbapenemase-producing enterobacteria (CPE) are a major
30 concern in clinical settings worldwide. Two fundamentally different processes shape
31 the epidemiology of CPE in hospitals: the dissemination of CPE clones from patient to
32 patient (between-patient transfer), and the transfer of carbapenemase-encoding
33 plasmids between enterobacteria in the gut microbiota of individual patients (within-
34 patient transfer). The relative contribution of each process to the overall dissemination
35 of carbapenem resistance in hospitals remains poorly understood. Here, we used
36 mechanistic models combining epidemiological data from more than 9,000 patients
37 with whole genome sequence information from 250 enterobacteria clones to
38 characterise the dissemination routes of the carbapenemase-encoding plasmid pOXA-
39 48 in a hospital setting over a two-year period. Our results revealed frequent between-
40 patient transmission of high-risk pOXA-48-carrying clones, mostly of *Klebsiella*
41 *pneumoniae* and sporadically *Escherichia coli*. The results also identified pOXA-48
42 dissemination hotspots within the hospital, such as specific wards and individual rooms
43 within wards. Using high-resolution plasmid sequence analysis, we uncovered the
44 pervasive within-patient transfer of pOXA-48, suggesting that horizontal plasmid
45 transfer occurs in the gut of virtually every colonised patient. The complex and
46 multifaceted epidemiological scenario exposed by this study provides new insights for
47 the development of intervention strategies to control the in-hospital spread of CPE.

48

49 **Introduction**

50 Antibiotic resistance is one of the most concerning health challenges facing modern
51 societies¹. Antibiotic resistance is of particular concern in clinical settings, where
52 resistant pathogens significantly increase the mortality rates of critically ill patients and
53 the costs associated with infection management and control^{1,2}. The spread of antibiotic
54 resistance genes between bacteria commonly associated with nosocomial infections
55 is mainly driven by the horizontal transfer of conjugative plasmids^{3,4}. However, the

56 frequency with which this occurs in the clinical settings and its importance for the
57 dissemination of resistance at a local level remain poorly defined.

58 One of the most clinically relevant groups of nosocomial pathogens are enterobacteria
59 that produce carbapenemases (β -lactamase enzymes able to degrade carbapenem
60 antibiotics). Among carbapenemase-producing enterobacteria (CPE), clones of
61 *Klebsiella pneumoniae* and *Escherichia coli* carrying plasmid-encoded
62 carbapenemases pose the highest clinical threat⁵. Despite their clinical relevance,
63 major gaps remain in our understanding of the epidemiology of CPE and of
64 carbapenemase-encoding plasmids. Previous work has highlighted the importance of
65 in-hospital CPE transmission from patient to patient^{6,7} (between-patient transfer).
66 However, the dissemination and evolution of CPE in hospitals present an additional
67 layer of complexity: the transfer of carbapenemase-encoding plasmids between
68 enterobacteria clones in the gut microbiota of individual patients (within-patient
69 transfer)^{8,9}. Understanding the relative importance of between-patient and within-
70 patient transfer is of central importance for understanding the epidemiology of CPE
71 and informing intervention strategies to control the spread of carbapenem resistance
72 in clinical settings.

73 One of the most frequent carbapenemases in enterobacteria is OXA-48¹⁰. OXA-48 was
74 first described in a *K. pneumoniae* strain isolated in Turkey in 2001¹¹ and is now
75 distributed worldwide, with particularly high prevalence in North Africa, Middle Eastern
76 countries, and Europe¹⁰. The *bla*_{OXA-48} gene is usually encoded in an IncL, broad-host-
77 range conjugative plasmid called pOXA-48⁸ (Figure S1). This plasmid is frequently
78 associated with *K. pneumoniae* high-risk clones¹², such as the sequence types 11
79 (ST11), ST15, ST101, and ST405^{6,13-15}, which are able to readily spread between
80 hospitalized patients producing outbreaks of infections^{16,17}. Previous epidemiological
81 studies strongly suggested the possibility of within-patient pOXA-48 transfer¹⁷⁻²¹,

82 indicating that pOXA-48 would be an ideal study system to investigate the nosocomial
83 dissemination of carbapenem resistance.

84 In the present study, we examined the between-patient and within-patient transfer
85 dynamics of plasmid pOXA-48 in a tertiary hospital over a two-year period. For our
86 analysis, we used a large and well-characterized collection of pOXA-48-carrying
87 enterobacteria generated at the *Hospital Universitario Ramon y Cajal* in Madrid as part
88 of the European project R-GNOSIS (Resistance of Gram-Negative Organisms:
89 Studying Intervention Strategies)^{22,23}. Using statistical models and combining
90 epidemiological data from more than 9,000 patients with whole-genome sequence
91 information from 250 enterobacteria clones, we aimed to define pOXA-48 transfer
92 dynamics at an unprecedented resolution. Specifically, we aimed to determine the
93 relative contribution of between-patient and within-patient plasmid transfer in the
94 epidemiology of pOXA-48, and to use these data to inform improved intervention
95 strategies to control the spread of carbapenem resistance in hospitals.

96

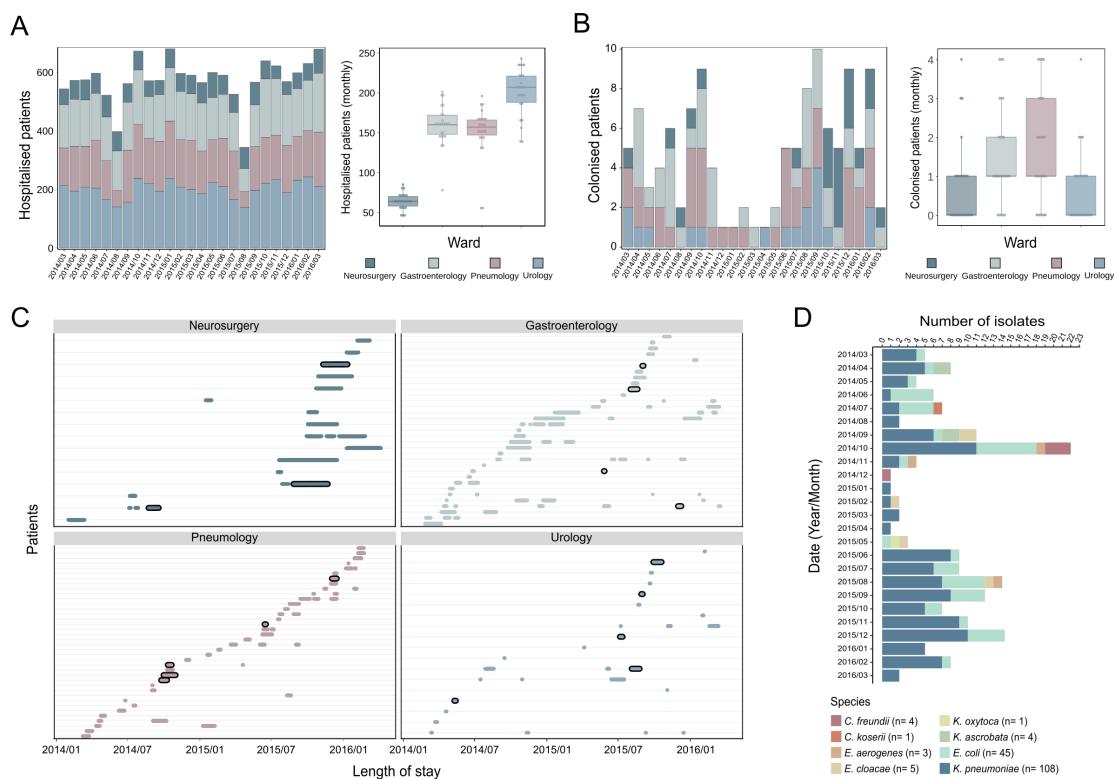
97 **Results**

98 *Patients colonised by pOXA-48-carrying enterobacteria in the hospital*

99 During the R-GNOSIS project, hospitalised patients were periodically sampled to
100 detect the presence of enterobacteria producing extended spectrum β -lactamases
101 (ESBL) and carbapenemases in their gut microbiota (see methods). The study enrolled
102 all patients admitted to two medical wards (gastroenterology and pneumology) and two
103 surgical wards (neurosurgery and urology) in the hospital. The full details of the R-
104 GNOSIS study in our hospital, including the study population and CPE
105 characterization, have been previously reported by Hernandez-Garcia *et al*^{18,23}. Briefly,
106 from March 2014 to March 2016, 28,089 rectal swabs were collected from 9,275
107 patients, and 171 pOXA-48-carrying enterobacteria strains were isolated and
108 characterised from 105 patients (Figure 1, Table S1). The proportion of patients who

109 were found to be colonized with pOXA-48-carrying enterobacteria on at least one
110 occasion during their hospital admission was 0.5% in urology (18/3,483), 1.3% in
111 gastroenterology (33/2,591), and 1.5% both in neurosurgery (16/1,068) and
112 pneumology (38/2,559), with the medical wards accounting for 68% of colonised
113 patients (71/105, Figure 1A-C).

114 In line with previous reports¹⁰, *K. pneumoniae* was the most frequent pOXA-48-
115 carrying species (n= 108). However, pOXA-48 was detected in an additional 7
116 enterobacterial species, with *E. coli* being the second most frequent carrier (n= 45,
117 Figure 1D, Table S1). In several pOXA-48 carrying patients (18/105), there was co-
118 colonisation of the gut microbiota with more than one species carrying the plasmid,
119 suggestive of within-patient plasmid transfer events (Figure 1C).



120
121 Figure 1. Study population, colonised patients, and pOXA-48-carrying enterobacteria.
122 (A) Patients sampled during the R-GNOSIS study. The left panel shows the number of
123 hospitalised patients in each ward over the 25-month study period. The right panel
124 shows the distribution of hospitalised patients per ward by month as a boxplot.

125 Horizontal lines inside boxes indicate median values, the upper and lower hinges
126 correspond to the 25th and 75th percentiles, and whiskers extend to observations
127 within 1.5 times the interquartile range. (B) Patients colonised by a pOXA-48-carrying
128 enterobacteria during the study. The left panel represents the number of colonised
129 patients in each ward over the 25-month study period. The right panel shows the
130 distribution of colonised patients per ward by month as a boxplot. (C) Distribution of
131 patients colonised by pOXA-48-carrying enterobacteria in the four wards under study
132 over the 25-month study period. Each row represents a patient, and the colour
133 segments represent the length of hospital stay (from admission to discharge). Black
134 outlining of colour segments indicates patient co-colonisation with more than one
135 pOXA-48-carrying species. (D) Enterobacteria isolates carrying pOXA-48 recovered
136 from the patients during the 25 months of the study. The species of the pOXA-48-
137 carrying isolates are colour-coded as indicated in the legend.

138 *Using epidemiological data to analyse pOXA-48 transfer dynamics.*

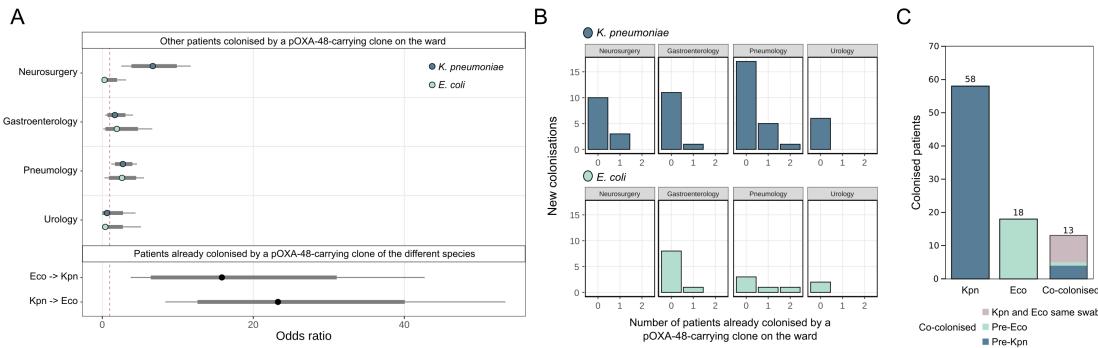
139 To investigate how pOXA-48 spread in the hospital, we analysed the epidemiological
140 data using a previously described model which enabled us to estimate the daily
141 probability of a patient acquiring pOXA-48-carrying enterobacteria and quantify the
142 effect of covariates on this probability (see methods and Supplementary Table 2)²⁴.
143 We performed this analysis independently for the two species with a large number of
144 isolates, *K. pneumoniae* and *E. coli*, and we included two covariates in the model. The
145 first covariate was the number of other patients colonized by pOXA48-carrying
146 enterobacteria in the ward on the same day, which we expect to be positively
147 associated with the daily risk of acquisition if between-patient bacterial transfer is
148 important. The second covariate was known pre-existing intestinal colonisation of the
149 patient by a pOXA48-carrying enterobacteria of a different species (*K. pneumoniae* or
150 *E. coli*). If within-patient plasmid transfer is important (from *K. pneumoniae* to *E. coli*
151 and vice versa, Figure 2), then we would expect this to also be positively associated

152 with the daily risk of a patient acquiring pOXA-48-carrying enterobacteria. We
153 considered different transmission models including and excluding these covariates and
154 performed model comparisons using the widely applicable information criterion (WAIC,
155 Supplementary Table 2). The model that best fitted our data was the one including
156 both covariates and permitting the transmission parameter β to vary by ward (see
157 Supplementary Table 3 for daily probability values and methods for details).

158 The baseline daily probabilities for becoming colonised with pOXA-48-carrying *K.*
159 *pneumoniae* or *E. coli* were 0.1% (95% credible interval [Crl] 0.08%, 0.12%) and 0.04%
160 (95% Crl, 0.02%, 0.05%), respectively (Supplementary Table 3). Results showed that
161 the probability of acquisition of a pOXA-48-carrying *K. pneumoniae* was higher if other
162 patients were already colonised with a pOXA-48-carrying clone in the wards of
163 neurosurgery (Odds ratio [OR] 6.7, 95% Crl 2.5, 11.7) and pneumology (OR 2.7, 95%
164 Crl 1.2, 4.6). In the wards of gastroenterology (OR 1.7, 95% Crl 0.4, 4.1) and urology
165 (OR 0.6, 95% Crl 0.01, 4.4) there were no clear effects. In contrast, the presence of
166 other patients colonised by pOXA-48-carrying clones was not associated with the
167 probability of acquiring a pOXA-48-carrying *E. coli* in the wards of neurosurgery (OR
168 0.23, 95% Crl 0.001, 2.0) or urology (OR 0.4, 95% Crl 0.002, 2.7), and there was only
169 weak evidence for a positive association in the wards of gastroenterology (OR 1.9,
170 95% Crl 0.4, 4.7) and pneumology (OR 2.6, 95% Crl 0.9, 4.5) (Figure 2A). This result
171 suggested that *K. pneumoniae* is more important for between-patient transfer than *E.*
172 *coli*.

173 The model also showed that prior colonisation with a pOXA-48-carrying clone of a
174 different species was associated with a dramatic increase in the probability of acquiring
175 a second pOXA48-carrying species (Figure 2A,C). This risk was high both when a
176 patient was first colonised by *K. pneumoniae* (OR 23.3, 95% Crl 8.3, 53.4) and when
177 initially colonized with *E. coli* (OR 15.8, 95% Crl 3.8, 42.7). This result underlines the
178 potential importance of within-patient plasmid transfer in the dissemination of pOXA-48,

179 a role supported by the high frequency of co-colonised patients (Figure 2C). However,
180 other explanations may be responsible for this observation, such as independent
181 colonisation events of predisposed patients by different pOXA-48-carrying clones.



182

183 Figure 2. Acquisition of pOXA-48-carrying enterobacteria by hospitalised patients. (A)
184 Posterior distribution of odds ratio for the daily risk of colonisation with a pOXA-48-
185 carrying *K. pneumoniae* or *E. coli*. Two covariates were included. The first is the
186 presence of other patients colonised by a pOXA-48-carrying clone on the ward, (upper
187 part, stratified by ward). If between-patient transfer of the plasmid is important, we
188 expect to see a positive association (odds ratio >1) with the daily probability of
189 acquiring a pOXA-48 clone. Second, pre-existing colonisation with a pOXA-48 clone
190 of a different species (lower part). This covariate measures how being previously
191 colonised by a pOXA-48-carrying *E. coli* is associated with the daily probability of
192 becoming colonized with a pOXA-48-carrying *K. pneumoniae* clone (Eco -> Kpn) and
193 vice versa (Kpn -> Eco). We expect to see a positive association if within-patient
194 transfer of pOXA-48 between different bacterial clones is important. Points represent
195 posterior medians; thick grey lines represent the 80% CrI and thinner black lines
196 represent the 95% CrI. (B) Number of previously uncolonised patients becoming
197 colonised by a pOXA-48-carrying *K. pneumoniae* (top row) or *E. coli* (bottom row) as
198 a function of the number of patients on the ward already colonised by a pOXA-48-
199 carrying clone. (C) Number of R-GNOSIS study patients colonised by pOXA-48-
200 carrying *K. pneumoniae* (Kpn) or *E. coli* (Eco) clones or both (co-colonised). For co-
201 colonised patients, the colour code indicates whether *K. pneumoniae* or *E. coli* were

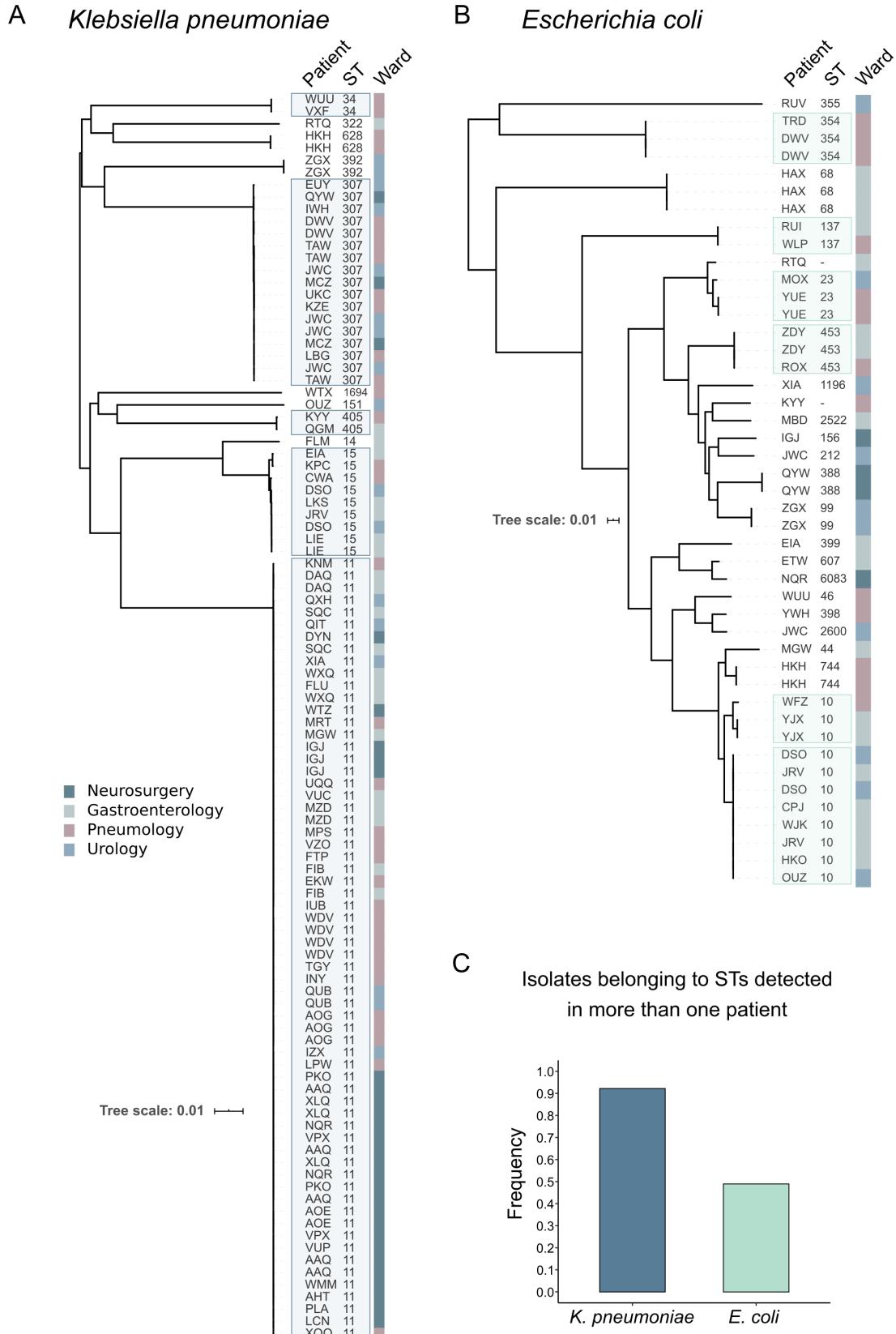
202 isolated first or whether both species were simultaneously isolated from the same
203 swab.

204 *Genomic analysis of pOXA-48-carrying enterobacteria*

205 A key limitation of our epidemiological model is that it is based solely on species
206 identification, which restricts the possibility of reconstructing the spread of specific
207 clones and plasmids. To track within-patient and between-patient plasmid transfer at
208 a higher level of resolution, we integrated genomic information by sequencing the
209 genomes of the 171 pOXA-48-carrying isolates represented in Figure 1D. In line with
210 previous studies^{22,25}, the sequencing results revealed that a small subset of isolates
211 initially identified as *K. pneumoniae* actually belonged to the species *Klebsiella*
212 *quasipneumoniae* (n= 2) and *Klebsiella variicola* (n= 3) (Supplementary Figure 2).

213 We analysed the genetic relatedness of isolates belonging to *K. pneumoniae* and to
214 *E. coli* separately by reconstructing the core genome phylogeny for each species
215 (Figure 3). For *K. pneumoniae* (n= 103), most of the isolates belonged to a few high-
216 risk sequence types: ST11 (n= 64), ST307 (n= 17), and ST15 (n= 9). In contrast, *E.*
217 *coli* (n= 45) showed a more diverse population structure, with only one sequence type,
218 ST10, comprising more than three isolates (n= 11).

219 We next considered the distribution of the different clonal groups (defined by the
220 different STs) across colonised patients (Figure 3A,B). Most *K. pneumoniae* isolates
221 belonged to STs present in more than one patient, whereas approximately half of *E.*
222 *coli* isolates belonged to STs present in only one patient (Figure 3C). This result,
223 together with the results of statistical analysis, suggested that a limited number of *K.*
224 *pneumoniae* high-risk clones are responsible for most of the between-patient transfer
225 events. However, we observed several cases of pOXA-48-carrying *E. coli* STs
226 colonising different patients, suggesting that *E. coli* is also responsible for sporadic
227 between-patient transmission events.



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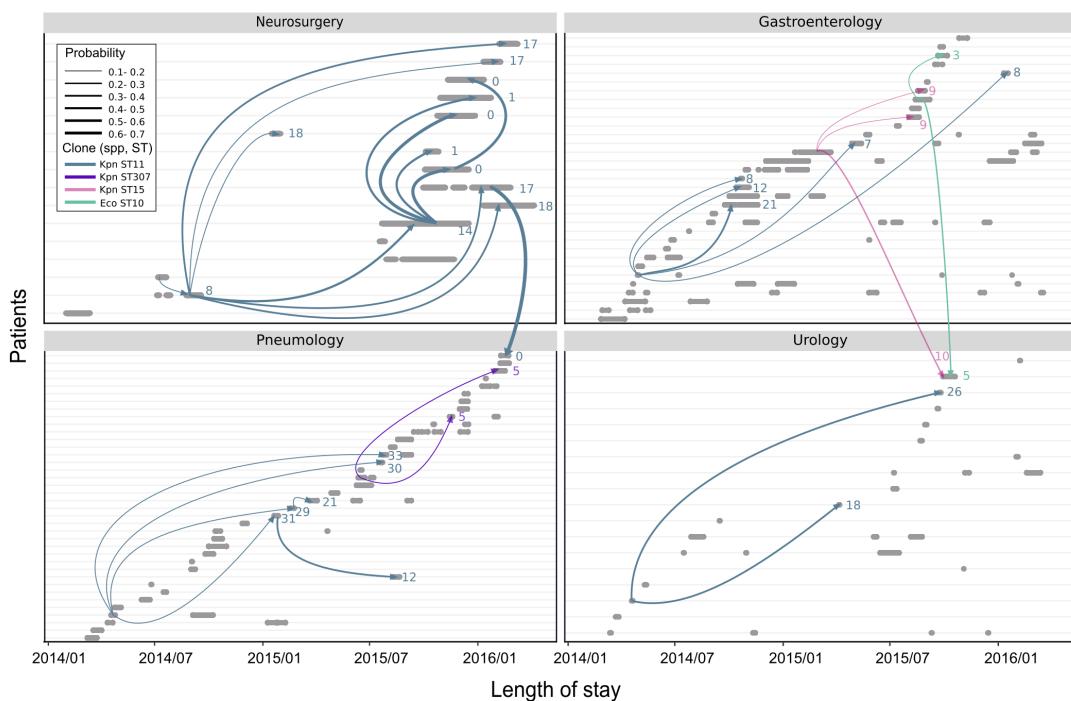
229 Figure 3. Phylogenetic analysis of pOXA-48-carrying *K. pneumoniae* and *E. coli*.
230 Genetic relationships among (A) *K. pneumoniae* (n= 103) and (B) *E. coli* (n= 45)

231 isolates carrying pOXA-48 and recovered during the R-GNOSIS study. Tree
232 construction is based on polymorphisms in the core genome (scale: single nucleotide
233 polymorphism [SNPs]/site). The columns to the right of the tree indicate patient code,
234 isolate sequence type (ST), and the ward where the isolate was recovered (colour
235 code in legend). Boxes with colour shading indicate recovery of isolates of the same
236 sequence type (ST) from multiple patients in the hospital. (C) Frequency of pOXA-48-
237 carrying *K. pneumoniae* and *E. coli* isolates belonging to STs detected in multiple
238 patients.

239 *Reconstruction of between-patient transfer dynamics of pOXA-48-carrying clones.*

240 To investigate the specific dissemination routes of pOXA-48-carrying clones, we
241 integrated epidemiological and genomic data using SCOTTI²⁶ (see methods). SCOTTI
242 is a structured coalescent-based tool for reconstructing bacterial transmission, which
243 accounts for bacterial diversity and evolution within hosts, non-sampled hosts, and
244 multiple infections of the same host. We analysed the spread of the dominant *K.*
245 *pneumoniae* and *E. coli* STs within and among the four wards under study (Figure 4,
246 Supplementary Figures 3-10). As expected from the genomic data (Figure 3A), clones
247 belonging to *K. pneumoniae* ST11 were responsible for most of the putative between-
248 patient transmission events. The analysis attributed transmission events of pOXA-48-
249 carrying ST11 on every single ward and even between wards, with neurosurgery being
250 the ward with the highest frequency and probability of ST11 transmission (Figure 4),
251 as suggested by the epidemiological model (Figure 2A). In light of these results, we
252 investigated *K. pneumoniae* ST11/pOXA-48 epidemiology in the neurosurgery ward in
253 more detail by looking at the spatiotemporal distribution of colonised patients
254 (Supplementary Figure 11). The neurosurgery ward includes 11 rooms with 20 beds
255 (9 double rooms and 2 single rooms). Of the 16 colonized patients, 6 had overlapping
256 stays in the same room, suggesting that this room acted as a hotspot for *K.*
257 *pneumoniae* ST11/pOXA-48 colonisation and transmission.

258 SCOTTI also predicted transmission events mediated by three further pOXA-48-
259 carrying clones. Two transmission events were attributed to *K. pneumoniae* ST307 in
260 the pneumology ward and three more to *K. pneumoniae* ST15: two in gastroenterology
261 and another one between the gastroenterology and urology wards. In line with the
262 genomic results (Figure 3B), SCOTTI also attributed two between-patient transfer
263 events to *E. coli* ST10, one on the gastroenterology ward and another one between
264 the gastroenterology and urology wards (Figure 4).



265

266 Figure 4. SCOTTI reconstruction of between-patient transfer of pOXA-48-carrying
267 enterobacteria. The charts represent SCOTTI-attributed between-patient transfer
268 events involving pOXA-48-carrying enterobacteria clones in the hospital, with
269 individual panels representing the distribution of patients colonized by pOXA-48-
270 carrying enterobacteria on the different wards. Each row represents an individual
271 patient, and the grey segments represent the length of stay (from admission to
272 discharge). Coloured arrows represent transmission events predicted by SCOTTI. Line
273 colour indicates the clone responsible for the transmission event, and line thickness
274 represents the probability of the SCOTTI-attributed transmission, as indicated in the

275 legend: Kpn, *K. pneumonia*; Eco, *E. coli*; ST, sequence type. Numbers to the right of
276 arrowheads indicate the number of SNPs differentiating the complete genomes of the
277 clone pair involved in the putative transmission event.

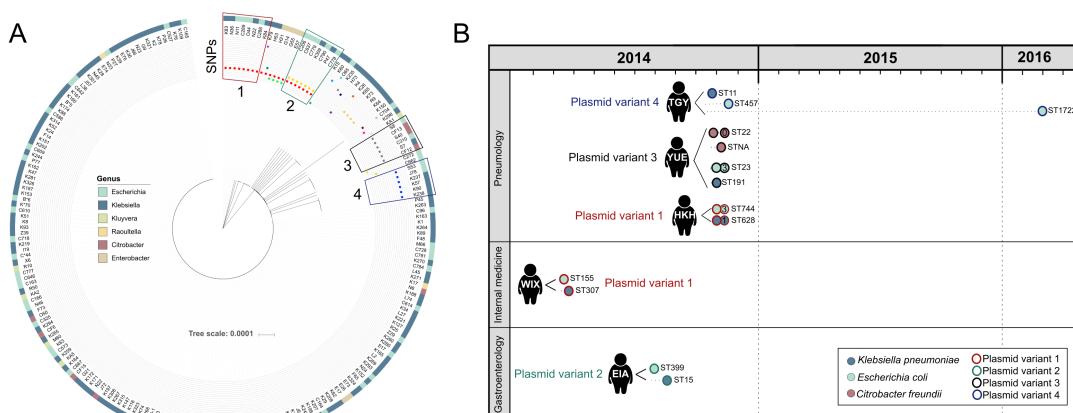
278 *Genetic analysis of pOXA-48 confirms pervasive within-patient plasmid transfer*

279 Our results suggest that the high frequency of patient colonisation by two plasmid-
280 carrying species could be due to within-patient pOXA-48 transfer (Figures 1 and 2).
281 However, although unlikely, another possibility is independent colonisation events
282 involving different plasmid-carrying clones. To distinguish between these possibilities,
283 we analysed the genetic sequence of plasmid pOXA-48 across all isolates with the aim
284 of using specific genetic signatures in the plasmid to provide evidence for or against
285 within-patient plasmid transfer. To increase the resolution of this analysis, we enriched
286 the R-GNOSIS collection by recovering and sequencing the complete genomes of all
287 the pOXA-48-carrying enterobacteria isolated from patients in our hospital since the
288 plasmid was first reported in 2012 (Supplementary Table 1). In total, we determined
289 and analysed the complete genomes of 250 strains, combining short-read and long-
290 read sequencing technologies (see methods and Supplementary Figure 12).

291 The results showed that pOXA-48 is highly conserved (Figure 5A). The core plasmid
292 sequence spanned more than 60 kb (>90% of plasmid sequence) in 218 of the 250
293 strains (Supplementary Table 1). Analysis of the core genome among these 218
294 plasmids revealed an identical sequence in 80% of them. In the remaining 20%, we
295 detected a total 21 SNPs, with each plasmid presenting 1 or 2 SNPs compared with
296 the most common variant (Figure 5A). This high degree of plasmid structural and
297 sequence conservation and the strong link between pOXA-48 and the *bla_{OXA-48}* gene
298 are important differences from previous analyses on the spread of plasmid-mediated
299 carbapenemases such as KPC^{8,9,27}.

300 Given the low plasmid-sequence variability, we could not track plasmid transmission
301 using the same tools used for bacterial transmission. Instead, we monitored plasmid

302 transfer by using the rare plasmid variants carrying specific core-region SNPs as
303 genetic fingerprints (Figure 5). We focused on instances where the same traceable
304 plasmid variant was present in different bacterial clones (belonging to different
305 species). We considered that isolation of different bacterial species carrying the same
306 rare plasmid variant from the same patient would be a very strong indicator of within-
307 patient plasmid transfer. We found four examples in which the same rare plasmid
308 variant was present in different bacterial species (Figure 5A). In all four, different
309 species carrying the same plasmid variant were isolated from the same patient (Figure
310 5B). For example, plasmid variant 3 was detected in 6 bacterial isolates belonging to
311 four clones (one *K. pneumoniae*, one *E. coli* and two *C. freundii*), and all of them were
312 recovered from a single patient in the hospital (patient code YUE). Crucially, the
313 chances of independent patient colonisation with the different bacterial clones carrying
314 these rare plasmid variants are extremely low (variant 1, 6.4×10^{-4} ; variant 2, 8.9×10^{-4} ;
315 variant 3, 1.1×10^{-8} ; variant 4, 2.1×10^{-5}), confirming that these were within-patient
316 plasmid transfer events.



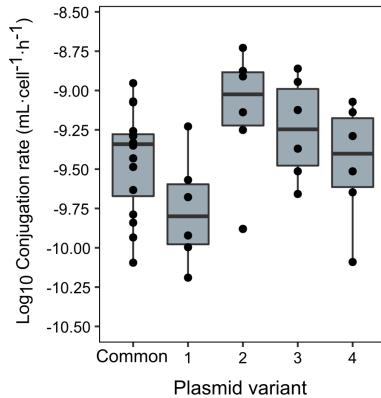
317
318 Figure 5. Pervasive within-patient pOXA-48 transfer. (A) Dendrogram constructed from
319 the 21 polymorphisms present in the core region of plasmid pOXA-48. The outermost
320 circle indicates plasmid-carrying clones genus according to the colour code in the
321 legend, the second circle indicates the clone names, and the remaining circles indicate
322 the presence of each plasmid SNP. Coloured boxes indicate the four plasmid variants

323 carrying a 'rare' SNP present in clones of different species and used as genetic
324 fingerprints. (B) Representation of patients colonized by clones carrying rare
325 (traceable) plasmid variants. Patients are represented with their corresponding three-
326 letter patient code. Circles represent clones isolated from the patient, with the fill colour
327 indicating the bacterial species and the outline colour indicating the plasmid variant
328 (see legend). The sequence type (ST) of each clone is indicated. Circles in the same
329 row indicate different isolates of the same clone; the number inside the second circle
330 indicates the number of SNPs accumulated in the complete genome relative to the first
331 isolation.

332 *High in vitro pOXA-48 conjugation rate*

333 Despite the limitations imposed by the sensitivity and frequency of the sampling
334 method, the four selected pOXA-48 variants with core-region SNPs demonstrated
335 pervasive within-patient plasmid transfer. However, the specific SNPs used as genetic
336 fingerprints might affect the conjugation ability of the plasmid, which would make it
337 impossible to generalize the results with these variants to the most common pOXA-48
338 variant. To exclude this possibility, we experimentally tested the conjugation rates of
339 the most common pOXA-48 variant and the four traceable variants by introducing the
340 plasmids independently into the *E. coli* strain J53 and determining the conjugation rate
341 of each variant in this isogenic background (Figure 6, see methods). Although
342 conjugation rates differed slightly (ANOVA; $F= 2.9$, $df= 4$, $P= 0.037$), they did not differ
343 significantly between the traceable SNP plasmid variants and the most frequent variant
344 (Tukey multiple comparisons of means, $P > 0.3$), indicating that all these plasmid
345 variants have similar within-patient transfer ability. We therefore conclude that
346 horizontal spread of pOXA-48 in the gut microbiota is the norm in colonized patients.
347 Interestingly, and as previously reported²⁸, the *in vitro* pOXA-48 conjugation rate was
348 extremely high, with a median frequency of 0.3 transconjugants per donor after only

349 one hour of mating (Supplementary Figure 13). The high pOXA-48 conjugation rate
350 helps to explain the frequent within-patient plasmid transfer reported here.



351

352 Figure 6. pOXA-48 conjugation rate. Conjugation rates of the most common pOXA-48
353 variant in the hospital (common, 12 biological replicates) and the four core-region SNP
354 variants used to track within-patient plasmid transfer (6 biological replicates). Plasmid
355 variant numbers correspond to those indicated in Figure 5. Horizontal lines inside
356 boxes indicate median values, the upper and lower hinges correspond to the 25th and
357 75th percentiles, and whiskers extend to observations within 1.5 times the interquartile
358 range.

359

360 **Discussion**

361 CPE are emerging as one of the most concerning threats to public health worldwide⁵.
362 Recent studies have highlighted the central relevance of hospitals as hotspots for the
363 dissemination of CPE among patients and for the dissemination of the
364 carbapenemase-encoding conjugative plasmids between enterobacteria clones⁶⁻⁸. In
365 this study, we performed a high-resolution epidemiological analysis to uncover the
366 dissemination routes of the carbapenemase-encoding plasmid pOXA-48 (both at the
367 bacterial and plasmid levels). By integrating epidemiological and genomic data, we
368 uncovered frequent between-patient bacterial transfer and pervasive within-patient
369 plasmid transfer.

370 In light of our results, we propose that in-hospital pOXA-48 dissemination generally
371 adheres to the following dynamics: high-risk pOXA-48-carrying enterobacteria clones,
372 mainly *K. pneumoniae* ST11, spread among hospitalised patients, colonising their gut
373 microbiota (Figures 2, 3 and 4). Once patients are colonised, the plasmid readily
374 spreads through conjugation towards other resident members of the gut microbiota
375 (enterobacteria such as *E. coli*, *C. freundii*, and *E. cloacae*, Figures 1 and 5). The
376 plasmid's high conjugation rate increases its chances of becoming established in the
377 gut microbiota because, even if the invading nosocomial clone is eliminated, pOXA-48
378 can survive in a different bacterial host. Moreover, the frequent plasmid transfer
379 provides a test bench for new bacterium-plasmid combinations, some of which may be
380 particularly successful associations able to persist and even disseminate towards new
381 human hosts⁴. An illustrative example of these general dynamics is the case of the
382 patient carrying plasmid variant 4 (Figure 5B; patient code TGY). This patient was first
383 colonised by *K. pneumoniae* ST11/pOXA-48 in October 2014, and 11 days later a
384 pOXA-48-carrying *E. coli* strain was isolated from the same patient (ST457). During a
385 new admission 17 months later, a different pOXA-48-carrying *E. coli* (ST1722) was
386 recovered the patient's gut microbiota. The pOXA-48 variant in all the clones carries a
387 traceable SNP, confirming that the patient was colonized throughout the period by a
388 pOXA-48-carrying enterobacteria, even though the plasmid had moved from its original
389 *K. pneumoniae* ST11 host to *E. coli* clones in the gut microbiota.
390 Another interesting observation emerging from this study is that most of the events
391 attributed to between-patient transmission originated from a small subset of patients
392 (Figure 4). This result highlights the potential role of super-spreader patients in the
393 nosocomial dissemination of CPE²⁹. Unfortunately, given the small number of super-
394 spreader patients, we were not able to associate them to any particular epidemiological
395 aspect, such as age or length of stay.

396 An important goal of this study is to inform new and improved intervention strategies
397 aimed at controlling the spread of carbapenem resistance in hospitals. Our results can
398 help in the design of interventions to control OXA-48 dissemination at two levels:
399 (i) *Between-patient*. We have shown that the spread of pOXA-48-carrying
400 enterobacteria between patients in the hospital is mainly mediated by high-risk clones
401 commonly associated with nosocomial infections. These clones reside in hospital
402 settings and are able to survive in the environment, creating stable reservoirs (often
403 involving sinks³⁰⁻³²). Moreover, our results also showed that there are specific
404 colonisation and transmission hotspots, such as individual rooms within wards
405 (Supplementary Figure 11). We therefore propose that measures to detect and control
406 environmental reservoirs and transfer hotspots could prevent between-patient OXA-
407 48 dissemination. Such measures could represent a useful addition to the strategies
408 based on patient surveillance and standard precautions already applied in hospitals,
409 and could complement and improve the outcome of contact isolation measures²².
410 (ii) *Within-patient*. A key finding of our study is the high prevalence of within-patient
411 pOXA-48 transfer, which in turn helps to establish long-term pOXA-48 gut carriers.
412 Preventing within-patient plasmid transfer and gut carriage is thus a particularly
413 promising strategy for containing carbapenem resistance. This goal could be achieved
414 either by blocking plasmid conjugation³³ or, ideally, by specifically clearing pOXA-48
415 from the gut microbiota of patients by targeted decontamination. Decontamination
416 strategies would aim to remove pOXA-48 plasmid or pOXA-48-carrying enterobacteria
417 from carriers while preserving the integrity of the gut microbiota. New biotechnological
418 advances are being made on this front. For example, CRISPR (clustered regularly
419 interspaced short palindromic repeats) based technology can be used for targeted
420 plasmid elimination³⁴, and the new toxin–intein antimicrobials could be engineered to
421 selectively remove pOXA-48-carrying clones from the microbiota³⁵. Further work is

422 urgently needed to tailor these emerging technologies into effective intervention
423 strategies against the threat of plasmid-mediated carbapenemases.

424

425 **Methods**

426 *Study design and data collection*

427 We studied samples collected from patients admitted in a Spanish university hospital
428 from March 4th, 2014, to March 31st, 2016, as part of an active surveillance-screening
429 program for detecting ESBL/carbapenemase-carriers (R-GNOSIS-FP7-HEALTH-F3-
430 2011-282512, www.r-gnosis.eu/)^{18,22,23,36}. This study was approved by the Ramón y
431 Cajal University Hospital Ethics Committee (Reference 251/13), which waived the
432 need for informed consent from patients on the basis that the study was assessing
433 ward-level effects and it was of minimal risk. This screening included a total of 28,089
434 samples from 9,275 patients admitted at 4 different wards (gastroenterology,
435 neurosurgery, pneumology and urology) in the Ramon y Cajal University Hospital
436 (Madrid, Spain). We used a randomly generated three-letters code for patient
437 anonymization. Rectal samples were obtained from patients within 72 h of ward
438 admission; weekly additional samples were recovered in patients hospitalised ≥ 7 days,
439 and a final sample at discharge was obtained in those patients with a hospital stay ≥ 3
440 days (swabbing interval: gastroenterology, median 2 days, IQR 1, 6 days; neurosurgery,
441 median 3 days, IQR 1, 7 days; pneumology, median 2 days, IQR 1, 6 days; urology,
442 median 1 day, IQR 1, 3 days). This protocol allowed us to obtain a time sequence for
443 each patient in the hospital.

444 In this paper we have focused on the subset of patients colonised by pOXA-48-carrying
445 enterobacteria within the R-GNOSIS project. Prevalence of colonisation by OXA-48-
446 carrying enterobacteria among patients from 2014 to 2016 was 1.13% (105/9,275
447 patients). pOXA-48-carrying enterobacteria were the most frequent CPE in the hospital
448 in this period, with 171 positive isolates (Supplementary Table 1). To better

449 characterise pOXA-48 diversity and dissemination, we included in the within-patient
450 pOXA-48 transfer analysis all the pOXA-48-carrying enterobacteria isolated from
451 patients in our hospital since it was first reported in 2012. Specifically, we included 79
452 additional pOXA-48 carrying enterobacteria not included in the R-GNOSIS project
453 (Supplementary Table 1).

454 *Bacterial characterisation*

455 Samples were initially characterised as previously described, following the RGNOSIS
456 protocol²³. Briefly, swabs were plated on Chromo ID-ESBL and Chrom-CARB/OXA-48
457 selective agar media (BioMérieux, France) and bacterial colonies able to grow on these
458 media were identified by MALDI-TOF MS (Bruker Daltonics, Germany). OXA-48
459 production was confirmed with KPC/MBL/OXA-48 Confirm Kit test (Rosco Diagnostica,
460 Denmark). The MicroScan automated system (Beckman Coulter, CA, USA) was used
461 for the antimicrobial susceptibility testing and the results were interpreted according to
462 EUCAST guidelines (EUCAST breakpoint v7.1, www.eucast.org). Furthermore,
463 specific *bla*_{OXA-48} resistance gene was identified by multiplex PCR³⁷, and the PCR
464 products were sequenced and compared with the GenBank database.

465 *Bacterial culture, DNA extraction, Illumina sequencing and PacBio sequencing*

466 All pOXA-48-carrying enterobacteria isolates (n=250) were grown in Lysogeny broth
467 (LB) medium at 37°C. Genomic DNA of all the strains was isolated using the Wizard
468 genomic DNA purification kit (Promega, Madison, WI, USA), following manufacturer's
469 instructions. Whole genome sequencing was conducted at the Wellcome Trust Centre
470 for Human Genetics (Oxford, UK), using the Illumina HiSeq4000 platform with 125
471 base pair (bp) paired-end reads. Furthermore, 2 isolates and 5 specific pOXA-48
472 plasmids (*K. pneumoniae* isolates k8 and k165, and plasmids from *K. pneumoniae*
473 isolates k2, k164, k187, k236-1 and k273) were sequenced using the PacBio platform
474 (The Norwegian Sequencing Centre; PacBio RSII platform using P5-C3 chemistry).

475 *Assembling and quality control (QC) analysis of sequence data*

476 Trimmomatic v0.33³⁸ was used to trim the Illumina sequence reads. SPAdes v3.9.0³⁹
477 was used to generate *de novo* assemblies from the trimmed sequence reads with the
478 –cov-cutoff flag set to 'auto'. QUAST v4.6.0⁴⁰ was used to generate assembly statistics.
479 All the *de novo* assemblies reached enough quality including total size of 5–7Mb, the
480 total number of contigs over 1 kb was lower than 200 and more than 90% of the
481 assembly comprised contigs greater than 1 kb. Prokka v1.5⁴¹ was used to annotate
482 the *de novo* assemblies with predicted genes.

483 *Phylogenetic analysis and identification of STs and clustering*

484 Mash v2.0⁴² was used to determine distances between genomes using the raw
485 sequence reads, and a phylogeny was constructed with mashtree v0.33⁴³. For the
486 analysis of the core genome (the set of homologous nucleotides present in all the
487 isolates when mapped against the same reference) and the core sequence of the
488 pOXA-48 plasmid, an alignment of the single nucleotide polymorphisms (SNPs)
489 obtained with Snippy v2.5 (<https://github.com/tseemann/snippy>) was used to infer a
490 phylogeny. A maximum-likelihood tree was generated using IQ-TREE with the feature
491 of automated detection of the best evolutionary model⁴⁴. All trees were visualised using
492 the iTOL tool⁴⁵. Recombination regions were identified with Gubbins⁴⁶.
493 The seven-gene ST of all the isolates was determined using the multilocus sequence
494 typing (MLST) tool (<https://github.com/tseemann/mlst>).

495 *Transmission mathematical modelling*

496 Our statistical model was designed based on the premises established by Crenen T, et
497 al²⁴. The objective of our model is to estimate the daily probability of acquisition of a
498 new pOXA-48-carrying enterobacteria by a patient in the hospital. Acquisition can
499 occur through pOXA-48-carrying bacteria acquisition (between-patient transfer), or
500 through pOXA-48 conjugation in the gut microbiota of the patient toward a new
501 enterobacteria host (within-patient transfer).

502 We tracked all the pOXA-48-carrying enterobacteria identified in the hospital during
503 the R-GNOSIS study period (Figure 1). This allows us to estimate and compare the
504 acquisition of the most prevalent species, *K. pneumoniae* and *E. coli* independently.
505 Each day in the ward, a patient can become colonised by a new pOXA-48-carrying *K.*
506 *pneumoniae* or *E. coli*. However, as we lacked swabbing results from each day, the
507 timing of new colonisation events with a pOXA-48-carrying clone are interval censored,
508 and the analysis needs to account for this interval censoring²⁴. If the probability of
509 becoming colonised on day i for patient j is p_{ij} , the probability of remaining uncolonized
510 is $(1-p_{ij})$. Therefore, in interval k for patient j consisting of N_{kj} days, the probability of
511 remaining uncolonized is:

$$512 \prod_{i=1}^{N_{kj}} (1 - p_{ij})$$

513 And the probability of becoming colonised (v_{kj}) is the complement:

$$514 v_{kj} = 1 - \prod_{i=1}^{N_{kj}} (1 - p_{ij})$$

515 The outcome for patient j in interval k (X_{kj}), is either that the patient acquired a new
516 pOXA-48-carrying enterobacteria ($X_{kj} = 1$) or did not ($X_{kj} = 0$). The likelihood is given by:

$$517 X_{kj} \sim Bernoulli(v_{kj})$$

518 The daily probability of becoming colonised (p_{ij}) is related by the logit link function to a
519 linear function of covariates (π_{ij}):

$$520 \pi_{ij} = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 \dots$$

$$521 p_{ij} = \exp(\pi_{ij}) / (\exp(\pi_{ij}) + 1)$$

522 Where x represents a vector of predictors (data) and β is a vector of slopes
523 (parameters) that are to be estimated. The β coefficient can be a single parameter, or
524 permitted to vary by ward. The range of values and the prior distributions of the different
525 parameters are described in Supplementary Table 2.

526 We developed and fitted models to study the probability of acquisition of pOXA-48-
527 carrying *K. pneumoniae* and, separately, *E. coli*. We included the probability of *K.*
528 *pneumoniae* and *E. coli* transferring the plasmid towards each other in the gut
529 microbiota of colonised patients. To that end, we introduced as covariates the number
530 of other patients already colonised by a pOXA-48-carrying enterobacteria each day, to
531 study the risk of between-patient transfer (β coefficient), and if a patient was previously
532 colonised with pOXA-48-carrying *E. coli* or *K. pneumoniae*, to study within patient
533 pOXA-48-transmission (γ coefficient).

534 We considered five different transmission models to assess transmission of pOXA-48-
535 carrying *K. pneumoniae* and *E. coli*:

536 1) Where the daily risk of acquiring pOXA-48-carrying *K. pneumoniae* and *E. coli*
537 is constant (intercept only).

538 2) A constant term plus a between-patient transmission parameter β , where the
539 explanatory variable (n_i) is the number of patients colonised by pOXA-48
540 enterobacteria in the four wards.

541 3) As (2) but permitting the transmission parameter β to vary by ward (β_w) and
542 considering the number of patients colonised by a pOXA-48 enterobacteria in each
543 ward (n_{wi}).

544 4) As (2) but including a γ parameter for the within-patient transmission, and an
545 explanatory variable (x_i), which indicates if a patient had been previously colonised by
546 a pOXA-48-carrying enterobacteria from a different species (yes, $x_i = 1$; no, $x_i = 0$).

547 5) As model (4) but permitting the transmission parameter β to vary by ward (β_w)
548 and considering the number of patients colonised by a pOXA-48 enterobacteria in each
549 ward (n_{wi}).

550 The probability of colonisation for individual j on day i for the respective models is
551 calculated from:

552 Transmission Model 1: $\text{logit}(p_{ij}) = \alpha$

553 Transmission Model 2: $\text{logit}(p_{ij}) = \alpha + \beta n_i$

554 Transmission Model 3: $\text{logit}(p_{ij}) = \alpha + \beta_w n_{wi}$

555 Transmission Model 4: $\text{logit}(p_{ij}) = \alpha + \beta n_i + \gamma x_i$

556 Transmission Model 5: $\text{logit}(p_{ij}) = \alpha + \beta_w n_{wi} + \gamma x_i$

557 We fitted the statistical models using Hamiltonian Markov chain Monte Carlo in Stan
558 (version 2.17.3) within the R environment (v. 3.4.3). Prior distributions were normal
559 distributions using weakly informative priors²⁴. Model comparison was performed with
560 widely applicable information criterion (WAIC, Supplementary Table 2). The model that
561 best fits our data is model number 5. We use 95% credible intervals (CrIs) to
562 summarise uncertainty in posterior distributions. Daily probabilities calculated with
563 model 5 are presented in Supplementary Table 3.

564 *Identification of transmission routes among patients*

565 We applied SCOTTI²⁶, a structured coalescent-based tool for reconstructing
566 transmission, to the dominant *K. pneumoniae* and *E. coli* STs (with more than four
567 isolates: *K. pneumoniae* ST11, ST15, ST307 and *E. coli* ST10), combining
568 epidemiological and genomic data. We used the genome alignment, avoiding the
569 recombination regions after the gubbins analysis⁴⁶, as input to SCOTTI, together with
570 the first date when an isolate was detected in a patient, and the start and end date of
571 each patient's infection risk period (Supplementary Table 1). Due to the possibility of
572 transmission events between wards, we established a hierarchical analysis. First, we
573 applied SCOTTI to the patients/genomes included in each ward to identify
574 transmission routes within each ward, and second, we analysed the data of the 4 wards
575 combined to identify additional transmission events between wards (Supplementary
576 Figures 3-10).

577 *Identification of within-patient transmission routes of specific plasmid variants*

578 In order to confirm within-patient plasmid transfer we studied specific pOXA-48 variants
579 across the different isolates. The sequences belonging to pOXA-48 plasmid were

580 mapped using the complete sequence of one of the plasmid sequenced by PacBio as
581 reference (from *K. pneumoniae* k8), and the different variants and SNPs were identified
582 using Snippy v2.5 (<https://github.com/tseemann/snippy>). We first analysed the degree
583 of genetic variation in the plasmid among all the 250 bacterial clones. We compared
584 the pOXA-48 variants sharing a core region of at least 60 kb (>90 % of the whole
585 sequence, n= 218, Supplementary Table 1). We investigated cases where a variant of
586 the plasmid carrying a “rare” traceable SNP is present in different clones (from different
587 species). We found four plasmid variants present in different bacterial species and, in
588 all cases, different species carrying the same plasmid variant were isolated from the
589 same patient (Figure 5B). For those patients, we estimated the probability of those
590 strains being acquired by independent subsequent transmissions events, assuming a
591 random distribution of plasmid-carrying strains across patients. Analyses were
592 performed using R (Version 3.4.2) (www.R-project.org).

593 *Conjugation assays*

594 An initial conjugation round was performed to introduce pOXA-48 plasmids variants
595 into *E. coli* J53⁴⁷ (a sodium azide resistant laboratory mutant of *E. coli* K-12). pOXA-
596 48-carrying wild type strains (donors) and *E. coli* J53 (recipient) were streaked from
597 freezer stocks onto solid LB agar medium with selective pressure (ertapenem 0.5
598 µg/ml and sodium azide 100 µg/ml, respectively) and incubated overnight at 37°C.
599 Three donor colonies and one recipient colony were independently inoculated in 2 ml
600 of LB in 15-ml culture tubes and incubated for 1.5 h at 37°C and 225 rpm. After growth,
601 donor and recipient cultures were collected by centrifugation (15 min, 1500 g) and cells
602 were re-suspended in each tube with 300 µl of sterile NaCl 0.9%. Then, the
603 suspensions were mixed in a 1:1 proportion, spotted onto solid LB medium and
604 incubated at 37°C for 1.5 hours. Transconjugants were selected by streaking the
605 conjugation mix on LB with ertapenem (0.5 µg/ml) and sodium azide (100 µg/ml). The
606 transconjugants were verified by *bla*_{OXA-48} gene amplification by PCR as previously

607 described¹¹. For the isogenic conjugation experiments, the five different *E. coli* J53
608 carrying pOXA-48 plasmid variants acted as independent donors, and a
609 chloramphenicol resistant version of J53 developed in our lab was used as the
610 recipient strain. 6 colonies of each donor and recipient strains were independently
611 inoculated in 2 ml of LB in 15-ml culture tubes and incubated overnight at 37 °C and
612 225 rpm. Each culture was used next day to inoculate 5 ml of LB in 50-ml culture tubes
613 (1:100 dilution). After 1 hour of incubation at 37°C and 225 r.p.m, the pellets were
614 collected by centrifugation (15 min, 1500 g) and cells were re-suspended in each tube
615 with 300 µl of sterile NaCl 0.9%. Donor and recipient suspensions were mixed in a 1:1
616 proportion and plated on a sterile nitrocellulose filter (0.45 µm) on LB agar medium and
617 incubated at 37°C for 1 hour. Simultaneously, each culture was plated on selecting
618 agar for donors, recipient and transconjugants as controls (carbenicillin 100 µg/ml,
619 chloramphenicol 50 µg/ml and a combination of both respectively). After 1 hour of
620 incubation at 37°C, the filter contents were re-suspended in 2 ml of sterile NaCl 0.9%,
621 diluted and plated on selective agar for donors, recipient and transconjugants.
622 Transconjugants were verified by PCR as described above. Conjugation rates were
623 determined using the end-point method^{48,49} (Figure 6), and the frequencies of
624 transconjugants per donor were calculated from the same data (Supplementary Figure
625 13).

626

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628

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776 **Acknowledgements**

777 This work was supported by the European Research Council under the European
778 Union's Horizon 2020 research and innovation programme (ERC grant agreement no.
779 757440-PLASREVOLUTION) and by the *Instituto de Salud Carlos III* (co-funded by
780 European Development Regional Fund "a way to achieve Europe") grants PI16-00860
781 and CP15-00012. The R-GNOSIS project received financial support from European
782 Commission (grant R-GNOSIS-FP7-HEALTH-F3-2011-282512). RC acknowledges
783 financial support from European Commission (R-GNOSIS) and *Plan Nacional de*
784 *I+D+i2013–2016* and *Instituto de Salud Carlos III, Subdirección General de Redes y*
785 *Centros de Investigación Cooperativa, Ministerio de Economía, Industria y*
786 *Competitividad, Spanish Network for Research in Infectious Diseases*
787 (REIPIRD16/0016/0011) co-financed by European Development Regional Fund "A
788 way to achieve Europe" (ERDF), Operative program Intelligent Growth 2014–2020.
789 BSC and TC acknowledge support from the UK Medical Research Council and
790 Department for International Development [grant number MR/K006924/1], and BSC
791 and PM acknowledge support under the framework of the JPIAMR - Joint
792 Programming Initiative on Antimicrobial Resistance. ASM is supported by a Miguel
793 Servet Fellowship (MS15-00012). JRB is a recipient of a Juan de la Cierva-
794 Incorporación Fellowship (IJC2018-035146-I) co-funded by *Agencia Estatal de*
795 *Investigación del Ministerio de Ciencia e Innovación*. MH-G was supported with a
796 contract from *Instituto de Salud Carlos III*, Spain (iP-FIS program, ref. IFI14/00022).
797 We thank the Oxford Genomics Centre at the Wellcome Centre for Human Genetics
798 (funded by Wellcome Trust grant reference 203141/Z/16/Z) for the generation and
799 initial processing of the sequencing data.

800 **Author contributions**

801 ASM, RLS and BC conceived the study. RC designed and supervised sampling and
802 collection of bacterial isolates. MHG, PRG collected the bacterial isolates and

803 performed bacterial characterization. CDA and NLF collected the epidemiological data
804 and performed preliminary analyses. R-GNOSIS WP5 Study Group designed sampling
805 protocols and facilitated the training and capacity building for the collection of bacterial
806 isolates and preliminary analyses. JdLF, JRB and CdIV performed the experimental
807 work and analysed the results. RLS, BC, PM and TC performed the data analysis.
808 ASM and RLS wrote the initial draft of the manuscript. ASM, RLS, JdLF, JRB, BC, PM,
809 and TC contributed to the final version of the manuscript. All authors read and
810 approved the manuscript.

811 **Competing interests**

812 Authors declare no competing interests.

813 **Data availability**

814 The sequences generated and analysed during the current study are available in the
815 Sequence Read Archive (SRA) repository, BioProject ID: PRJNA626430,
816 <http://www.ncbi.nlm.nih.gov/bioproject/626430>.

817 **Code availability**

818 The code generated during the current study is available in GitHub,
819 http://www.github.com/leonsampedro/transmission_stan_code.

820 **Supplementary tables**

821 Supplementary Table 2. Transmission models to study the daily probability of
822 acquisition of pOXA-48-carrying enterobacteria by hospitalised patients.

Model	Parameters	Priors	<i>K. pneumoniae</i>		<i>E. coli</i>	
			Posterior Median (95% CrI) ¹	WAIC ²	Posterior Median (95% CrI) ¹	WAIC ²
1	α (intercept)	Normal (0, 10)	-6.787 (-6.9797, -6.6019)	1411	-7.633 (-7.9330, -7.3640)	705
2	α (intercept) β (between-patient transfer)	Normal (0, 10) Normal (0, 10)	-6.897 (-7.1103, -6.6990) 1.0611 (0.4981, 1.4857)	1402	-7.716 (-8.0609, -7.4121) 0.8333 (-0.7172, 1.5460)	704
3	α (intercept) β (between-patient transfer) M (beta mean) Σ (beta standard deviation)	Normal (0, 10) Normal (μ , σ) Normal (0, 10) Normal (0, 5)	-6.893 (-7.1098, -6.7027) Varies by ward 0.6697 (-2.3853, 2.6142) 1.5789 (0.4050, 6.3881)	1397	-7.699 (-8.0255, -7.4054) Varies by ward 0.0665 (-4.5453, 2.3773) 1.4629 (0.2166, 7.6397)	706
4	α (intercept) β (between-patient transfer) γ (within-patient transfer)	Normal (0, 10) Normal (0, 10) Normal (0, 10)	-6.919 (-7.1521, -6.7088) 1.0724 (0.3978, 1.4903) 2.7390 (1.2877, 3.7049)	1394	-7.827 (-8.1749, -7.5305) 0.6875 (-0.9421, 1.4782) 3.034 (2.0509, 3.8361)	682
5	α (intercept) β (between-patient transfer) M (beta mean) Σ (beta standard deviation) γ (within-patient transfer)	Normal (0, 10) Normal (μ , σ) Normal (0, 10) Normal (0, 5) Normal (0, 10)	-6.924 (-7.1401, -6.7253) Varies by ward 0.7198 (-3.0451, 2.9985) 1.5960 (0.3937, 6.7936) 2.7360 (1.2051, 3.7623)	1388	-7.836 (-8.1921, -7.5285) Varies by ward -0.2390 (-5.9522, 2.3138) 1.9315 (0.3361, 8.3896) 3.124 (2.1074, 3.9739)	682

823

824 The table shows the parameters, prior and posterior distributions along with the WAIC
825 (model comparison measure where lower values indicate a better fit to data). See
826 methods for equations. Normal prior distributions show the mean and standard
827 deviation respectively within brackets. ¹ 95% Credible interval. ² Widely applicable
828 information criterion (WAIC).

829

830 Supplementary Table 3. Risk factors and the daily probability for acquisition of pOXA-
831 48-carrying enterobacteria by hospitalised patients.

Variable	Daily Probability of Acquisition ¹ Posterior Median (95% CrI ²)	
	<i>K. pneumoniae</i>	<i>E. coli</i>
Baseline	0.00098 (0.00079, 0.00120)	0.00040 (0.00028, 0.00054)
Other patients colonised (β) ³		
Neurosurgery	0.00650 (0.00276, 0.01089)	0.00012 (2.39E-9, 0.00117)
Gastroenterology	0.00163 (0.00034, 0.00385)	0.00072 (4.86E-5, 0.00254)
Pneumology	0.00268 (0.00115, 0.00445)	0.00099 (0.00012, 0.00216)
Urology	0.00056 (3.34E-7, 0.00381)	0.00016 (5.88E-9, 0.00178)
Previously colonised patient (γ) ⁴	0.01489 (0.00330, 0.04009)	0.00889 (0.00336, 0.01917)

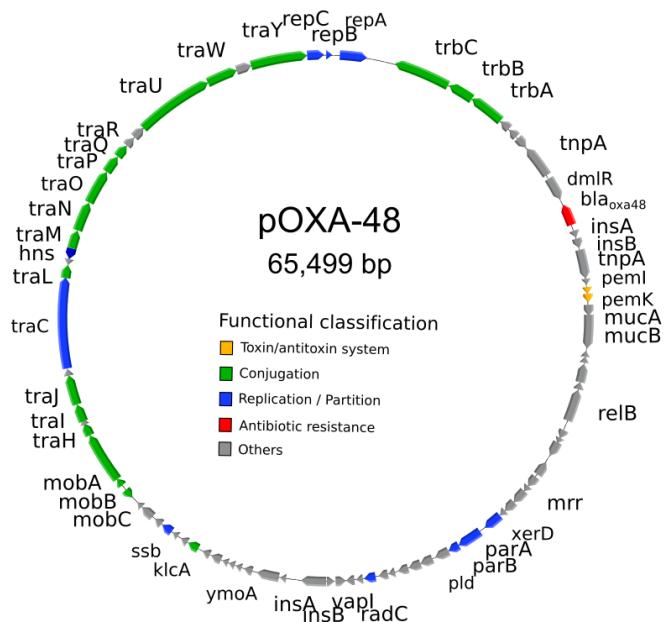
832

833 Daily probability of acquisition of pOXA-48-carrying *K. pneumoniae* or *E. coli* among
834 patients admitted to the four different wards during the study period of the R-GNOSIS
835 project. ¹ Probability of acquisition is the posterior intercept plus the posterior
836 coefficients transformed onto the probability scale. ² Credible Interval of Posterior
837 Distribution. ³ Risk of between-patient transfer: other patients already colonised by a
838 pOXA-48-carrying enterobacteria each day, divided by ward. ⁴ Risk of within-patient
839 transfer: patient previously colonised by a pOXA-48-carrying enterobacteria of the
840 different species.

841 **Supplementary figures**

842 **Supplementary Figure 1.** Plasmid pOXA-48.

843

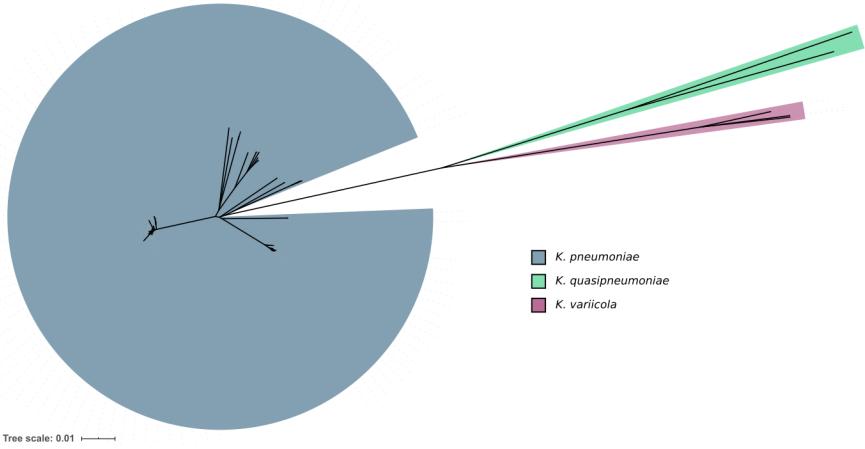


844

845 Schematic representation of plasmid pOXA-48. The reading frames for genes are
846 shown as arrows, with the direction of transcription indicated by the arrowhead. Arrow
847 colours indicate the functional classification of the gene (see legend). The *bla*_{OXA-48}
848 gene is indicated in red.

849 Supplementary Figure 2. Phylogenetic analysis of isolates preliminary identified as *K.*
850 *pneumoniae*.
851

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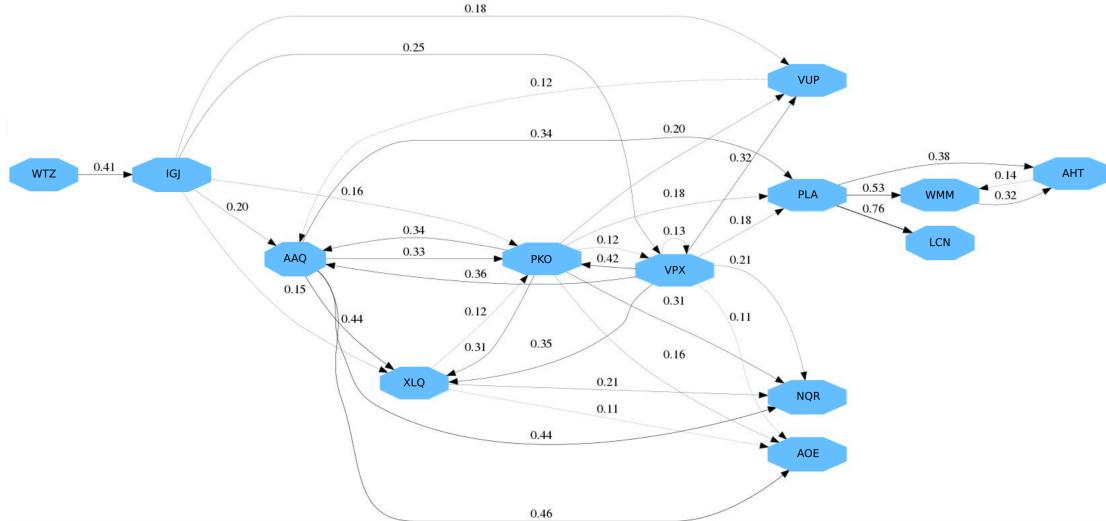


853

854 Unrooted phylogeny of 108 whole genome assemblies from the clones phenotypically
855 identified as *K. pneumoniae*. Branch length gives the mash distance (a measure of k-
856 mer similarity) between assemblies. Note the three distinct clusters, which are
857 considered to be separate species (distance > 0.05): *K. pneumoniae* (n= 103), *K.*
858 *quasipneumoniae* (n= 2) and *K. variicola* (n= 3).

859 Supplementary Figure 3. *K. pneumoniae* ST11 between-patient transfer dynamics in
860 the neurosurgery ward.

861



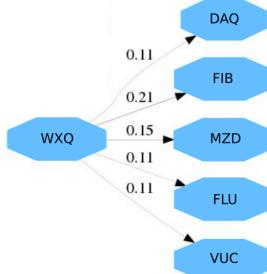
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863

864 Transmission events of *K. pneumoniae* ST11 carrying plasmid pOXA-48 predicted by
865 SCOTTI in the neurosurgery ward. Blue boxes represent patients, with patient codes
866 indicated within the box. Lines represent the predicted between-patient transfer
867 events, and the number above the lines indicate the probability of the transfer event.

868 Supplementary Figure 4. *K. pneumoniae* ST11 between-patient transfer dynamics in
869 the gastroenterology ward.

870



871

872

873 Transmission events of *K. pneumoniae* ST11 carrying plasmid pOXA-48 predicted by
874 SCOTTI in the gastroenterology ward. Blue boxes represent patients, with patient
875 codes indicated within the box. Lines represent the predicted between-patient transfer
876 events, and the number above the lines indicate the probability of the transfer event.

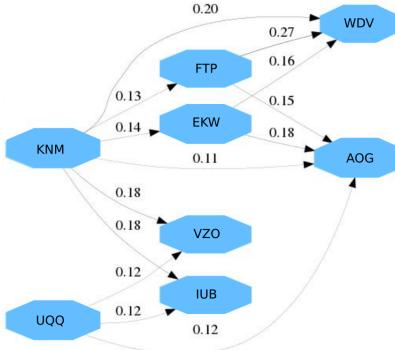
877 Supplementary Figure 5. *K. pneumoniae* ST11 between-patient transfer dynamics in
878 the pneumology ward.

879

880

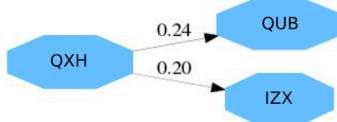
881

882 Transmission events of *K. pneumoniae* ST11 carrying plasmid pOXA-48 predicted by
883 SCOTTI in the pneumology ward. Blue boxes represent patients, with patient codes
884 indicated within the box. Lines represent the predicted between-patient transfer
885 events, and the number above the lines indicate the probability of the transfer event.



886 Supplementary Figure 6. *K. pneumoniae* ST11 between-patient transfer dynamics in
887 the urology ward.

888



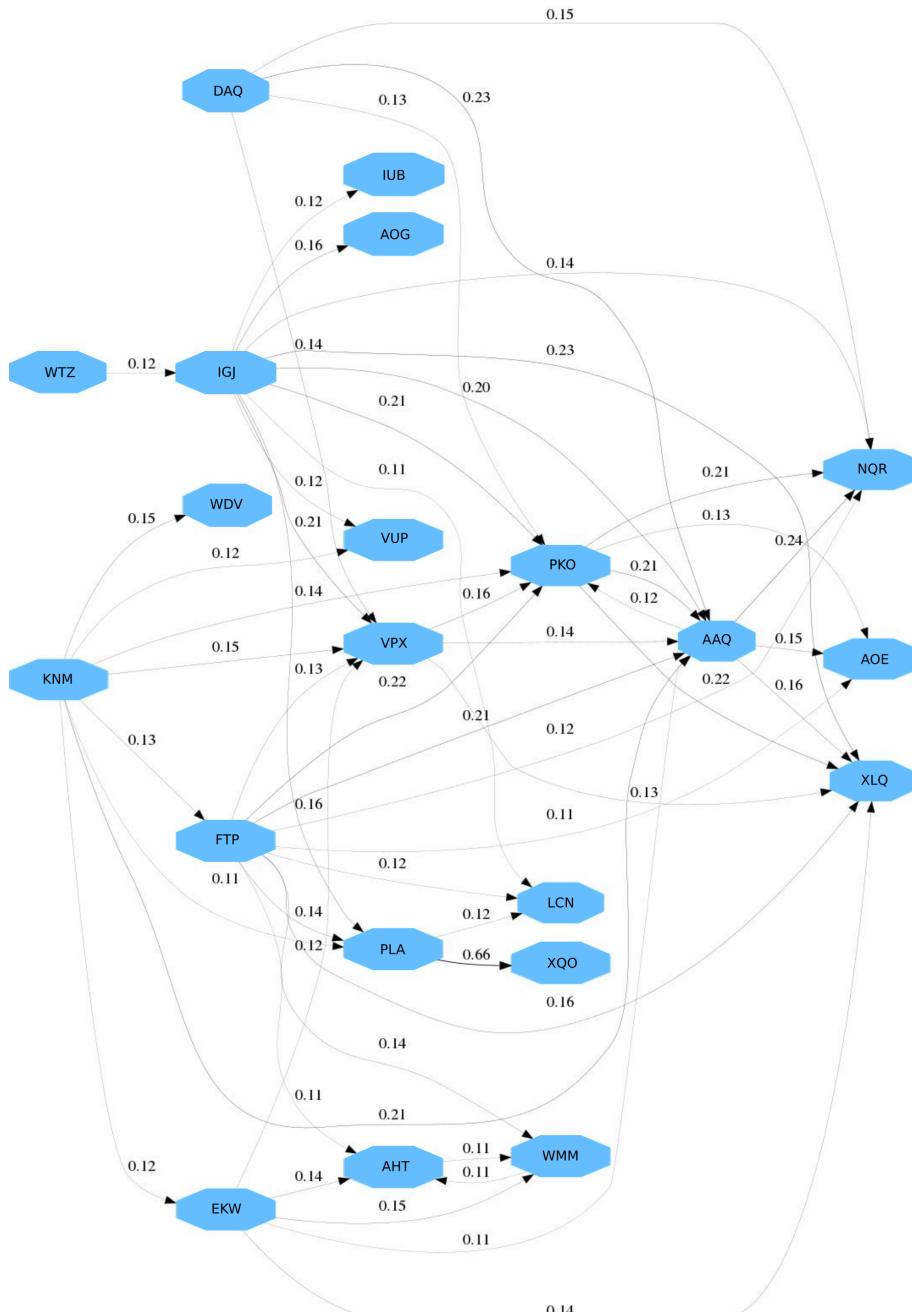
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891

892 Transmission events of *K. pneumoniae* ST11 carrying plasmid pOXA-48 predicted by
893 SCOTTI in the urology ward. Blue boxes represent patients, with patient codes
894 indicated within the box. Lines represent the predicted between-patient transfer
895 events, and the number above the lines indicate the probability of the transfer event.

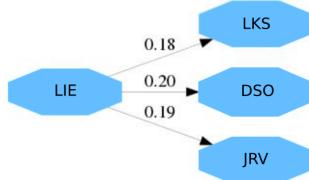
896 Supplementary Figure 7. *K. pneumoniae* ST11 between-patient transfer dynamics
897 across all the wards.



904 Supplementary Figure 8. *K. pneumoniae* ST15 between-patient transfer dynamics

905 across all the wards.

906



907

908

909 Transmission events of *K. pneumoniae* ST15 carrying plasmid pOXA-48 predicted by
910 SCOTTI when combining patients from the four wards. Blue boxes represent patients,
911 with patient codes indicated within the box. Lines represent the predicted between-
912 patient transfer events, and the number above the lines indicate the probability of the
913 transfer event.

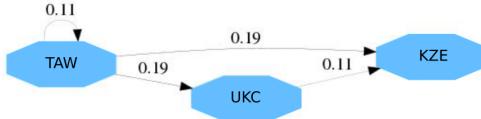
914 Supplementary Figure 9. *K. pneumoniae* ST307 between-patient transfer dynamics in
915 the pneumology ward.

916

917

918

919 Transmission events of *K. pneumoniae* ST307 carrying plasmid pOXA-48 predicted by
920 SCOTTI in the pneumology ward. Blue boxes represent patients, with patient codes
921 indicated within the box. Lines represent the predicted between-patient transfer
922 events, and the number above the lines indicate the probability of the transfer event.



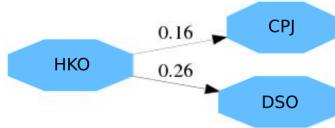
923 Supplementary Figure 10. *E. coli* ST10 between-patient transfer dynamics across all
924 the wards.

925

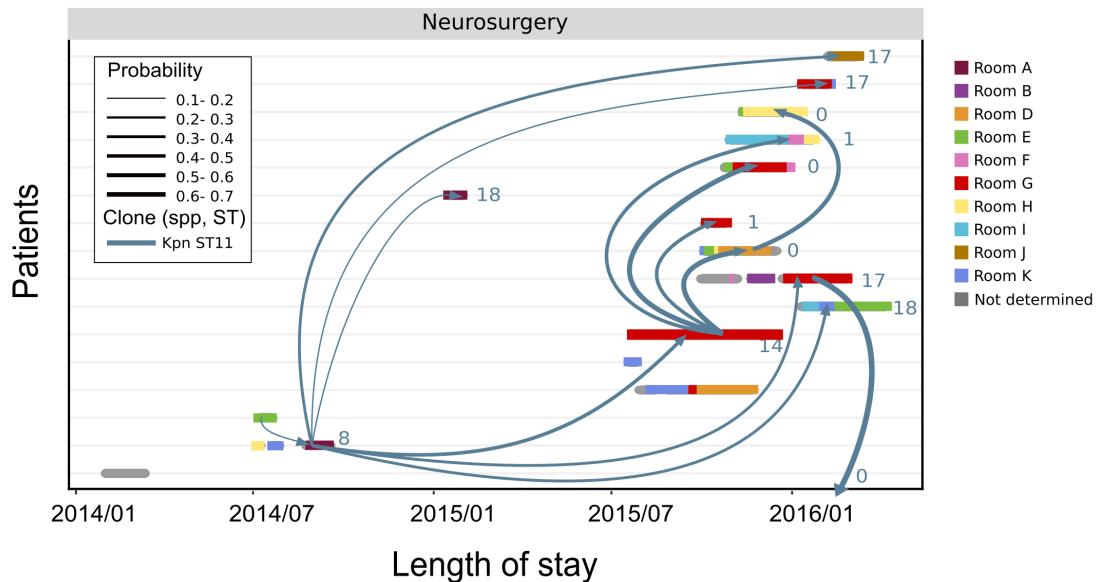
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928 Transmission events of *E. coli* ST10 carrying plasmid pOXA-48 predicted by SCOTTI
929 when combining patients from the four wards. Blue boxes represent patients, with
930 patient codes indicated within the box. Lines represent the predicted between-patient
931 transfer events, and the number above the lines indicate the probability of the transfer
932 event.



933 Supplementary Figure 11. Spatiotemporal distribution of patients colonised by *K.*
934 *pneumoniae* ST11 in the neurosurgery ward.



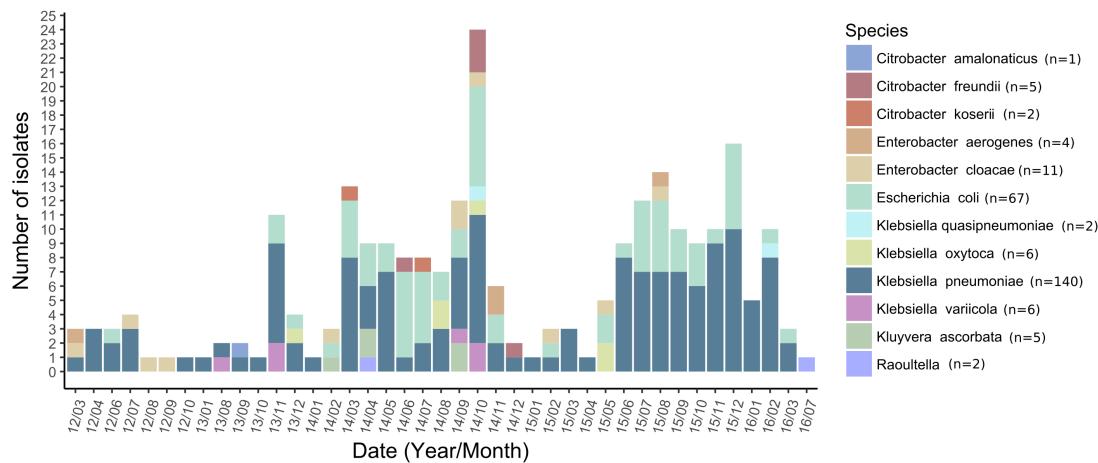
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936

937 Distribution of patients colonised by pOXA-48-carrying *K. pneumoniae* ST11 in the
938 neurosurgery ward. Each row represents a patient and the colour segments represent
939 the length of stay in the hospital (from admission to discharge). The colours of the
940 segments represent the different rooms within the ward (see legend). Arrows represent
941 transmission events predicted by SCOTTI. Line thickness represents the probability of
942 the transmission predicted by SCOTTI. The number to the right of the arrowhead
943 indicates the number of SNPs between the complete genomes of the pair of clones
944 involved in the putative transmission event. Note that 6 out of 16 patients shared room
945 G in overlapping stays.

946 Supplementary Figure 12. pOXA-48-carrying enterobacteria analysed in this study.

947



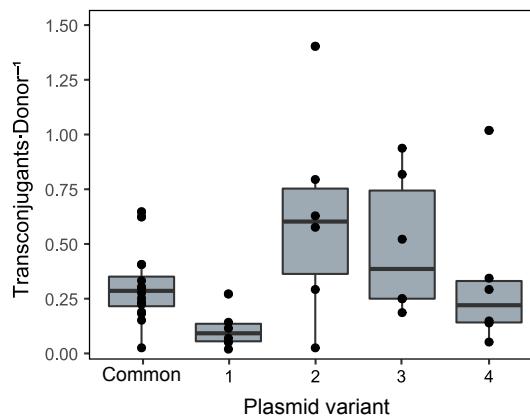
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949

950 Representation of the 250 pOXA-48-carrying clones isolated in the hospital from the
951 first description till the end of the study period. The colour code indicates the species
952 of the pOXA-48-carrying enterobacteria as indicated in the legend.

953 Supplementary Figure 13. Frequency of conjugation of plasmid pOXA-48.

954



955

956

957 Conjugation frequencies (transconjugants per donor) of the most common pOXA-48
958 variant in the hospital (common, n= 12 biological replicates) and the four variants with
959 SNPs in the core region used to track within-patient plasmid transfer (n= 6 biological
960 replicates). Plasmid variant numbers correspond to those indicated in Figure 5. The
961 line inside the box marks the median. The upper and lower hinges correspond to the
962 25th and 75th percentiles and whiskers extend to observations within 1.5 times the
963 interquartile range. The data presented here is the same as in figure 6, but represented
964 as conjugation frequency instead of rate.