

1 **Major role of the high-pathogenicity island (HPI) in the intrinsic**
2 **extra-intestinal virulence of *Escherichia coli* revealed by a**
3 **genome-wide association study**

4 **Marco Galardini^{1,6,*}, Olivier Clermont², Alexandra Baron², Bede Busby³, Sara Dion²,**
5 **Sören Schubert⁴, Pedro Beltrao¹, Erick Denamur^{2,5,*}**

6 ¹EMBL-EBI, Wellcome Genome Campus, Cambridge, United Kingdom

7 ²Université de Paris, IAME, UMR1137, INSERM, Paris, France

8 ³Genome Biology Unit, EMBL, Heidelberg, Germany

9 ⁴Max von Pettenkofer Institute of Hygiene and Medical Microbiology, Faculty of
10 Medicine, LMU Munich, Germany

11 ⁵APHP, Hôpitaux Universitaires Paris Nord Val-de-Seine, Site Bichat, Paris, France

12 ⁶Current address: Biological Design Center, Boston University, Boston, MA 02215, USA

13 ***Corresponding authors:** mgala@bu.edu and erick.denamur@inserm.fr

14 **Abstract:**

15 The bacterium *Escherichia coli* is not only an important gut commensal, but also a
16 common pathogen involved in both diarrheic and extra-intestinal diseases. To
17 characterize the genetic determinants of extra-intestinal virulence we carried out an
18 unbiased genome-wide association study (GWAS) on 234 commensal and
19 extra-intestinal pathogenic strains representative of the species phylogenetic diversity,
20 tested in a mouse model of sepsis. We found that the high-pathogenicity island (HPI), a
21 ~35 kbp gene island encoding the yersiniabactin siderophore, is highly associated with
22 death in mice, surpassing all other genetic factors by far. We validated the association *in*
23 *vivo* by deleting key components of the HPI in strains in two phylogenetic backgrounds,
24 and found that virulence is correlated with growth in the presence of various compounds
25 including several antimicrobials, which hints at collateral sensitivities associated with
26 intrinsic pathogenicity. This study points at the power of unbiased genetic approaches to
27 uncover virulence determinants and the use of phenotypic data to generate new
28 hypothesis on pathogenicity and phenotypic characteristics associated with it.

29 Introduction

30 *Escherichia coli* is both a commensal of vertebrates¹ and an opportunistic pathogen²
31 involved in a wide range of intestinal and extra-intestinal infections. Extra-intestinal
32 infections in humans represent a considerable burden³, bloodstream infections
33 (bacteraemia) being the most severe with a high attributable mortality of between
34 10-30%⁴⁻⁶. The regular increase over the last 20 years of *E. coli* bloodstream incidence⁷
35 and antibiotic resistance⁸ is particularly worrisome. The factors associated with high
36 mortality are mainly linked to host conditions such as age, the presence of underlying
37 diseases and to the portal of entry, with the urinary origin being more protective. These
38 factors outweighing those directly attributable to the bacterial agent^{4-6,9}.

39 Nevertheless, the use of animal models has shown a great variability in the intrinsic
40 extra-intestinal virulence potential of natural *E. coli* isolates. In a mouse model of sepsis
41 where bacteria are inoculated subcutaneously, it has been clearly shown that the
42 intrinsic virulence quantified by the number of animal deaths over the number of
43 inoculated animals for a given strain is dependant on the number of virulence factors
44 such as adhesins, toxins, protectins and iron capture systems¹⁰⁻¹³. One of the most
45 relevant virulence factors is the so-called high-pathogenicity island (HPI), a 36 to 43 kb
46 region encoding the siderophore yersiniabactin, a major bacterial iron uptake system¹⁴.
47 The deletion of the HPI results in a decrease in the intrinsic virulence in the mouse
48 model but in a strain-dependent manner¹³⁻¹⁶, indicating complex interactions between
49 the genetic background of the strains and the HPI.

50 The limitation of these gene KO studies is that they target specific candidate genes.
51 Recently, the development of new approaches in bacterial genome-wide association
52 studies (GWAS)¹⁷⁻²⁰ allows searching in an unbiased manner for genotypes associated
53 to specific phenotypes such as drug resistance or virulence. In this context, we
54 conducted a GWAS in 234 commensal and extra-intestinal pathogenic strains of *E. coli*,
55 representing the species phylogenetic diversity, to search for traits associated to
56 virulence in the mouse model of sepsis²¹. The strains belong to three large strain
57 collections that span the species' major phylogroup diversity; the ECOR²², IAI¹⁰ and
58 NILS²³ collections. All three collections contain commensal as well as extra-intestinal
59 pathogenic *E. coli* (ExPEC), being defined as strains that possessed currently
60 recognized extra-intestinal virulence factors and/or demonstrated enhanced virulence in
61 an appropriate animal model of extra-intestinal infection²⁴. Most importantly, strains from
62 these collections have been recently sequenced and phenotyped across hundreds of
63 growth conditions, including antibiotics and other chemical and physical stressors²⁵.
64 This data could then be used to find phenotype associations with virulence and to
65 generate hypotheses on the function of genetic variants associated with the ExPEC
66 phenotype and potential collateral sensitivities associated with them.

67 Results

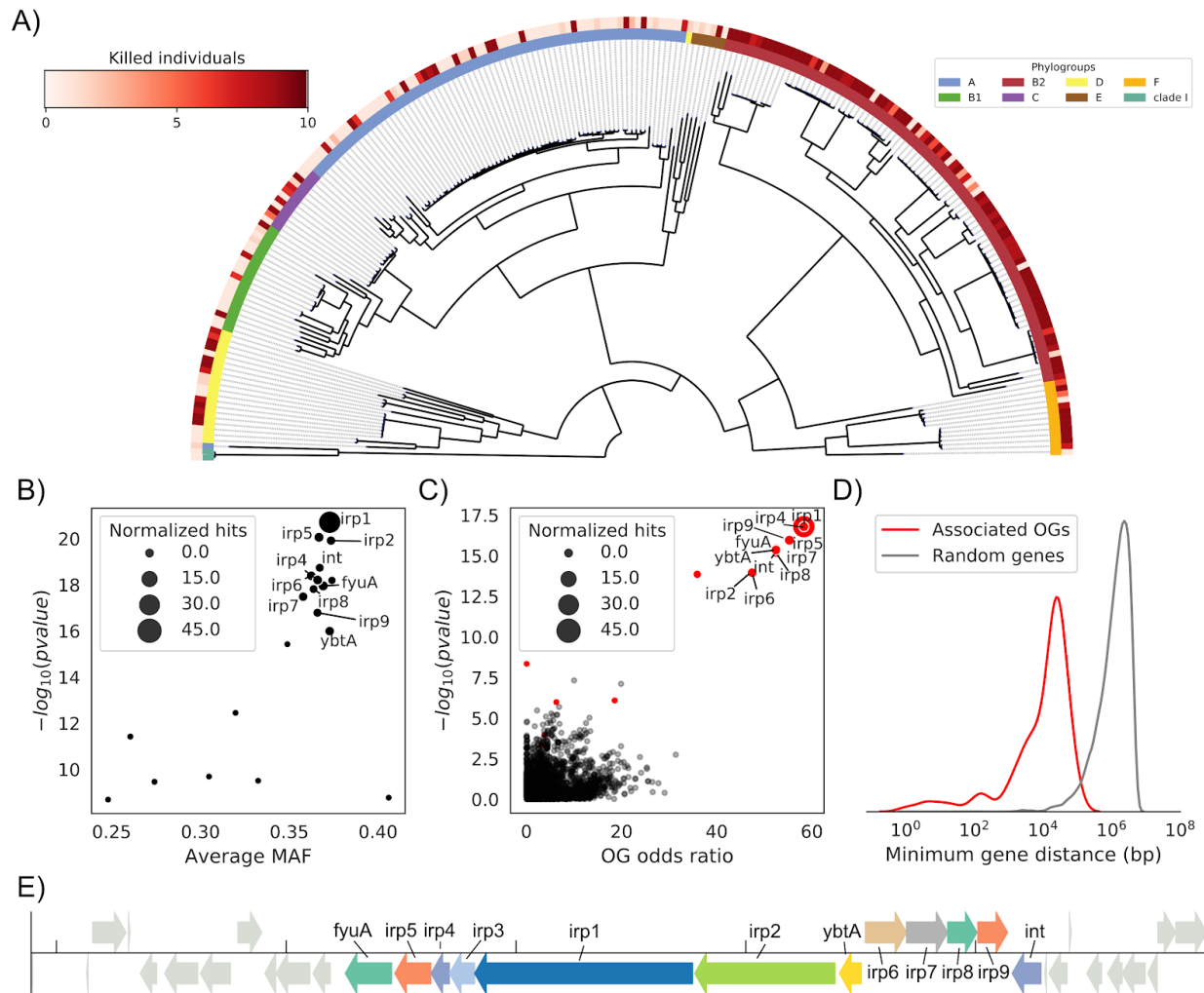
68 GWAS identifies the high-pathogenicity island as the strongest driver of the 69 extra-intestinal virulence phenotype

70 We studied three strain collections representative of the *E. coli sensu lato* phylogenetic
71 diversity, i.e., *Escherichia* clade I in addition to phylogroup A, B1, C, D, E and F
72 strains²⁶. These strains encompass 90 commensal strains and 144 strains isolated in
73 various extra-intestinal infections, mainly urinary tract infections and septicemia^{10,22,23}.
74 To avoid any bias linked to host conditions, we assessed the strain virulence as its
75 intrinsic extra-intestinal pathogenic potential using a well-calibrated mouse model of
76 sepsis^{10,21}, expressed as the number of killed mice over the 10 inoculated per strain. In
77 accordance with previous data, phylogroups B2, D and F have a higher proportion of
78 virulent strains, as compared to phylogroups A and B1 (Figure 1A, Supplementary Table
79 1).

80 We used a bacterial GWAS method to associate *k*-mers to the virulence phenotype,
81 allowing us to simultaneously test the contribution of core and accessory genome
82 variation to pathogenicity¹⁹. It is generally understood that such methods require large
83 sample sizes to have sufficient power, partly due to the need to break the long clonal
84 frames typical of bacterial genomes; the appropriate sample size is also a function of
85 the penetrance of the associated variants^{18,27}. We ran simulations with an unrelated set
86 of complete *E. coli* genomes and verified that our sample size was appropriate for
87 variants with high penetrance (i.e. odds ratio above 5, Supplementary Figure 1,
88 Methods). We reasoned that the genetic determinants of virulence are likely to have a
89 relatively high penetrance, and that the strains used were genetically diverse, enough to
90 break the clonal frame.

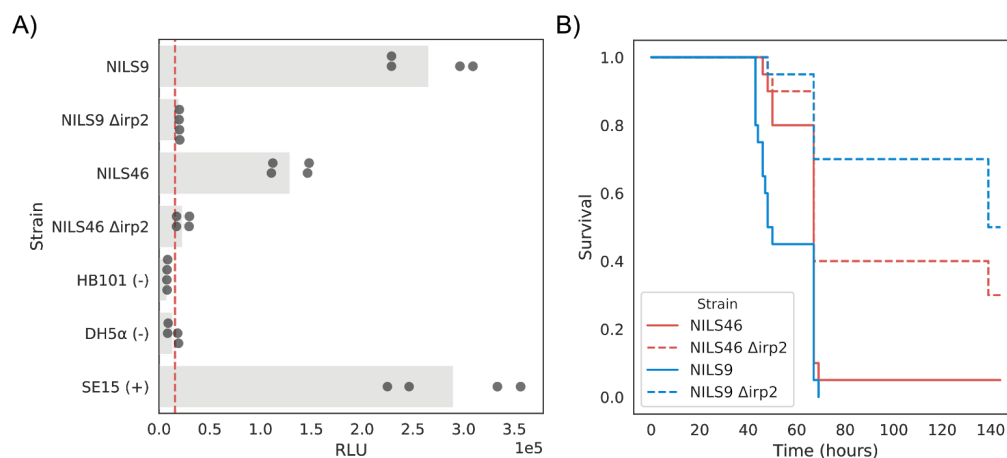
91 We uncovered a statistically significant association between 47,598 *k*-mers and the
92 virulence phenotype, which were mapped back to 86 genes across the strains'
93 pangenome (Figure 1B, Methods). A separate association using genes' presence
94 absence patterns showed that the genes to which the associated *k*-mers mapped to
95 have an odds ratio that far exceeds the required threshold we estimated from
96 simulations (Figure 1C). Since the average minimum allele frequency (MAF) of
97 associated *k*-mers is consistently around 36% (Figure 1B) and the distance between the
98 genes they map to across all strains is around 1 kbp (Figure 1D), we concluded that the
99 virulence phenotype is associated to the presence of a gene island. In fact, all genes
100 belonging to the HPI had the vast majority of associated *k*-mers mapped to them
101 (normalized hits ≥ 0.1 , Figure 1E). Moreover, we found that the HPI structure was
102 highly conserved across the 151 genomes that encode it (Supplementary Figure 2). We
103 also observed that the distribution of known virulence factors doesn't match the
104 virulence phenotype as closely as the HPI or has *k*-mers passing the association

105 threshold, further reinforcing the association results that the HPI is one of the main
 106 genetic factors behind virulence across phylogroups (Supplementary Figure 3).



107 **Figure 1. The HPI is strongly associated with the extra-intestinal virulence phenotype**
 108 **assessed in the mouse sepsis assay.** A) Core genome phylogenetic tree of the *E. coli* strains
 109 used in this study rooted on *Escherichia* clade I strains. Outer ring reports virulence as the
 110 number of killed mice over the 10 inoculated per strain, inner ring the phylogroup each strain
 111 belongs to. B) Results of the k-mer association analysis: for each gene the minimum association
 112 p-value and average minimum allele frequency (MAF) across all mapped k-mers are reported.
 113 The normalized hits are computed by dividing the number of mapped kmers by the length of the
 114 gene. C) Results of the gene association analysis; each gene tested is represented. Genes from
 115 the k-mer association analysis are highlighted in red. D) The associated genes (normalized hits
 116 ≥ 0.1) belong to a gene cassette. OGs: orthologous groups. E) The HPI gene cassette
 117 structure in strain IAI39; all associated genes are highlighted.

118 **KO gene experiments validate the role of the HPI in the extra-intestinal phenotype**
119 The studies on the role of the HPI in experimental virulence gave contrasting results
120 according to the strains' genetic background¹³. Among B2 phylogroup strains, HPI
121 deletion in the 536 (ST127) strain did not have any effect in the mouse model of sepsis²⁸
122 whereas this deletion in the NU14 (ST95) strain dramatically attenuated virulence¹³. Two
123 strains from this study belonging to B2 phylogroup/ST141 (IAI51 and IAI52) deleted in
124 *irp1* have attenuated virulence in the same model¹⁵. To have a broader view of the role
125 of the HPI in various genetic backgrounds, we constructed *irp2* deletion gene mutants in
126 two strains of phylogroup D (NILS46) and A (NILS9) belonging to STs frequently
127 involved in human bacteraemia (ST69 and ST10, respectively)²⁹. We first verified that
128 the wild-type strains strongly produced yersiniabactin, whereas the *irp2* mutants did not
129 (Figure 2A). We then tested them in the mouse sepsis model and saw an increase in
130 survival for both mutated strains (log-rank test p-value < 0.0001 and 0.0217 for strain
131 NILS9 and NILS46, respectively, Figure 2B, Supplementary Table 2) with no significant
132 difference between the survival profiles for the two mutants (log-rank test p-value > 0.1).
133 We have therefore validated *in vivo* the causal link between the HPI and the virulence
134 phenotype detected by the means of an unbiased association approach, which
135 demonstrates the power and accuracy of bacterial GWAS.



136 **Figure 2. Phenotypic consequences of HPI's deletion.** A) Deletion of HPI leads to a
137 decrease in production of yersiniabactin. Production of yersiniabactin is measured using a
138 luciferase-based reporter (Methods). Strains marked with a "-" and "+" sign indicates a negative
139 and positive control, respectively. The red dashed line indicates an arbitrary threshold for
140 yersiniabactin production, derived from the average signal recorded from the negative controls
141 plus two standard deviations. RLU, relative light units. B) Deletion of HPI leads to an increase in
142 survival after infection. Survival curves for wild-type strains and the corresponding *irp2* deletion
143 mutant, built after infection of 20 mice for each strain.

144 **High-throughput phenotypic data sheds light on HPI's function**

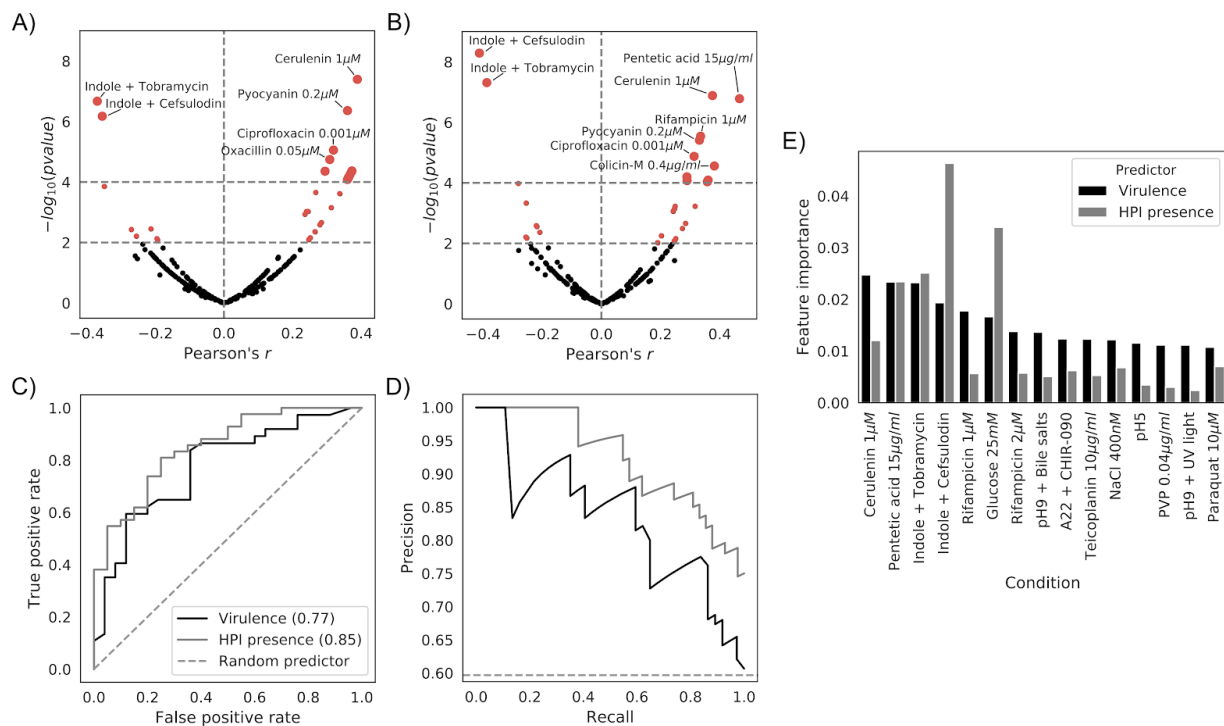
145 The main function encoded by the HPI cassette is iron scavenging through the
146 expression of the siderophore yersiniabactin¹⁵, which has been previously validated in
147 *E. coli* through knockout experiments¹³. In order to investigate other putative functions,
148 we leveraged a previously-generated high-throughput phenotypic screening in an *E. coli*
149 strain panel that largely overlaps with the strains used here (186 over 234)²⁵. We
150 observed a relatively high correlation between growth profiles in certain conditions and
151 both virulence and presence of the HPI cassette (Figure 3A and 3B, Supplementary
152 Table 3); given the strong association between the two, we observed similar conditions
153 being correlated.

154 As expected, we found a correlation between growth on the iron-sequestering agent
155 pentetic acid³⁰ and both HPI presence and virulence (Pearson's r: 0.47 and 0.36,
156 respectively). We similarly, observed a correlation between pyocyanin, a redox-active
157 phenazine compound being able to reduce Fe³⁺ to Fe²⁺³¹, and both HPI presence and
158 virulence (Pearson's r: 0.36 and 0.29, respectively)

159 Interestingly, we found similarly strong correlations with growth on sub-inhibitory
160 concentrations of several antibiotics, such as rifampicin, ciprofloxacin, amoxicillin and
161 oxacillin, as well as other antimicrobial agents such as cerulenin and colicin. This might
162 be due to the presence of resistance alleles and/or genes that are strongly associated
163 with pathogenic strains, or might point to the role of iron homeostasis in intrinsic
164 resistance to antibiotics³². As an example, quinolones bind Fe³⁺ on its pyridione ring,
165 which is also involved in the interaction with its target, DNA gyrase³³. Cell envelope
166 permeability can also be modified in response to the presence of iron via
167 two-component systems, rendering the cell more resistant³². On the other hand we
168 found that growth in presence of indole at 2 mM in association with sub-inhibitory
169 concentrations of cefsulodin and tobramycin antibiotics, but not with each of these
170 compounds alone, was negatively correlated with both HPI presence and virulence.
171 This might indicate a synergy between antibiotics and indole. In lysogeny broth, sub
172 lethal concentrations of antibiotics increased the endogenous production of indole by
173 the cells³⁴ and, at very high concentration (5 mM), indole induces the production of
174 reactive oxygen species and is toxic for the cells³⁵. This toxicity has been shown to be
175 partly iron mediated due to the Fenton reaction³⁶, explaining that cells with increased
176 import of extracellular iron due to the HPI might be more sensitive to these compounds.
177 These associations suggest the potential for collateral sensitivities related to both
178 intrinsic pathogenicity and the presence of the HPI.

179 Given the relatively large number of conditions correlated with both pathogenicity
180 and HPI presence, we tested whether both features could be predicted from growth
181 data. We used the commonly-used random forests machine learning algorithm with
182 appropriate partitioning of input data to tune hyperparameters and reduce overfitting,

183 leading to two classifiers for virulence and presence of the HPI cassette with high
 184 predictive power (Figure 3C and 3D and Methods). We noted that prediction of HPI
 185 presence performs slightly better than virulence, possibly reflecting the complex nature
 186 of the latter phenotype. As expected, we found that conditions with relatively high
 187 correlation with both features have a higher weight in both classifiers (Figure 3E,
 188 Supplementary Table 4), which suggests that a subset of phenotypic tests might be
 189 sufficient to classify pathogenic strains. These results show how phenotypic data can be
 190 used to generate hypotheses over gene function and pathogenesis.



191 **Figure 3. Growth profiles can predict virulence and HPI presence.** A-B) Volcano plots for
 192 the correlation between the strains' growth profiles and: A) virulence levels and B) presence of
 193 the HPI. C-D) Use of the strains' growth profiles to build a predictor of virulence levels and
 194 presence of the HPI. C) Receiver operating characteristic (ROC) curve and D) Precision-Recall
 195 curve for the two tested predictors. E) Feature importance for the predictors, showing the top 15
 196 conditions contributing to the virulence levels predictor.

197 Discussion

198 With the steady decline in the price of genomic sequencing and the increasing
 199 availability of molecular and phenotypic data for bacterial isolates, it has finally become
 200 possible to use statistical genomics approaches such as GWAS to uncover the genetic
 201 determinants of relevant phenotypes. Such approaches have the advantage of being
 202 unbiased, and can then be used to confirm previous targeted findings and potentially

203 uncover new factors, given sufficient statistical power. The accumulation of other
204 molecular and phenotypic data can on the other hand uncover variables correlated with
205 phenotype, which can be used to generate testable hypothesis on the function of
206 genomic hits and potential collateral sensitivities associated with them. Given the rise of
207 both *E. coli* extra-intestinal infections and antimicrobial resistance, we reasoned that the
208 intrinsic virulence assessed in a calibrated mouse model of sepsis^{10,21} is a phenotype
209 worth exploring with such an unbiased approach.

210 We were able to confirm earlier reports on the importance of the HPI in
211 extra-intestinal virulence^{13–16,37}, which showed the strongest signal in both the *k*-mer and
212 accessory genome association analysis, and whose importance was validated *in vitro*
213 and in an *in vivo* model. The distribution of the HPI within the species resulting from
214 multiple horizontal gene transfers via homologous recombination³⁸ has probably
215 facilitated its identification using GWAS. Additional genetic factors might have been
216 overlooked by this analysis, due to the relatively small sample size; we however
217 estimate that those putative additional factors might have a relatively low penetrance,
218 based on our simulations in an independent dataset. As sequencing of bacterial isolates
219 is becoming more common in clinical settings^{39–41}, we expect to be able to uncover
220 these additional genetic factors in future studies.

221 The association between both the intrinsic virulence phenotype and the presence of
222 the HPI and previously collected growth data allowed us to generate testable
223 hypotheses on mechanism of pathogenesis and putative additional functions of the HPI.
224 In particular we observed a relatively strong correlation between growth on various
225 antimicrobial agents and both pathogenicity and HPI presence, which confirms the
226 pressure to acquire resistance for these isolates, but also on the potential role of HPI
227 and iron homeostasis on antimicrobial resistance³². *E. coli* mutants of *fur*, a
228 transcriptional regulator that represses iron uptake systems, which accumulate high
229 level of intracellular iron, have been shown to increase resistance to quinolones,
230 aminoglycoside, tetracycline, rifampicin and amoxicillin⁴². The negative correlation with
231 growth profiles in the presence of the indole associated to antibiotics points to the
232 possible deleterious role of iron in the effect of sublethal doses of antibiotics. A vicious
233 circle is rapidly established as antibiotics increase the production of indole³⁴, which in
234 turn destabilise the membrane³⁵, further increasing the penetration of the antibiotics.
235 The deletion of TonB, an iron transporter, increase resistance to the antibiotic, showing
236 the role of reactive oxygen species generated by the Fenton reaction in the presence of
237 iron³⁶. Altogether, these data bring new light on the “liaisons dangereuses” between iron
238 and antibiotics that could potentially be targeted³².

239 We also demonstrate how growth data across several conditions can accurately
240 distinguish pathogenic from non-pathogenic isolates, which could lead to the
241 development of growth-based tests, which could complement and validate existing

242 diagnostic tools based on molecular and phenotypic data⁴³⁻⁴⁵. Taken together this
243 analysis demonstrates how a data-centric approach can increase our knowledge of
244 complex bacterial phenotypes and guide further empirical work on gene function and its
245 relationship to intrinsic pathogenicity.

246 **Materials and methods**

247 **Strains used**

248 The full list of the 234 strains used in the association analysis, together with their main
249 characteristics is reported in Supplementary Table 1. The genome sequences of all 234
250 strains is available through Figshare⁴⁶.

251 The construction of the *irp2* deletion mutants of the NILS9 and NILS46 strains was
252 achieved following a strategy adapted from Datsenko and Wanner⁴⁷. Primers used in
253 the study are listed in Supplementary Table 5. In brief, primers used for gene disruption
254 included 44-46 nucleotide homology extensions to the 5'- and 3' regions of the target
255 gene, respectively, and additional 20 nucleotides of priming sequence for amplification
256 of the resistance cassette on the template plasmids pKD4. The PCR product was then
257 transformed into strains carrying the helper plasmid pKOBEG expressing the lambda
258 red recombinase under control of an arabinose-inducible promoter⁴⁸. Kanamycin
259 resistant transformants were selected and further screened for correct integration of the
260 resistance marker by PCR. For elimination of the antibiotic resistance gene, helper
261 plasmid pCP20 was used according to the published protocol. PCR followed by Sanger
262 sequencing of the mutants were performed to verify the deletion and the presence of
263 the expected scar.

264 **Yersiniabactin detection assay**

265 Production of the siderophore yersiniabactin was detected and quantified using a
266 luciferase reporter assay as described elsewhere^{13,49}. Briefly, bacterial strains were
267 cultivated in NBD medium for 24 hours at 37°C. Next, bacteria were pelleted by
268 centrifugation and the supernatant was added to the indicator strain WR 1542
269 harbouring plasmid pACYC5.3L. All the genes necessary for yersiniabactin uptake are
270 located on the plasmid pACYC5.3L, i.e. *irp6*, *irp7*, *irp8*, *fyuA*, *ybtA*. Furthermore, this
271 plasmid is equipped with a fusion of the *fyuA* promoter region with the luciferase
272 reporter gene. The amount of yersiniabactin can be quantified semi-quantitatively, as
273 yersiniabactin-dependant upregulation of *fyuA* expression is determined by luciferase
274 activity of the *fyuA-luc* reporter fusion.

275 **Mouse virulence assay**

276 Ten female mice OF1 of 14-16 g (4 week-old) from Charles River® (L'Arbresle, France)
277 received a subcutaneous injection of 0.2 ml of bacterial suspension in the neck ($2 \cdot 10^8$

278 colony forming unit). Time to death was recorded during the following 7 days. Mice
279 surviving more than 7 days were considered cured and sacrificed¹⁰. In each experiment,
280 the *E. coli* CFT073 strain was used as a positive control killing all the inoculated mice
281 whereas the *E. coli* K-12 MG1655 strain was used as a negative control for which all the
282 inoculated mice survive²¹. For the mutant assays, 20 mice per strain were used to
283 obtain statistical relevant data. The data was analysed using the lifeline package
284 v0.21.0⁵⁰.

285 **Association analysis**

286 All genome-wide association analysis were carried out using pyseer, version v1.2.0¹⁹.
287 All input genomes were re-annotated using prokka, version v1.13.3⁵¹, to ensure uniform
288 gene calls and excluding contigs whose size was above 200 base pairs. The core
289 genome phylogenetic tree was generated using ParSNP⁵² to generate the core genome
290 alignment and gubbins v2.3.1⁵³ to generate the phylogenetic tree. The strain's
291 pangenome was estimated using roary v3.12.0⁵⁴. K-mers distributions from the input
292 genome assemblies were computed using fsm-lite¹⁸, with a minimum and maximum *k*
293 value of 9 and 100, respectively. The association between both k-mers and pangenome
294 and phenotype (expressed as number of mice killed post-infection) was carried out
295 using the FastLMM⁵⁵ linear mixed-model implemented in pyseer, using a kinship matrix
296 derived from the phylogenetic tree as population structure. For both association analysis
297 we used the number of unique presence/absence patterns to derive an appropriate
298 p-value threshold for the association likelihood ratio test ($2.90E^{-09}$ and $7.03E^{-06}$ for the
299 *k*-mers and pangenome analysis, respectively). *K*-mers significantly associated with the
300 phenotype were mapped back to each input genome using bwa mem v0.7.17-r1188⁵⁶
301 and betools v.2.27.1⁵⁷, using the pangenome analysis to collapse gene hits to individual
302 groups of orthologs. A sample protein sequence for each groups of orthologs where at
303 least on *k*-mer with size 20 or higher was mapped was extracted giving priority to strain
304 IAI39 when available, given it was the only strain with a complete genome available;
305 those sample sequences where used to search for homologs in the uniref50 database
306 from uniprot⁵⁸ using blast v2.7.1+⁵⁹. Each group of orthologs was then given a gene
307 name using both available literature information and the results of the homology search.
308 Distances between each pair of associated groups of orthologs was computed using the
309 annotation files, using an equal number of random pairs as background.

310 **Power simulations**

311 Statistical power was estimated using an unrelated set of 548 complete *E. coli* genomes
312 downloaded from NCBI RefSeq using ncbi-genome-download v0.2.9 on May 24th 2018.
313 Each genome was subject to the same processing as the actual ones used in the real
314 analysis (re-annotation, phylogenetic tree construction, pangenome estimation). The

315 gene presence/absence patterns were used to run the simulations, in a similar way as
316 described in the original SEER implementation¹⁸. Briefly, for each sample size, a
317 random subset of strains was selected, and the likelihood ratio test p-value threshold
318 was estimated by counting the number of unique gene presence/absence patterns in
319 the reduced roary matrix. For each odds ratio tested, a binary case-control phenotype
320 vector was constructed for the strains subset using the following formulae:

$$321 \quad P_{case|variant} = \frac{D_e}{MAF}$$

$$322 \quad P_{case|novariant} = \frac{\frac{S_r}{S_r+1} D_e}{1 - MAF}$$

323 Were S_r is the ratio of case/controls (set at 1 in these simulations), MAF as the
324 minimum allele frequency of the target gene in the strains subset, and D_e the number of
325 cases. pyseer's LMM model was then applied to the presence/absence vector of the
326 target gene and the likelihood ratio test p-value was compared with the empirical
327 threshold. The randomization was repeated 100 times and power was defined as the
328 proportion of randomizations for each sample size and odds ratio whose p-value was
329 below the threshold. The *pks2* and *fabG* genes were used as gene targets in the
330 simulations, and both gave very similar results.

331 **Correlations with growth profiles**

332 The previously generated phenotypic data²⁵ for 186 over 234 strains were used to
333 compute correlations with both the number of mice killed after infection and
334 presence/absence of the HPI. The data was downloaded from the ecoref website
335 (<https://evocellnet.github.io/ecoref/download/>) and the pearson correlation with the
336 s-scores was computed together with the correlation p-value. Two predictors, one for
337 virulence (number of killed mice post-infection) and one for presence of the HPI were
338 built using the random forest classifier algorithm implemented in scikit-learn v.020.2⁶⁰,
339 using the s-scores as predictors. The input was column imputed, and 33% of the
340 observations were kept as a test dataset, using a "stratified shuffle split" strategy. The
341 remainder was used to train the classifier, using a grid search to select the number of
342 trees and the maximum number of features used, through 10 rounds of stratified shuffle
343 split with validation set size of 33% the training set and using the F1 measure as score.
344 The performance of the classifiers on the test set were assessed by computing the area
345 under the receiver operating characteristic curve (ROC-curve).

346 **Code and data availability**

347 All input data and code used to run the analysis and generate the plots is available
348 online at https://github.com/mgalardini/2018_ecoli_pathogenicity. Code is mostly based
349 on the Python programming language and the following libraries: numpy v1.16.1⁶¹, scipy
350 v1.2.1⁶², biopython v1.71^{63,64}, pandas v0.24.1⁶⁵, pybedtools v0.8.0⁶⁶, dendropy 4.4.0⁶⁷,
351 ete3 v3.1.1⁶⁸, statsmodels v0.9.0⁶⁹, matplotlib v3.0.2⁷⁰, seaborn v0.9.0⁷¹, jupyterlab
352 v0.34.11⁷² and snakemake v4.5.0⁷³.

353 **Ethics statement**

354 All animal experimentations were conducted following European (Directive 2010/63/EU
355 on the protection of animals used for scientific purposes) and national recommendations
356 (French Ministry of Agriculture and French Veterinary Services, accreditation A
357 75-18-05). The protocol was approved by the Animal Welfare Committee of the
358 Veterinary Faculty in Lugo, University of Santiago de Compostela (AE-LU-002/12/INV
359 MED.02/OUTROS 04).

360 **Acknowledgements**

361 We are grateful to Ivan Matic for discussion on the effect of indole. This work was
362 partially supported by the “Fondation pour la Recherche Médicale” (Equipe FRM 2016,
363 grant number DEQ20161136698).

364 **References**

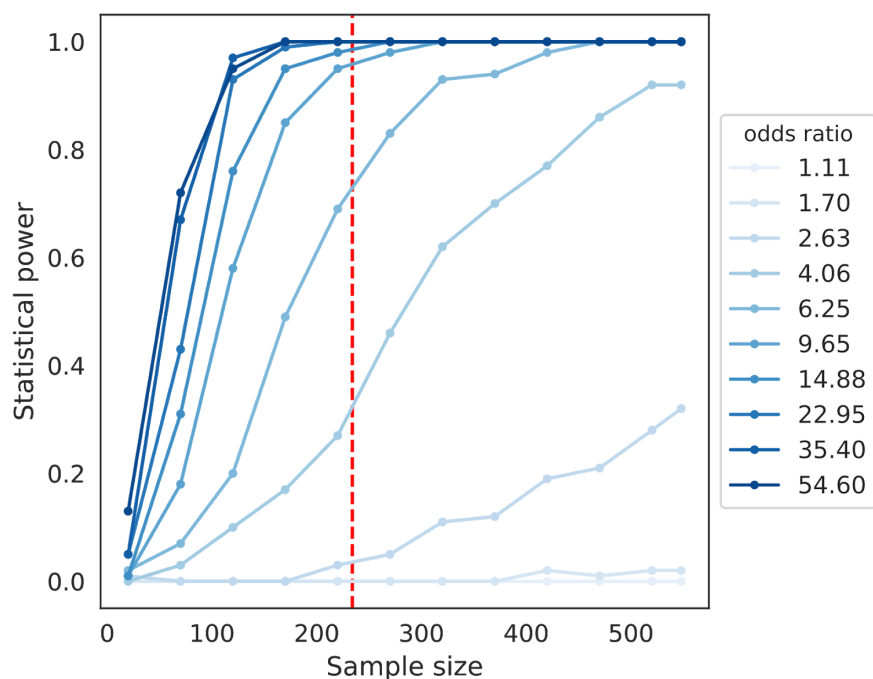
- 365
- 366 1. Tenaillon, O., Skurnik, D., Picard, B. & Denamur, E. The population genetics of commensal
367 *Escherichia coli*. *Nat. Rev. Microbiol.* **8**, 207–217 (2010).
 - 368 2. Croxen, M. A. & Brett Finlay, B. Molecular mechanisms of *Escherichia coli* pathogenicity. *Nature*
369 *Reviews Microbiology* **8**, 26–38 (2010).
 - 370 3. Russo, T. A. & Johnson, J. R. Medical and economic impact of extraintestinal infections due to
371 *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes Infect.* **5**, 449–456
372 (2003).
 - 373 4. Lefort, A. *et al.* Host Factors and Portal of Entry Outweigh Bacterial Determinants To Predict the
374 Severity of *Escherichia coli* Bacteremia. *Journal of Clinical Microbiology* **49**, 777–783 (2011).
 - 375 5. Burdet, C. *et al.* *Escherichia coli* bacteremia in children: age and portal of entry are the main
376 predictors of severity. *Pediatr. Infect. Dis. J.* **33**, 872–879 (2014).
 - 377 6. Abernethy, J. K. *et al.* Thirty day all-cause mortality in patients with *Escherichia coli* bacteraemia in
378 England. *Clin. Microbiol. Infect.* **21**, 251.e1–8 (2015).
 - 379 7. Vihta, K.-D. *et al.* Trends over time in *Escherichia coli* bloodstream infections, urinary tract infections,
380 and antibiotic susceptibilities in Oxfordshire, UK, 1998–2016: a study of electronic health records.
381 *The Lancet Infectious Diseases* **18**, 1138–1149 (2018).
 - 382 8. Cassini, A. *et al.* Attributable deaths and disability-adjusted life-years caused by infections with
383 antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level
384 modelling analysis. *Lancet Infect. Dis.* **19**, 56–66 (2019).
 - 385 9. Baudron, C. R. *et al.* *Escherichia coli* bacteraemia in adults: age-related differences in clinical and
386 bacteriological characteristics, and outcome. *Epidemiology & Infection* **142**, 2672–2683 (2014).
 - 387 10. Picard, B. *et al.* The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection.
388 *Infect. Immun.* **67**, 546–553 (1999).
 11. Johnson, J. R. & Kuskowski, M. Clonal origin, virulence factors, and virulence. *Infect. Immun.* **68**,

- 389 424–425 (2000).
- 390 12. Tourret, J., Diard, M., Garry, L., Matic, I. & Denamur, E. Effects of single and multiple pathogenicity
391 island deletions on uropathogenic *Escherichia coli* strain 536 intrinsic extra-intestinal virulence. *Int. J.*
392 *Med. Microbiol.* **300**, 435–439 (2010).
- 393 13. Smati, M. *et al.* Strain-specific impact of the high-pathogenicity island on virulence in extra-intestinal
394 pathogenic *Escherichia coli*. *Int. J. Med. Microbiol.* **307**, 44–56 (2017).
- 395 14. Schubert, S., Cuenca, S., Fischer, D. & Heesemann, J. High-pathogenicity island of *Yersinia pestis* in
396 enterobacteriaceae isolated from blood cultures and urine samples: prevalence and functional
397 expression. *J. Infect. Dis.* **182**, 1268–1271 (2000).
- 398 15. Schubert, S., Picard, B., Gouriou, S., Heesemann, J. & Denamur, E. *Yersinia* high-pathogenicity
399 island contributes to virulence in *Escherichia coli* causing extraintestinal infections. *Infect. Immun.* **70**,
400 5335–5337 (2002).
- 401 16. Johnson, J. R. & Russo, T. A. Molecular Epidemiology of Extraintestinal Pathogenic *Escherichia coli*.
402 *EcoSal Plus* **8**, (2018).
- 403 17. Earle, S. G. *et al.* Identifying lineage effects when controlling for population structure improves power
404 in bacterial association studies. *Nature Microbiology* **1**, 1–8 (2016).
- 405 18. Lees, J. A. *et al.* Sequence element enrichment analysis to determine the genetic basis of bacterial
406 phenotypes. *Nat. Commun.* **7**, 12797 (2016).
- 407 19. Lees, J., Galardini, M., Bentley, S. D. & Weiser, J. N. pyseer: a comprehensive tool for microbial
408 pangenome-wide association studies. *bioRxiv* (2018).
- 409 20. Jaillard, M. *et al.* A fast and agnostic method for bacterial genome-wide association studies: Bridging
410 the gap between k-mers and genetic events. *PLoS Genet.* **14**, e1007758 (2018).
- 411 21. Johnson, J. R. *et al.* Experimental mouse lethality of *Escherichia coli* isolates, in relation to accessory
412 traits, phylogenetic group, and ecological source. *J. Infect. Dis.* **194**, 1141–1150 (2006).
- 413 22. Ochman, H. & Selander, R. K. Standard reference strains of *Escherichia coli* from natural
414 populations. *J. Bacteriol.* **157**, 690–693 (1984).
- 415 23. Bleibtreu, A. *et al.* The *rpoS* gene is predominantly inactivated during laboratory storage and
416 undergoes source-sink evolution in *Escherichia coli* species. *J. Bacteriol.* **196**, 4276–4284 (2014).
- 417 24. Russo, T. A. & Johnson, J. R. Proposal for a new inclusive designation for extraintestinal pathogenic
418 isolates of *Escherichia coli*: ExPEC. *J. Infect. Dis.* **181**, 1753–1754 (2000).
- 419 25. Galardini, M. *et al.* Phenotype inference in an *Escherichia coli* strain panel. *Elife* **6**, 1–19 (2017).
- 420 26. Clermont, O., Christenson, J. K., Denamur, E. & Gordon, D. M. The Clermont *Escherichia coli*
421 phylo-typing method revisited: improvement of specificity and detection of new phylo-groups.
422 *Environ. Microbiol. Rep.* **5**, 58–65 (2013).
- 423 27. Power, R. A., Parkhill, J. & de Oliveira, T. Microbial genome-wide association studies: lessons from
424 human GWAS. *Nat. Rev. Genet.* **18**, 41–50 (2016).
- 425 28. Diard, M. *et al.* Pathogenicity-associated islands in extraintestinal pathogenic *Escherichia coli* are
426 fitness elements involved in intestinal colonization. *J. Bacteriol.* **192**, 4885–4893 (2010).
- 427 29. Kallonen, T. *et al.* Systematic longitudinal survey of invasive *Escherichia coli* in England
428 demonstrates a stable population structure only transiently disturbed by the emergence of ST131.
429 *Genome Res.* (2017). doi:10.1101/gr.216606.116
- 430 30. Pippard, M. J., Jackson, M. J., Hoffman, K., Petrou, M. & Modell, C. B. Iron chelation using
431 subcutaneous infusions of diethylene triamine penta-acetic acid (DTPA). *Scand. J. Haematol.* **36**,
432 466–472 (1986).
- 433 31. Cornelis, P. & Dingemans, J. *Pseudomonas aeruginosa* adapts its iron uptake strategies in function
434 of the type of infections. *Front. Cell. Infect. Microbiol.* **3**, 75 (2013).
- 435 32. Ezraty, B. & Barras, F. The ‘liaisons dangereuses’ between iron and antibiotics. *FEMS Microbiol. Rev.*
436 **40**, 418–435 (2016).
- 437 33. Uivarosi, V. Metal complexes of quinolone antibiotics and their applications: an update. *Molecules* **18**,
438 11153–11197 (2013).
- 439 34. Mathieu, A. *et al.* Discovery and Function of a General Core Hormetic Stress Response in *E. coli*
440 Induced by Sublethal Concentrations of Antibiotics. *Cell Rep.* **17**, 46–57 (2016).
- 441 35. Garbe, T. R., Kobayashi, M. & Yukawa, H. Indole-inducible proteins in bacteria suggest membrane
442 and oxidant toxicity. *Arch. Microbiol.* **173**, 78–82 (2000).

- 443 36. Giroux, X., Su, W.-L., Bredeche, M.-F. & Matic, I. Maladaptive DNA repair is the ultimate contributor
444 to the death of trimethoprim-treated cells under aerobic and anaerobic conditions. *Proc. Natl. Acad.*
445 *Sci. U. S. A.* **114**, 11512–11517 (2017).
- 446 37. Johnson, J. R. *et al.* Contribution of yersiniabactin to the virulence of an Escherichia coli sequence
447 type 69 ('clonal group A') cystitis isolate in murine models of urinary tract infection and sepsis.
448 *Microb. Pathog.* **120**, 128–131 (2018).
- 449 38. Schubert, S. *et al.* Role of Intraspecies Recombination in the Spread of Pathogenicity Islands within
450 the Escherichia coli Species. *PLoS Pathog.* **5**, e1000257 (2009).
- 451 39. Fricke, W. F. & Rasko, D. A. Bacterial genome sequencing in the clinic: bioinformatic challenges and
452 solutions. *Nat. Rev. Genet.* **15**, 49–55 (2014).
- 453 40. Quainoo, S. *et al.* Whole-Genome Sequencing of Bacterial Pathogens: the Future of Nosocomial
454 Outbreak Analysis. *Clin. Microbiol. Rev.* **30**, 1015–1063 (2017).
- 455 41. Tagini, F. & Greub, G. Bacterial genome sequencing in clinical microbiology: a pathogen-oriented
456 review. *Eur. J. Clin. Microbiol. Infect. Dis.* **36**, 2007–2020 (2017).
- 457 42. Nichols, R. J. *et al.* Phenotypic landscape of a bacterial cell. *Cell* **144**, 143–156 (2011).
- 458 43. Tsalik, E. L., Bonomo, R. A. & Fowler, V. G., Jr. New Molecular Diagnostic Approaches to Bacterial
459 Infections and Antibacterial Resistance. *Annu. Rev. Med.* **69**, 379–394 (2018).
- 460 44. Břinda, K., Callendrello, A., Cowley, L. & Charalampous, T. Lineage calling can identify antibiotic
461 resistant clones within minutes. *bioRxiv* (2018).
- 462 45. Bradley, P. *et al.* Rapid antibiotic-resistance predictions from genome sequence data for
463 Staphylococcus aureus and Mycobacterium tuberculosis. *Nat. Commun.* **6**, 10063 (2015).
- 464 46. Galardini, M. Escherichia coli pathogenicity GWAS: input genome sequences. (2019).
465 doi:10.6084/m9.figshare.8866259.v1
- 466 47. Datsenko, K. A. & Wanner, B. L. One-step inactivation of chromosomal genes in Escherichia coli
467 K-12 using PCR products. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 6640–6645 (2000).
- 468 48. Chaveroche, M. K., Ghigo, J. M. & d'Enfert, C. A rapid method for efficient gene replacement in the
469 filamentous fungus Aspergillus nidulans. *Nucleic Acids Res.* **28**, E97 (2000).
- 470 49. Martin, P. *et al.* Interplay between Siderophores and Colibactin Genotoxin Biosynthetic Pathways in
471 Escherichia coli. *PLoS Pathogens* **9**, e1003437 (2013).
- 472 50. Davidson-Pilon, C. *et al.* *CamDavidsonPilon/lifelines: v0.21.0.* (2019). doi:10.5281/zenodo.2638135
- 473 51. Seemann, T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**, 2068–2069 (2014).
- 474 52. Treangen, T. J., Ondov, B. D., Koren, S. & Phillippy, A. M. The Harvest suite for rapid core-genome
475 alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biol.* **15**, 524
476 (2014).
- 477 53. Croucher, N. J. *et al.* Rapid phylogenetic analysis of large samples of recombinant bacterial whole
478 genome sequences using Gubbins. *Nucleic Acids Res.* **43**, e15 (2015).
- 479 54. Page, A. J. *et al.* Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* **31**,
480 3691–3693 (2015).
- 481 55. Lippert, C. *et al.* FaST linear mixed models for genome-wide association studies. *Nature Methods* **8**,
482 833–835 (2011).
- 483 56. Li, H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. *arXiv*
484 [*q-bio.GN*] (2013).
- 485 57. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features.
486 *Bioinformatics* **26**, 841–842 (2010).
- 487 58. UniProt Consortium. UniProt: a hub for protein information. *Nucleic Acids Res.* **43**, D204–12 (2015).
- 488 59. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. Basic local alignment search tool.
489 *Journal of molecular biology* **215**, 403–410 (1990).
- 490 60. Pedregosa, F. *et al.* Scikit-learn: Machine Learning in Python. *J. Mach. Learn. Res.* **12**, 2825–2830
491 (2011).
- 492 61. Van Der Walt, S., Colbert, S. C. & Varoquaux, G. The NumPy array: a structure for efficient numerical
493 computation. *Comput. Sci. Eng.* **13**, 22–30 (2011).
- 494 62. Jones, E., Oliphant, T. & Peterson, P. SciPy: Open source scientific tools for Python. [http://www.
495 scipy.org/](http://www.scipy.org/) (2001).
- 496 63. Cock, P. J. A. *et al.* Biopython: freely available Python tools for computational molecular biology and

- 497 bioinformatics. *Bioinformatics* **25**, 1422–1423 (2009).
- 498 64. Talevich, E., Invergo, B. M., Cock, P. J. & Chapman, B. a. Bio.Phylo: A unified toolkit for processing,
499 analyzing and visualizing phylogenetic trees in Biopython. *BMC Bioinformatics* **13**, 209 (2012).
- 500 65. McKinney, W. & Others. Data structures for statistical computing in Python. in *Proceedings of the 9th*
501 *Python in Science Conference* **445**, 51–56 (2010).
- 502 66. Dale, R. K., Pedersen, B. S. & Quinlan, A. R. Pybedtools: a flexible Python library for manipulating
503 genomic datasets and annotations. *Bioinformatics* **27**, 3423–3424 (2011).
- 504 67. Sukumaran, J. & Holder, M. T. DendroPy: a Python library for phylogenetic computing. *Bioinformatics*
505 **26**, 1569–1571 (2010).
- 506 68. Huerta-Cepas, J., Serra, F. & Bork, P. ETE 3: Reconstruction, Analysis, and Visualization of
507 Phylogenomic Data. *Mol. Biol. Evol.* **33**, 1635–1638 (2016).
- 508 69. Seabold, S. & Perktold, J. Statsmodels: Econometric and statistical modeling with python. in
509 *Proceedings of the 9th Python in Science Conference* **57**, 61 (SciPy society Austin, 2010).
- 510 70. Hunter, J. D. Matplotlib: A 2D Graphics Environment. *Computing in Science Engineering* **9**, 90–95
511 (2007).
- 512 71. Waskom, M. *et al. mwaskom/seaborn: v0.9.0 (July 2018)*. (2018). doi:10.5281/zenodo.1313201
- 513 72. Kluyver, T. *et al.* Jupyter Notebooks—a publishing format for reproducible computational workflows. in
514 *ELPUB* 87–90 (2016).
- 515 73. Köster, J. & Rahmann, S. Snakemake—a scalable bioinformatics workflow engine. *Bioinformatics* **34**,
516 3600 (2018).

517 **Supplementary Figures**



518 **Supplementary Figure 1.** Simulations of statistical power on an unrelated set of
519 complete *E. coli* genomes, using the *pks2* gene as target. The dotted red line indicate
520 the sample size used in the actual analysis.

Phylogroup

A

B1

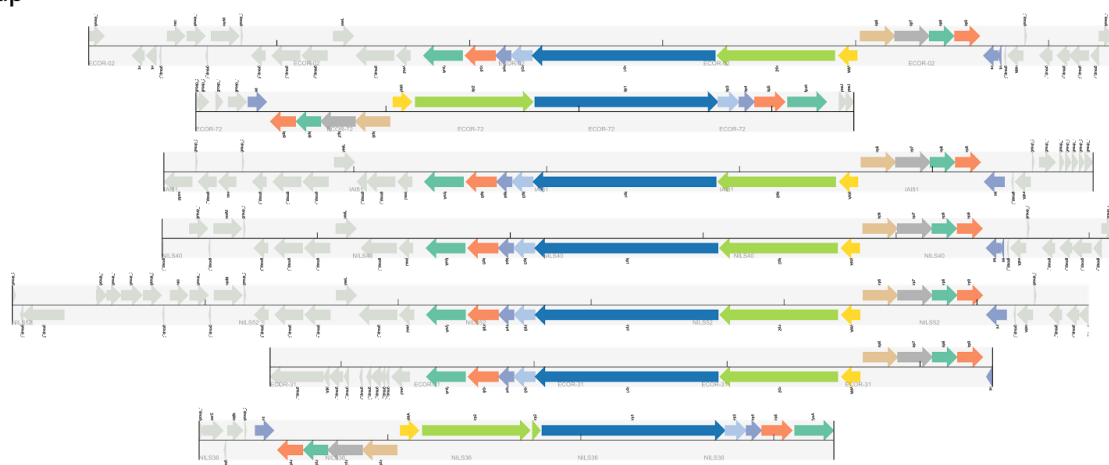
B2

C

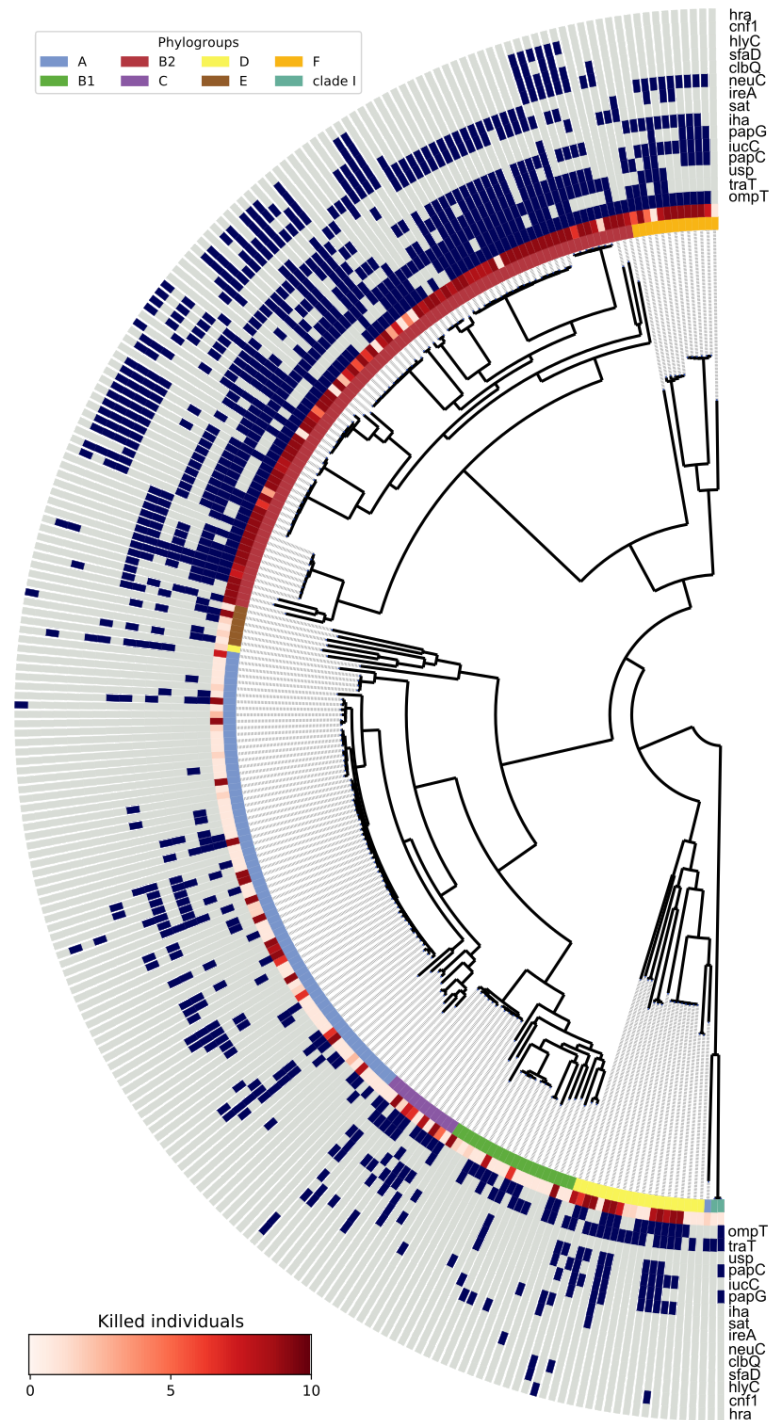
D

E

F



521 **Supplementary Figure 2.** HPI structure conservation across strains. One strain per
522 phylogroup is shown, using the same color scheme as Figure 1E for each gene.



523 **Supplementary Figure 3.** Presence/absence patterns of known virulence factors other than genes belonging to the HPI. Blue indicates presence, light grey indicates absence.
524
525 Phenotypes (number of killed mice) and phylogroup of each strain are reported as in
526 Figure 1A.

527 **Supplementary Information**

528 **Supplementary Table 1:** Strains' information, including virulence phenotype

529 **Supplementary Table 2:** Survival analysis for NILS9 and NILS46 wild-type and HPI
530 mutants

531 **Supplementary Table 3:** Correlation between growth on stress conditions (s-scores)
532 and both virulence and presence of the HPI

533 **Supplementary Table 4:** Feature importance for each growth condition in the random
534 forests predictor for virulence and HPI presence

535 **Supplementary Table 5:** List of PCR primers used in this study