Poultry-Associated Nitrofurantoin-Resistant and Pre-Resistant *Escherichia coli* Clones are Found in Multiple Countries and One-Health Compartments

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Abstract

Escherichia coli is the most common cause of urinary tract infection (UTI) in humans. The nitrofuran-class antibacterial drug nitrofurantoin is a frequent UTI therapy, with resistance rarely observed. Here we show that nitrofurantoin resistant (NFT-R) *E. coli* are sometimes excreted by dogs fed a raw meat diet in the city of Bristol, United Kingdom, and that NFT-R and pre-resistant (one mutation away from NFT-R) *E. coli* can be found contaminating chicken meat sold for human consumption and chicken-based raw dog food in the same city. Using whole genome sequencing, we identified multiple NFT-R or pre-resistant *E. coli* clones spanning several phylogroups. These clones were dominated by isolates from poultry farms and poultry meat in Europe, Canada, the United States and Japan, and we identified instances where closely related NFT-R and pre-resistant isolates have colonised humans and caused UTIs. The origins of these poultry-associated NFT-R and pre-resistant *E. coli* clones are uncertain, but nitrofuran-class antibacterials (particularly furazolidone, furaltadone, and nitrofurazone) were used in poultry production during the 1970s and 80s, though this practice has been banned since the 1990s. It is possible, therefore, that this caused an initial selective pressure for the emergence of NFT-R and pre-resistant *E. coli* clones on poultry farms. Our findings have potentially important implications for domestic hygiene, particularly among people receiving nitrofurantoin therapy.

36 ## 1. Introduction

37 Nitrofurantoin is a nitrofuran-class antibacterial recommended as first line treatment
38 for uncomplicated urinary tract infection (UTI) in adults in the UK, most of which are
39 caused by *Escherichia coli* [1]. Despite widespread use, nitrofurantoin resistance is
40 rare [2].

41 The ability of nitrofurantoin to kill relies on either of two oxygen-insensitive
42 nitroreductases produced in the bacterium, NfsA and NfsB, which reduce nitrofurantoin
43 to liberate highly reactive intermediates [3]. Nitrofurantoin resistance occurs
44 predominately by loss of function mutations, deletions or insertional inactivation of both
45 *nfsA* and *nfsB*, and in rare cases, by mutation of *ribE* or carriage of the plasmid-
46 mediated *oqxAB* efflux pump genes [2, 4-6]. We have recently identified the presence
47 of nitrofurantoin "pre-resistant" urinary *E. coli*, where one gene (more commonly *nfsA*)
48 is disrupted, but the other remains intact. The nitrofurantoin MIC increases, but not
49 above the breakpoint defining clinical resistance [2].

50 The requirement for mutations in two genes – *nfsA* and *nfsB* – may form a barrier to
51 nitrofurantoin resistance emerging among *E. coli* in an infection [7] and there is
52 currently no evidence for nitrofurantoin-resistant (NFT-R) *E. coli* clones circulating in
53 the human population [2]. If such clones emerge, this could spark a rise in NFT-R UTIs.

54 Nitrofuran-class antibacterials (especially furazolidone and furaltadone, though
55 nitrofurantoin itself was never licenced) were used in food producing animals in
56 Europe, particularly poultry, until the 1990s when the European Union banned this due
57 to the risks associated with toxic chemical residues in meat. Similar bans are in place
58 in the USA, Australia, the Philippines, Thailand and Brazil [8]. In some countries,
59 nitrofurantoin and other nitrofuran-class antibacterials are used to treat ornamental

60 birds, homing pigeons and fish, and as an off-label therapy for recurrent UTI in dogs,
61 cats and horses [9], however, in the UK, nitrofurantoin is not licensed for use in any
62 animal [10].

63 Surveys of NFT-R bacteria from animals are scarce, and in the few studies that have
64 considered NFT-R *E. coli*, resistance was very rare or non-existent [11-14], but
65 emergence of NFT-R *E. coli* in dogs during treatment of UTI has been reported [15].

66 We have established a 50 x 50 km study region in the South West of England in which
67 we have sequenced resistant *E. coli* from human infections [2,16-18], and from the
68 faeces of farmed [17,19], companion [20,21], and zoo animals [22]. In the work
69 reported here, we investigated whether NFT-R *E. coli* are excreted by dogs in our
70 study region or are found on meat being sold in stores in the area, and used whole
71 genome sequence (WGS) data to test whether these NFT-R *E. coli* are shared across
72 One Health compartments, including human infections. In doing so, we have identified
73 a reservoir of NFT-R and pre-resistant *E. coli* clones from numerous sequence types
74 (STs) on poultry meat, poultry farms and in dogs fed raw meat. Members of these
75 poultry-associated clones have caused human UTIs.

76

77 **2. Materials and Methods**

78 2.1. Canine faecal sample collection and processing

79 Ethical approval was obtained from the Faculty of Health Research Ethics Committee,
80 University of Bristol (ref: 89282) alongside Ethical Approval of an Investigation
81 Involving Animals (ref: UB/19/057). Details of canine faecal sample collection are
82 published [20,21], but briefly, 297 dogs were recruited at dog-walking sites in the city
83 of Bristol between September 2019 and September 2020. All owners gave informed

consent to participate. Faecal samples were collected and a standardized questionnaire was provided to collect demographic data and variables chosen as being potentially associated with carriage of ABR *E. coli* in dogs. Samples were refrigerated and processed within 48 h. Faecal sample aliquots were weighed (0.1–0.5 g) and PBS was added at 10 mL/g before vortexing and mixing with an equal volume of 50% sterile glycerol. Samples were stored at -70°C.

2.2. Meat sample collection and processing

Details of meat sample collection and processing are published [23], but briefly, we sampled beef, chicken, lamb and pork meat collected from large chain grocery stores in the city of Bristol between October and November 2022, totalling 15 samples per type of meat, except for pork where due to availability only 13 were collected. Samples were either processed on the day or kept at 4°C and processed within the use by date. For raw chicken sold as raw dog food (RDF), one of each brand sold across five RDF stores in Bristol was purchased. A total of 15 packets were collected in September 2023, covering 11 brands of RDF.

Under sterile conditions, 200g of each meat/RDF sample was placed into a stomacher bag with 200 mL of EC broth (Oxoid ltd). Bags were sealed and incubated for 5 h at 37°C, 180 rpm. Each liquid culture was aliquoted into Eppendorf tubes containing sterile glycerol (to 25% v/v final) and stored at -70°C.

2.3. Microbiology

106 Twenty microlitres of each thawed liquid culture aliquot (above) was spread onto
107 Tryptone Bile X-Glucuronide agar plates (Sigma) containing Nitrofurantoin (64 mg/L)
108 based on EUCAST resistance breakpoints for human infections [24] and incubated
109 overnight at 37°C. The media were quality control checked by plating known NFT-R
110 and susceptible isolates. Putative NFT-R *E. coli* were re-streaked using the same
111 medium to confirm resistance. Isolates were stored on cryobeads at -70°C. Resistance
112 was confirmed by MIC testing using broth microdilution according to EUCAST
113 methodology [24].

114

115 2.4. WGS and phylogenetic analysis

116 WGS was performed by MicrobesNG to achieve a minimum 30-fold coverage.
117 Preparation of isolates was carried out following MicrobesNG strain submission
118 procedures as described [25]. Genomes were sequenced on an Illumina NovaSeq
119 6000 (Illumina, San Diego, USA) using a 250 bp paired end protocol. Sequencing data
120 were analysed using ResFinder 4.4.2 [26], Hound [27] and MLST 2.0 [28].

121 Sequence alignment and phylogenetic analysis was carried out using the
122 computational facilities of the Advanced Computing Research Centre, University of
123 Bristol (http://www.bristol.ac.uk/acrc/) and the Bioconda environment management
124 system [29]. Alignment with reference sequences (**Table S1**) was performed using
125 Snippy and Snippy-Core software (https://github.com/tseemann/snippy) and SNP
126 distances were determined using SNP-dists (https://github.com/tseemann/snp-dists).
127 Maximum likelihood phylogenetic trees were produced using RAxML and the
128 GTRGAMMA model of rate of heterogeneity [30]. Phylogenetic trees were illustrated
129 using Microreact [31]. Where only raw sequence reads were available on Enterobase

130 [32], SPAdes [33] was used to assemble these sequences prior to inclusion into the
131 phylogenetic analysis.

132

133 **3. Results**

134 3.1. NFT-R *E. coli* excreted by dogs being fed raw (uncooked) meat.

135 We tested faecal samples from 297 healthy dogs living in the city of Bristol. Two dogs
136 excreted NFT-R *E. coli* at detectable levels. The NFT-R *E. coli* were ST6805 and
137 ST919 and both had loss-of-function mutations in *nfsA* and *nfsB* (**Table 1**).

138 We collected owner-completed survey data about the lives of these 297 Bristol dogs
139 [20,21]. Since only two dogs excreted NFT-R *E. coli* it is not possible to be confident
140 about any statistical assessment of associations between excretion and any lifestyle
141 practice. Nonetheless, we noted (**Table S2**) that both dogs were fed raw meat at the
142 time of faecal sampling, which was a practice observed for only 31/297 dogs (Fisher
143 Exact p=0.011).

144

145 3.2. NFT-R *E. coli* in uncooked chicken meat and chicken-based commercial RDF.

146 In the UK, raw meat fed to dogs is sourced as commercial RDF or meat sold primarily
147 for human consumption (once cooked) [34]. Accordingly, to investigate whether raw
148 meat potentially being fed to dogs in the city of Bristol was contaminated with NFT-R
149 *E. coli*, we tested 73 samples of meat from across the city. These were 15 packets
150 each of beef, chicken and lamb, and 13 of pork purchased from large-chain grocery
151 stores, and 15 packets of chicken-based commercial RDF of various brands.

152 Two out of 58 (3%) samples of meat from large-chain grocery stores tested positive
153 for NFT-R *E. coli*; both were chicken meat. WGS showed that these two isolates had
154 loss of function mutations in *nfsA* and *nfsB* and were ST665 and ST8874 (**Table 1**).
155 Out of fifteen packets of chicken-based commercial RDF, one (7%) was positive for
156 NFT-R *E. coli.* WGS showed this to be ST8874, the same ST found in one packet of
157 chicken meat (**Table 1**).

158

159 3.3. Poultry-associated NFT-R and NFT pre-resistant *E. coli* clones found across
160 multiple countries and One Health compartments, including human UTI

161 The NFT-R ST8874 and ST919 isolates found on meat or excreted by one dog in
162 Bristol had the same mutations in *nfsA* and in *nfsB* and none of them carried acquired
163 resistance genes (**Table 1**). These STs are single-locus variants: *adk*38 in ST8874
164 versus *adk*861 in ST919 but otherwise they share the same multi-locus ST alleles.

165 We found 71 ST919 single-locus variant genomes with available sequence data on
166 Enterobase (search date, 2nd May 2025) (**Table S3**). Of these, 16 carried *nfsA/B*
167 mutations identical to the Bristol ST919/ST8874 isolates reported above, with the rest
168 being wild-type (**Table S3**). One of the *nfsA/B* mutants was an ST8874 isolate from a
169 chicken yolk sac infection in Czechia [35]. The other 15 *nfsA/B* mutants were ST919,
170 of which one isolate is a published NFT-R *E. coli* from a human UTI from London, UK
171 [6]. Of the remaining *nfsA/B* mutant ST919 genomes on Enterobase, all are from
172 chicken caecal contents/faeces or chicken meat. They are from the UK, Canada, the
173 USA and Japan (**Table S3**).

174 We performed a core genome alignment of the ST919/ST8874 *nfsA*/*B* mutant isolates
175 and present a matrix of single nucleotide polymorphism (SNP) distances between

176 pairs of isolates (**Table S4**) and a phylogenetic tree (**Fig. 1**). The two Bristol ST8874
177 meat isolates and the Czech yolk sac infection isolate were very closely related to
178 each other (<60 SNPs across a core genome alignment), and to an ST919 UK poultry
179 isolate on Enterobase (<100 SNPs) which was only 33 SNPs different from the NFT-
180 R human UTI isolate from London, UK. The ST8874 clone appears to have branched
181 off from this UK ST919 clone (**Fig. 1**). However, the ST919 isolate excreted by a dog
182 in Bristol was more closely related to chicken isolates from the USA and Canada (49-
183 73 SNPs) than to the ST919 UK chicken isolate (172 SNPs). The ST919 isolates from
184 North America were variable in their relatedness but did noticeably cluster together
185 (**Fig. 1**). The most diverse isolate was from Japan (**Table S4**, **Fig. 1**).

186 After establishing the existence of a poultry-associated global NFT-R ST919/ST8874
187 clone (**Fig. 1**), we next considered whether the ST665 and ST6805 NFT-R *E. coli* from
188 meat and dogs in Bristol (**Table 1**) also cluster into widely dispersed clones. We found
189 95 ST665 sequences on Enterobase. Of 77 with a defined origin, 46 (60%) were from
190 poultry meat or poultry farms and 12 (16%) from humans. Two of these human isolates
191 and three isolates from chicken faeces, all from the UK had the same *nfsA* (138FS)
192 and *nfsB* (101Stop) mutations as the NFT-R ST665 isolate from chicken meat in Bristol
193 (**Table S5**).

194 Multiple human-derived ST665 isolates from across Europe and into the Middle East
195 had loss-of-function mutations in *nfsA* but wild-type *nfsB* (**Table S5**) and so are
196 considered nitrofurantoin pre-resistant [2]. In some cases this was the same 138FS
197 *nfsA* mutation seen in the UK NFT-R isolates, with other common *nfsA* mutations being
198 46FS and 49FS. These same three mutations were observed among ST665 isolates
199 from poultry and wild birds in multiple countries (**Table S5**).

200 A SNP distance analysis revealed that the six UK NFT-R *nfsA* (138FS), *nfsB* (101Stop)
201 isolates are diverse. For example, the Bristol chicken meat isolate is 1773 SNPs away
202 from the closest UK chicken faeces isolate and 2095 away from the closest UK human
203 isolate. Furthermore, the chicken faeces *nfsA* (138FS), *nfsB* (101Stop) isolates were
204 at least 1964 SNPs away from the human isolates (**Table S6**). This divergence was
205 reflected in the resulting phylogenetic tree (**Fig. 2**). In other regards the isolates from
206 Enterobase phylogenetically clustered based on their *nfsA* allele (**Fig. 2**). Indeed, it
207 appears from this analysis that the NFT-R ST665 NFT-R *nfsA* (138FS), *nfsB* (101Stop)
208 isolates have evolved from three divergent 138FS lineages (**Fig. 2**).

209 For all three main ST665 nitrofurantoin pre-resistant lineages (46FS, 49FS or 138FS
210 *nfsA* alleles but having wild-type *nfsB*) there are instances of likely sharing between
211 poultry, wild birds and humans (**Table S6, Fig. 2**). For example, the 46FS human
212 isolates from the Netherlands and France are 94 and 91 SNPs, respectively, different
213 from a chicken isolate from the Netherlands. A Spanish human 138FS isolate was 42
214 SNPs different from an isolate from a wild Stork, also from Spain. Finally, a Polish
215 human 49FS isolate was 207 SNPs different from a Hungarian chicken isolate.

216 Among four ST6805 isolates on Enterobase, one Danish poultry isolate
217 (SAMEA112328101) shared the same *nfsA* mutation as a Bristol dog isolate (**Table**
218 **1**), but it had a wild type *nfsB*, hence is also considered pre-resistant. These two
219 isolates are 2281 SNPs apart, however, so there is no evidence of a circulating clone.

220 Based on the above findings, we suspected that there might be additional
221 nitrofurantoin pre-resistant clones present on chicken meat being sold in Bristol. To
222 test this, we sequenced 92 nitrofurantoin susceptible *E. coli* from RDF and 76 from
223 uncooked chicken meat, cultured from the same samples as the NFT-R isolates

224 (above), but which did not grow on nitrofurantoin agar at a concentration required to
225 define them as resistant. We found 17 isolates from 12/15 samples of chicken meat
226 and 10 isolates from 4/15 samples of chicken-based RDF that had obvious loss of
227 function in *nfsA* but wild-type *nfsB* and so are defined as pre-resistant. These isolates,
228 accounting for 16.1% of all isolates from chicken meat or RDF sequenced, spanned
229 11 STs (**Table S7**). They included ST665 isolates from two chicken and one RDF
230 samples with identical *nfsA* alleles (46FS). These had pairwise SNP distances ranging
231 from 110 to 355, and are part of the widely disseminated 46FS pre-resistant clone,
232 defined above, with one chicken and one RDF isolate being 84 and 96 SNPs different,
233 respectively, from a human isolate from the Netherlands (**Table S6; Fig. 2**).

234 Pre-resistant ST69 isolates with identical *nfsA* mutations were identified from six
235 chicken meat and one RDF samples collected in Bristol with SNP distances of 10 to
236 41 between them (**Table S7**). The other STs found to include *nfsA* mutants were ST10,
237 ST57, ST58, ST155, ST162 and ST2705. Furthermore, for ST7529 and ST752 *nfsA*
238 mutants found on the chicken meat/RDF in Bristol, we found overlap with human
239 isolates. For ST7529, one human clinical Enterobase isolate from the UK was found
240 to be 103 SNPs different from the two Bristol RDF isolates, which were themselves
241 identical. All carried the same *nfsA* loss of function mutation (**Table S7**).

242 Of ST752 isolates on Enterobase (n=503), the majority were from poultry (33.0%) or
243 human (28.2%) sources. Of these, 91.9% of human isolates and 84.9% of poultry
244 isolates carried the same *nfsA* mutation as also seen in the Bristol chicken meat
245 isolates suggesting they are nitrofurantoin pre-resistant. We selected ST752 *nfsA*
246 mutant Enterobase isolates from poultry and humans (n=10 for each) for comparison
247 with the six isolates from chicken meat in Bristol (**Table S7**). Two human isolates, one
248 from the UK and the other from the Netherlands were 43 and 61 SNPs different,

249 respectively, from one of the Bristol chicken meat isolates (**Table S7; Table S8**).
250 Furthermore, there were four incidences where ST752 isolates from humans were
251 <100 SNPs from Enterobase poultry isolates, with the closest pair being 48 SNPs
252 apart (**Table S7; Table S8**).

253

254 **4. Discussion**

255 Because nitrofurantoin is not licenced for use in dogs in the UK, we were surprised to
256 find NFT-R *E. coli* in faeces excreted by two dogs out of a cohort of 297 based on
257 testing one faecal sample per dog with a limit of detection of approximately 1000 viable
258 bacteria per gramme of faeces. The NFT-R *E. coli* from one dog is clonally related to
259 those found in poultry meat and poultry farm environment samples in the UK, Canada,
260 Czechia and Japan, including two single-locus variant STs (**Fig. 1**). Given that we also
261 found NFT-R *E. coli* from this same clone in uncooked chicken meat, and chicken-
262 based RDF purchased in the same city, a possible explanation for these dogs
263 excreting poultry-associated NFT-R *E. coli* is the fact – reported by their owners – that
264 they were being raw fed at the time of sampling. Whilst we have shown compelling
265 evidence that feeding raw meat to dogs is associated with them excreting
266 fluoroquinolone resistant *E. coli* [21], the evidence for NFT-R *E. coli* presented here is
267 much weaker. This may be related to the relative degrees of contamination of meat
268 with *E. coli* resistant to these different agents. For example, we identified here 10%
269 (3/30) of chicken meat or chicken-based RDF samples were positive for NFT-R *E. coli*,
270 but we have also reported that 47% (14/30) of these same samples were positive for
271 fluoroquinolone-resistant *E. coli* [23].

272 Several studies have reported sharing of resistant *E. coli* between dogs and their
273 owners [36-39], so it is possible that dogs excreting NFT-R *E. coli* may pass these on
274 to their owners. This is in addition to any associated risks of accidental ingestion when
275 handling and preparing RDF or other uncooked poultry, which we have shown here
276 can be contaminated with numerous NFT-R or pre-resistant *E. coli* clones, particularly
277 ST665 and ST752, which are also found in clinical samples from humans. Accordingly,
278 it would seem prudent for those at risk of UTI, and particularly those who are taking
279 nitrofurantoin, to be cautious when handling uncooked meat or interacting with pets
280 fed uncooked meat, and practice enhanced hygiene regimes. This is also important
281 for people taking other antibiotics, and particularly ciprofloxacin, but since the number
282 of nitrofurantoin courses dispensed in the community in some countries is very high,
283 and since resistance rates among urinary *E. coli* from humans are very low, the
284 additional zoonotic burden exposed here is potentially important.

285 Whilst nitrofuran antibacterial use in poultry production was banned in many countries
286 decades ago due to toxicity concerns if residues are found in meat [11], it is notable
287 that SNP distances between isolates from the various NFT-R and pre-resistant clones
288 reported here are large, possibly because they emerged many years ago.

289 It remains unclear how prevalent NFT-R or pre-resistant *E. coli* are in meat sold for
290 human consumption throughout the UK and around the world, and whether other
291 poultry-associated NFT-R or pre-resistant clones exist, but the novel findings of this
292 study should stimulate interest in answering that question through widespread
293 surveillance in multiple One Health compartments.

294

295

296 **Data Availability**

297 Novel WGS data are deposited with ENA under accessions PRJEB74489 (Isolates
298 from meat) and PRJEB74488 (isolates from canine faeces).

299

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307

308 **Author Contributions**

309 Conceived the Study: J.E.S., M.B.A.

310 Collection of Data: J.E.S, B.A.

311 Cleaning and Analysis of Data: J.E.S. O.M.

312 Initial Drafting of Manuscript: J.E.S., M.B.A.

313 Corrected and Approved Manuscript: All authors

314

315

316

Declaration of Competing Interest

M.B.A. is married to the owner of a veterinary practice that sells various mass-manufactured dog foods amounting to a value less than 5% of total turnover. Otherwise, none of the above named authors is declaring any conflict of interest in relation to the above titled submitted manuscript.

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Table 1 NFT-R *E. coli* isolates excreted by dogs, and from raw meat in Bristol, UK

			Effect of non-synonymous mutations relative to *E. coli* K12		
Isolate	ST	Phylo-group	NfsA	NfsB	Other resistance genes
RCM1	ST8874	B2	I117T, K141E, G187D, 212Stop	46Stop	None
RCM2	ST665	A	138FS	M75I, V93A, 101Stop	*aadA1*, *aph(3")-Ib*, *aph(6)-Id*, *sul1*, *sul2*, bla_{TEM-1B}, *tet*(A)
RDF15	ST8874	B2	I117T, K141E, G187D, 212Stop	46Stop	None
DOG1030	ST919	B2	I117T, K141E, G187D, 212Stop	46Stop	None
DOG1110	ST6805	A	Insertional inactivation after codon 225	M75I, 79Stop	*aadA5*, *dfrA17*, *sul2*, *tet*(A)

Isolates were from raw chicken meat sold for human consumption (RCM), chicken raw dog food (RDF), and canine faeces (DOG), sequence type (ST) and missense and nonsense mutations, deletions and insertions affecting NfsA and NfsB sequences are noted. Green represents those variants predicted to be functionally wild-type. Red represents those conferring loss of function [2].

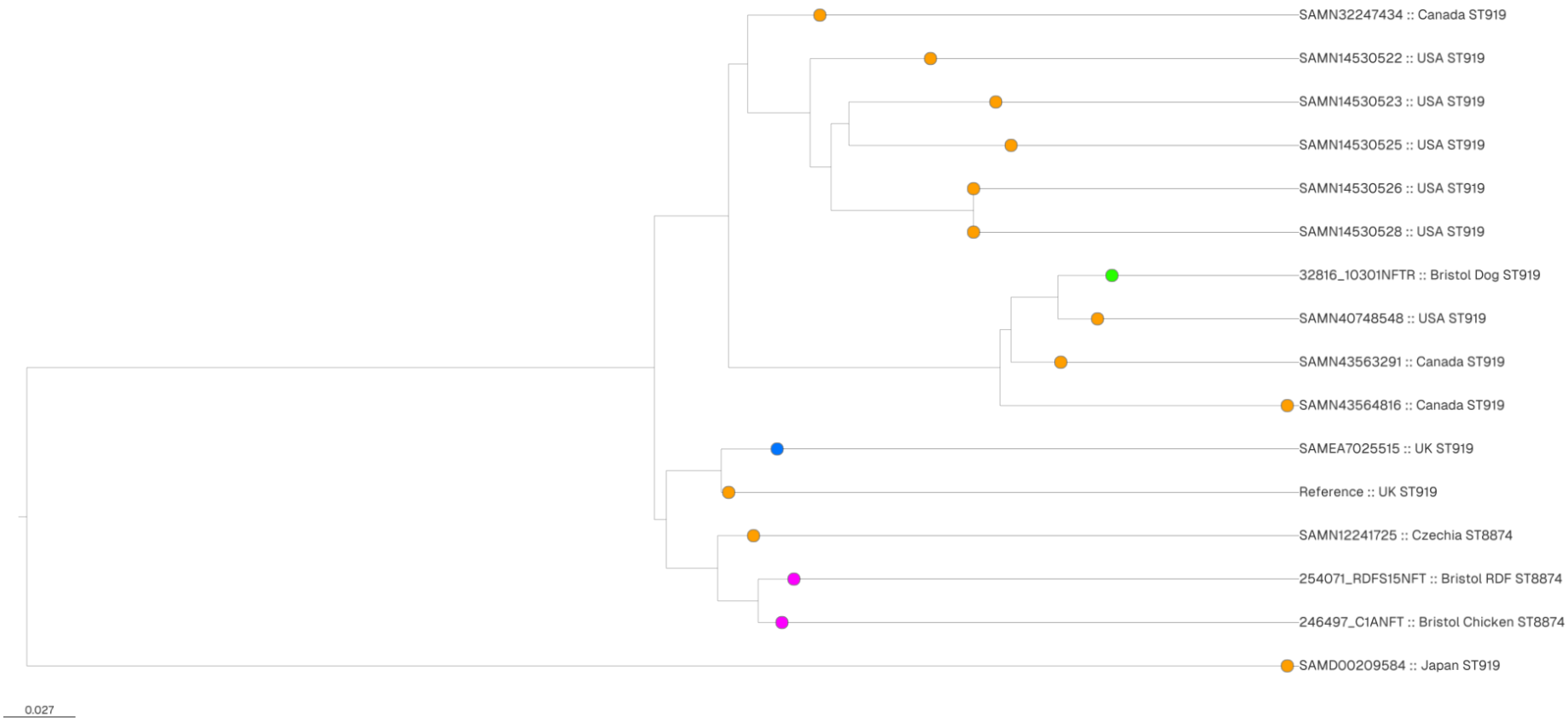

Figure 1. Maximum Likelihood phylogenetic tree based on a core genome alignment illustrating the evolutionary relationship between NFT-R ST919/ST8874 NfsA/NfsB mutant isolates. Core genomes from Enterobase isolates (from poultry sources, orange; from human sources, blue) and chicken meat/RDF (pink) or dogs (green) from Bristol were aligned using Snippy and Phylogenetic trees were constructed based on RaXML analysis. Isolate origins as reported in Enterobase and their STs are noted.

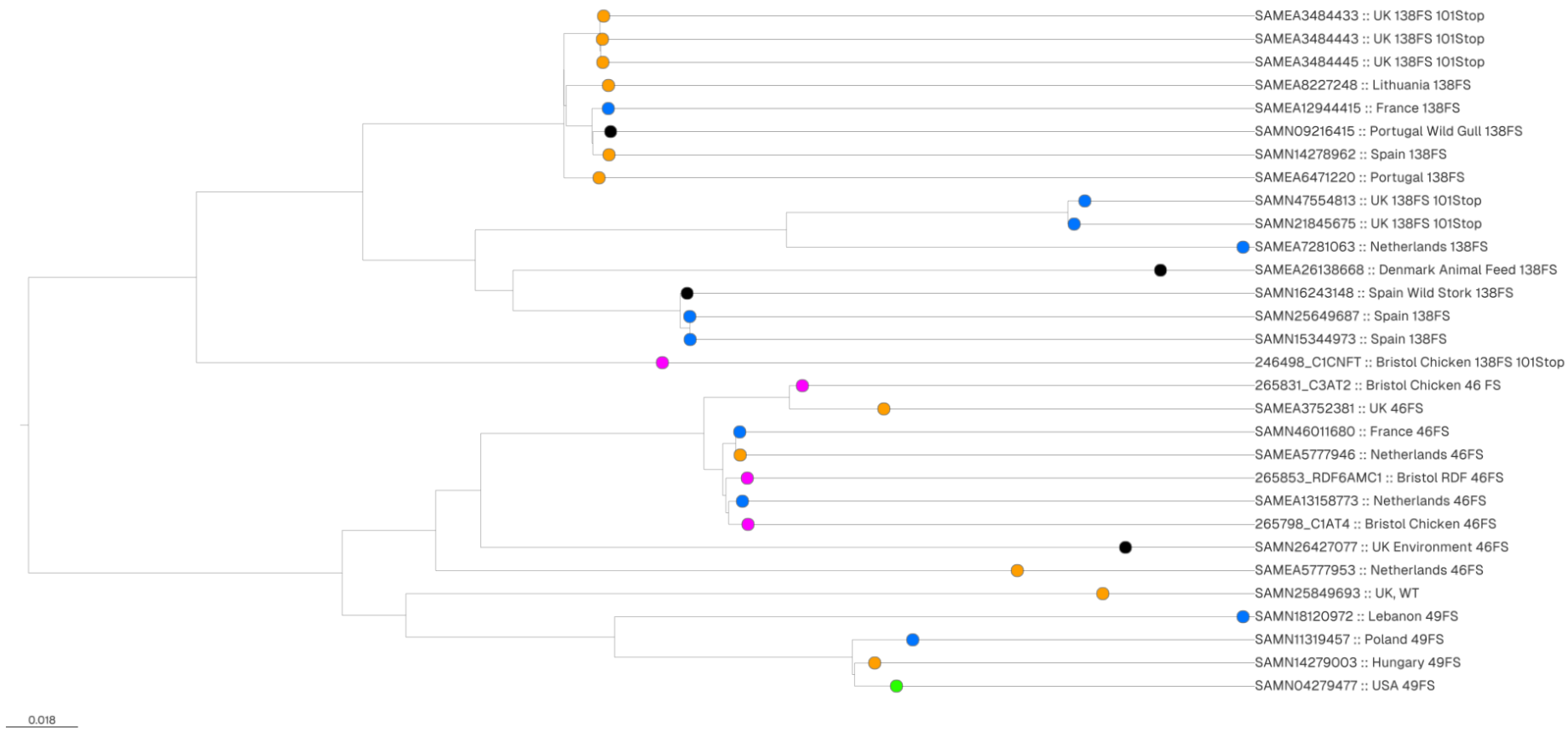

Figure 2. Maximum Likelihood phylogenetic tree based on a core genome alignment illustrating the evolutionary relationship between NFT-R and pre-resistant ST665 isolates. Core genomes from Enterobase isolates (from poultry sources, orange; from human sources, blue; from dogs, green; from other sources as noted, black) and chicken meat/RDF isolates from Bristol (pink) were aligned using Snippy and Phylogenetic trees were constructed based on RaXML analysis. Isolate origins as reported in Enterobase and the NfsA mutations (plus NfsB 101Stop where relevant) are noted.

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