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and blaTEM-190 in a dog from the United Kingdom**

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Occurrence and characterisation of *Escherichia coli* Sequence Type 410 co-harbouring *bla*_{NDM-5}, *bla*_{CMY-42} and *bla*_{TEM-190} in a dog from the United Kingdom

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Running title: NDM-5 producing *Escherichia coli* ST410 in a UK dog

26 SYNOPSIS

27 Background/ Objectives

28 Carbapenemase-producing Enterobacteriaceae (CPE) are a public health threat, and have
29 been found in humans, animals and the environment. Carbapenems are not authorised for use
30 in EU or UK companion animals, and the prevalence of carbapenem-resistant Gram-negative
31 bacilli (CRGNB) in this population is unknown.

32 Methods

33 We investigated CRGNB isolated from animal specimens received by one diagnostic
34 laboratory from 34 UK veterinary practices (Sept 2015-Dec 2016). Any Gram-negative
35 isolates from clinical specimens showing reduced susceptibility to fluoroquinolones and/or
36 aminoglycosides and/or cephalosporins were investigated phenotypically and genotypically
37 for carbapenemases. A complete genome assembly (Illumina/Nanopore) was generated for
38 the single isolate identified to investigate the genetic context for carbapenem resistance.

39 Results

40 One ST410 *Escherichia coli* isolate [(CARB35); 1/191, 0.5%], cultured from a wound in a
41 springer spaniel, harboured a known carbapenem-resistance gene (bla_{NDM-5}). The gene was
42 located in the chromosome on an integrated 100kb IncF plasmid, also harbouring other drug
43 resistance genes (*mrx*, *sul1*, *ant1*, *dfrA*). The isolate also contained bla_{CMY-42} and $bla_{TEM-190}$
44 on two separate plasmids (IncI1 and IncFII, respectively), which showed homology with
45 other publicly available plasmid sequences from Italy and Myanmar.

46 Conclusions

47 Even though the use of carbapenems in companion animals is restricted, the concurrent
48 presence of bla_{CMY-42} and other antimicrobial resistance genes could lead to co-selection of

49 carbapenemase genes in this population. Further studies investigating the selection and flow
50 of plasmids carrying important resistance genes amongst humans and companion animals are
51 needed.

52

53 ***Introduction***

54 Carbapenemase-producing Enterobacteriaceae (CPE) are a serious public health problem due
55 to limited therapeutic options for CPE-associated infections.¹ Increased carbapenem use,
56 especially for treating infections caused by ESBL-producers, is a significant driver of CPE
57 emergence in human medicine.² In contrast, carbapenems are not authorised for veterinary
58 use^{3,4} except for the management of MDR Gram-negative infections under the prescribing
59 cascade

60 ([https://www.gov.uk/guidance/the-cascade-prescribing-unauthorised-medicines#special-](https://www.gov.uk/guidance/the-cascade-prescribing-unauthorised-medicines#special-considerations-for-the-responsible-use-of-antibiotics-under-the-cascade)
61 [considerations-for-the-responsible-use-of-antibiotics-under-the-cascade](https://www.gov.uk/guidance/the-cascade-prescribing-unauthorised-medicines#special-considerations-for-the-responsible-use-of-antibiotics-under-the-cascade)).

62 Furthermore, there is limited carbapenem resistance testing and no UK national surveillance
63 of CPE prevalence in companion animals. Consequently, occurrence of carbapenemase-
64 producing bacteria in animals may remain undetected. Here, we report surveillance data from
65 a UK Veterinary Diagnostics Laboratory which introduced carbapenem-resistance screening
66 for Gram-negative bacteria. We also describe the molecular characterisation of a NDM-5
67 producing *E. coli* isolates isolated from a wound in a dog.

68

69 ***Materials and methods***

70 Bacterial isolates cultured from clinical specimens submitted September 2015-December
71 2016 to one UK diagnostic laboratory were included in this study. Clinical specimens were

72 received from 34 veterinary practices across England (n=29), Wales (n=4) and Ireland (n=1)
73 and included swabs, urine, tissues, sterile fluids, bronchoalveolar lavages, faecal samples
74 (cats, dogs) and bovine milk samples. To increase detection of all carbapenemase-producing
75 isolates (including OXA-48 producers), any Gram-negative bacteria with reduced
76 susceptibility to fluoroquinolones, aminoglycosides and/or cephalosporins cultured were
77 tested using chromID Carba SMART agar bi-plates (bioMérieux, Basingstoke, UK).

78

79 Each half of the bi-plates was inoculated with 10µl of fresh, pure culture (0.5 MacFarland
80 suspension), and incubated aerobically (37±2°C, 22-24 hours). *Klebsiella pneumoniae*
81 NCTC13368 (SHV-18 [ESBL]) was used as a negative control and *K. pneumoniae*
82 NCTC13438 (KPC-3), NCTC13440 (VIM-1) and NCTC13442 (OXA-48) as positive
83 controls. Isolates exhibiting characteristic growth were identified using either API
84 (20E/20NE, bioMérieux UK Ltd) or MALDI-TOF (Laboklin, Germany).

85

86 Susceptibility testing for the carbapenem-resistant isolate identified in this study was
87 performed by broth microdilution (TREK Diagnostic System, West Sussex, UK), interpreted
88 according to the **European Committee on Antimicrobial Susceptibility testing guidelines**
89 (version v7.0, at:

90 [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_B](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf)
91 [reakpoint_Tables.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf)).

92

93 Bacterial DNA was extracted by heat lysis and centrifugation, and used to screen for:
94 carbapenemases (*bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{KPC}); ESBLs (*bla*_{CTX-M}, *bla*_{TEM},
95 *bla*_{SHV}, *bla*_{GES}, *bla*_{PER}, *bla*_{VEB}); plasmid-mediated pAmpC-group genes,^{5,6} and colistin

96 resistance (*bla*_{MCR-1}, *bla*_{MCR-2}; [https://www.eurl-ar.eu/CustomerData/Files/Folders/21-](https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/278_mcr-multiplex-pcr-protocol-v2-oct16.pdf)
97 [protocols/278_mcr-multiplex-pcr-protocol-v2-oct16.pdf](https://www.eurl-ar.eu/CustomerData/Files/Folders/21-protocols/278_mcr-multiplex-pcr-protocol-v2-oct16.pdf)) as previously described.

98

99 *Whole genome sequencing and analysis*

100 The carbapenem-resistant *E. coli* isolate was re-cultured from stock; DNA was extracted
101 using the Qiagen Genomic-tip 100/G kit (Qiagen, Hilden, Germany). Aliquots of the same
102 DNA extract were sequenced on both the Illumina HiSeq 4000 and Oxford Nanopore
103 Technologies' MinION (library preparation kit: SQK-LSK208, flowcell: FLO-MIN106 R9.4,
104 as in Phan HTT *et al.*⁷ Sequence data have been deposited in the NCBI (BioProject:
105 PRJNA473397).

106

107 A hybrid, complete genome assembly was constructed from the two sequencing datasets
108 using Unicycler (v4.1; parameters: --no_correct --min_component_size 500 --
109 min_dead_end_size 500 --verbosity 1 --mode bold). The Unicycler assembly was also
110 compared with a hybridSPAdes (v.3.6; default parameters, "careful" option) assembly to
111 verify the genome structure using a different assembly method.

112

113 In silico MLST typing was performed using BLASTn against the PubMLST allele databases
114 (available at <https://pubmlst.org/general.shtml>). Plasmid typing, insertion sequence typing
115 and resistance gene characterisation were carried out using PlasmidFinder, the ISFinder
116 database and an in-house script (ResistType), as previously described.⁷ The chromosomally
117 integrated plasmid sequence and multi-drug resistance region harbouring *bla*_{NDM-5} were
118 compared with publicly available plasmid sequences in GenBank using BLASTn, with

119 default settings. Data visualisations were created using the GenomeDiagram module in
120 Biopython.

121

122 **Results**

123 One hundred and ninety-one Gram-negative isolates from dogs (n=158), cats (n=27), cattle
124 (n=4), a rabbit and a guinea pig, were sub-cultured onto chromID Carba SMART bi-plates; of
125 these, 28 isolates generated moderate-heavy growth, where *Acinetobacter* spp. (n=4) and
126 *Pseudomonas* spp. (n=23) grew on one or both halves, whilst *Escherichia coli* (1/191) grew
127 on the CARB side only.

128

129 No *bla*_{ESBL}/*pAmpC* or carbapenem-resistance genes were identified in cultured *Pseudomonas*
130 spp. and *Acinetobacter* spp. which were most likely selected due to their intrinsic resistance
131 (decreased permeability and/or expression of efflux pumps) to the agents included in the
132 CARBA-SMART plates. However, the *E. coli* isolate (isolate CARB35) harbored *bla*_{NDM}
133 (confirmed on sequencing to be *bla*_{NDM-5}), *bla*_{CMY} and *bla*_{TEM}. The NDM-producing *E. coli*
134 was cultured (pure growth) from a foreleg wound on the 5th digit of a 7-year-old English
135 springer spaniel. The dog had a history of foot lacerations, dog bite wounds and urinary tract
136 infections, for which he received multiple courses of amoxicillin/clavulanate, cefovecin,
137 doxycycline and enrofloxacin in the preceding six years.

138

139 The NDM-producing *E. coli* was resistant to ampicillin, cefoxitin, aztreonam, cefazolin,
140 cefepime, cefpodoxime (all >16µg/mL), amoxicillin/clavulanate (>32 µg/mL),
141 piperacillin/tazobactam, ticarcillin/clavulanate (>64 µg/mL), meropenem, imipenem

142 (4µg/mL), ciprofloxacin(2µg/mL), levofloxacin (>8 µg/mL), and
143 trimethoprim/sulfamethoxazole (>4 µg/mL). The isolate remained susceptible to gentamicin,
144 amikacin (≤1 and ≤4 µg/mL, respectively), tigecycline (≤0.25 µg/mL), and
145 colistin/polymyxin B (≤0.25 µg/mL).

146

147 The two assemblers agreed on a complete assembly for the NDM-5-producing *E. coli* isolate
148 which included a chromosome (~4.9Mb; ST410) and five plasmids (~3kb, 4kb, 59kb [IncI1],
149 89kb [IncFII], 90kb [IncY). *bla*_{NDM-5} was chromosomally integrated into the *E. coli* genome,
150 flanked by multiple IS elements, in an 18.6kb configuration harbouring other drug resistance
151 genes (*mrx*, *sull*, *ant1*, *dfrA*) (Fig.1). A similar flanking sequence for *bla*_{NDM-5} has been
152 observed in only a handful of publicly available but largely unpublished sequences, including
153 submissions from the US, China and Germany (Fig.S1). This multi-drug resistance region
154 was nested within a 100kb region encoding plasmid-associated genes, including IncFII,
155 IncFIA and IncFIB replicons, and flanked by two *IS150* insertion sequences in the same
156 orientation, most consistent with it representing an integrated plasmid (Fig. 1). This 100kb
157 region had 99% sequence identity over 86% of its length to the reference plasmid sequences
158 CP024860.1 (172kb; submitted Nov-2017 by NIH, USA; isolation source unknown) and
159 KP789020.1 (*E. coli* WCHEC13-8 plasmid pCTXM15 harbouring *bla*_{NDM-1} and *bla*_{CMY-42}
160 from Chengdu, China; 56kb; submitted Nov-2015; human clinical isolate).

161

162 The IncI1 plasmid (59kb), carrying *bla*_{CMY-42}, had a 99% sequence match to *E. coli* plasmid
163 tig00001287_pilon (GenBank accession: CP021882.1, 68kb);the IncFII plasmid (89kb),
164 carrying *bla*_{TEM-190}, shared 99% sequence identity over 93% of the sequence query with two
165 *E. coli* plasmids (GenBank accessions: KY463220.1, AP018147.1).

166

167 Genes/gene mutations encoding resistance to spectinomycin/streptomycin (*aadA2* -
168 chromosome, positions: 194104-194895), trimethoprim/trimethoprim-sulfamethoxazole
169 (*dfrA12* - chromosome, positions: 193199-193696; *sulI* - chromosome, positions: 195400-
170 196239; *folP* - chromosome, positions: 680079-680927), macrolides-lincosamide-
171 streptogramin (*mphA* - chromosome, positions: 202749-203654 and plasmid pCARB35-2,
172 positions: 83836-84741), nitrofurantoin (in *nfsA*: chromosome, positions: 3510946-3511599)
173 and fluoroquinolones (*gyrA* - chromosome, positions: 1676727-1679354; *parC* -
174 chromosome, positions: 841664-843922; *parE* - chromosome, positions: 831111-833003),
175 and *fimE* (chromosome, positions: 4197047-4197643) and *usp* (chromosome, positions:
176 2077529-2077957, 2620969-2621403, and 3475153-3475581) virulence factors, were also
177 identified.

178

179 **Discussion**

180 ESBL/AmpC-producing Enterobacteriaceae have emerged in food-producing and companion
181 animals over the past two decades.⁸ Although still rare, carbapenemase-producers, mainly
182 NDM-1 *Acinetobacter* spp., VIM-1 *E. coli* and *Salmonella* spp., have been reported
183 worldwide in livestock.⁹ However, there are very few reports of CPE in companion animals.
184 NDM-1-producing *E. coli* was first described in dogs and cats from the US in 2013, only four
185 years after the first description of NDM-1-producing bacteria in humans.¹⁰ OXA-48-
186 producing *E. coli* and *Klebsiella* spp. were first reported in dogs from Germany and
187 subsequently also in clinical canine isolates in the US, including pandemic strains such as *E.*
188 *coli* ST648.^{11, 12} However, a low prevalence (0.6%, n=160) of CPE, consisting of a single
189 VIM-1-producing *K. pneumoniae* isolate, was found amongst companion animals from

190 Spain.¹³ More recently, IMP-4-producing *Salmonella* Typhimurium was isolated from a cat
191 with persistent haemorrhagic diarrhoea in Australia¹⁴ and OXA-23-producing *A. baumannii*
192 associated with urinary tract infection was detected in a cat from Portugal.¹⁵

193

194 NDM-5 differs from NDM-1 by two amino acid substitutions and has been described in
195 Enterobacteriaceae from both humans and livestock, mainly in Asian countries, including
196 Myanmar.¹⁶ NDM-5-producing *E. coli* was also recently reported in clinical isolates from
197 Italian patients, one of which had a history of travel to Thailand,¹⁷ and also in Spain in a
198 patient who had not travelled abroad.¹⁸ In companion animals, NDM-5-producing *E. coli*
199 ST1284 was isolated from a rectal swab in a dog from Algeria; molecular characterisation
200 suggested that *bla*_{NDM-5} was likely to be chromosomally located.¹⁹

201

202 The NDM-5-producing *E. coli* isolate in our study was ST410, and harboured *bla*_{NDM-5} on a
203 plasmid integrated into the chromosome. ST410 is an emerging clone with worldwide
204 distribution, associated with MDR human infections, including bloodstream infections and
205 demonstrating potential for nosocomial spread.²⁰ *bla*_{TEM-190} was present on an IncFII plasmid
206 highly similar to IncFII *bla*_{NDM-5} plasmids found in human clinical isolates from Italy (ST405)
207 and Myanmar (ST410).^{16, 18} Our isolate also harboured *bla*_{CMY-42} located on an IncI1 plasmid,
208 similarly present in the Italian NDM-5 producing *E. coli* isolates,¹⁷ suggesting a shared
209 plasmid population amongst which *bla*_{NDM-5}, *bla*_{TEM-190} and *bla*_{CMY-42} are circulating.

210

211 Although *bla*_{NDM-1} is common amongst human carbapenem-resistant isolates in the UK,
212 *bla*_{NDM-5} has been reported on only a small number of occasions: once, in 2011, also on an
213 IncFII plasmid in a ST648 *E. coli* recovered from a patient recently hospitalised in India,²¹

214 and in 2014, in four ST410 isolates, for which there are limited additional metadata, and in
215 which the genetic location of *bla*_{NDM-5} could not be determined.²⁰ Given the similarity of its
216 genetic background with previously described human isolates, the low prevalence of CPE in
217 animals, and the increasing evidence of environmental contamination with CPE by human
218 hospital effluents,⁸ it is possible that the NDM-5-*E. coli* isolated in this study might be of
219 human origin. Our study was limited with respect to the sampling frame and lack of available
220 epidemiological data, but suggests that further detailed studies on the selection and flow of
221 important resistance genes, including carbapenemases, amongst humans and animals are
222 needed.

223
224 Although the use of carbapenems in companion animals is uncommon, the concurrent
225 presence of *bla*_{CMY-42} and *bla*_{TEM-190} on common plasmids could lead to rapid co-selection of
226 *bla*_{NDM} in this population. In addition, the detection of a carbapenemase producing *E. coli*
227 ST410 recently described as a high-risk MDR clone with increased potential for inter-species
228 transmission,²² in companion animals is concerning. Hence, improved antimicrobial
229 stewardship as well as introducing routine detection of carbapenem-resistance in animal
230 isolates is warranted to reduce the risk of zoonotic transmission and will contribute to
231 concerted “One Health” efforts in containing the spread of resistance to last resort
232 antimicrobials.

233
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243

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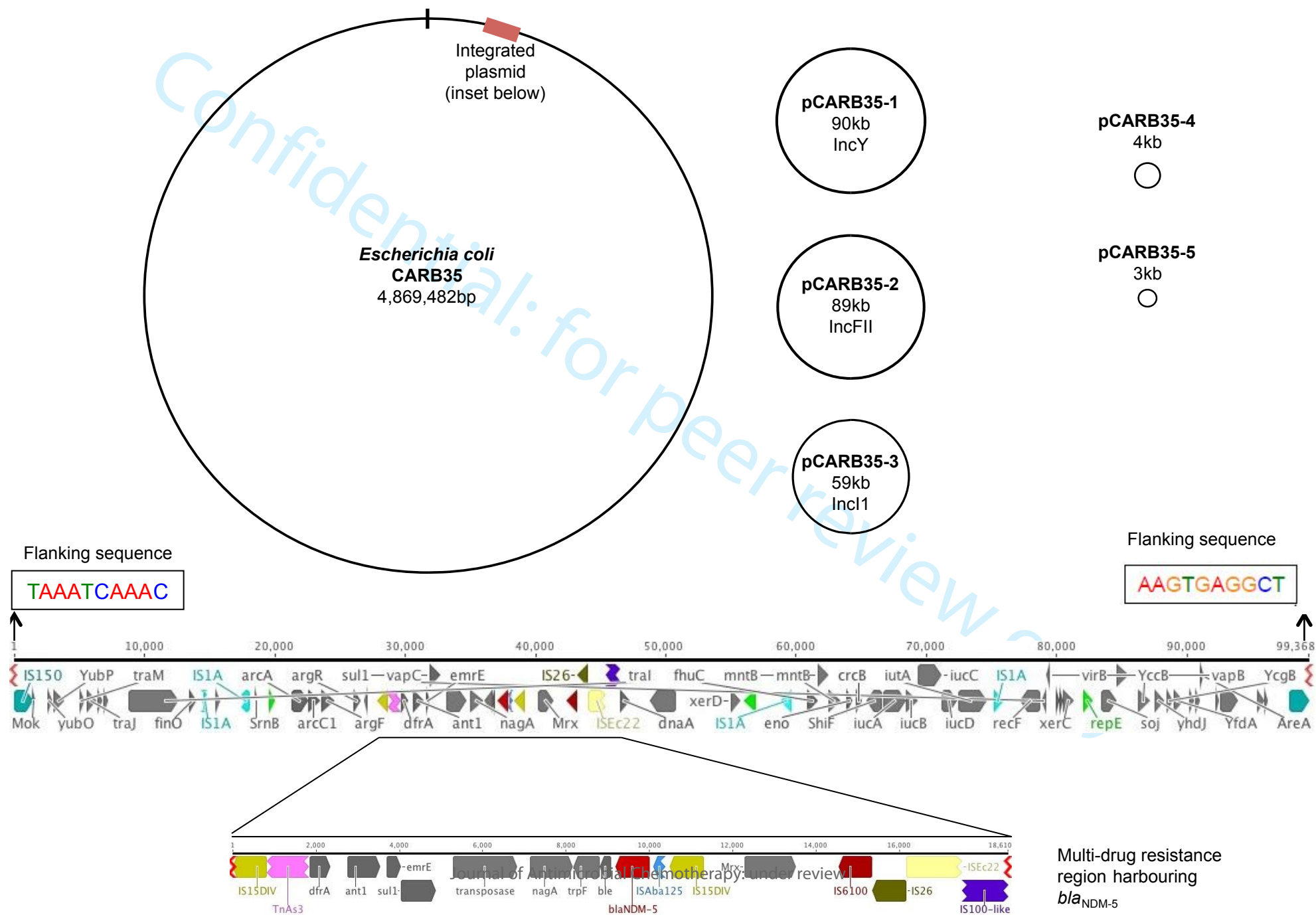
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348 Fig 1: Genomic structure of *Escherichia coli* CARB35, with the *bla*_{NDM-5}-containing plasmid-
349 like structure integrated into the chromosomal sequence. Insets show the structures flanking
350 the *bla*_{NDM-5} gene in greater detail, highlighting the presence of multiple other co-localised
351 drug resistance genes and insertion sequences (This figure appears in colour in the online
352 version of *JAC* and in black and white in the printed version of *JAC*)



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355 Fig S1. Blastn alignments to multi-drug-resistance region of pCARB35-1 (last accessed: 10/Aug/2018) (last accessed: 10/Aug/2018)

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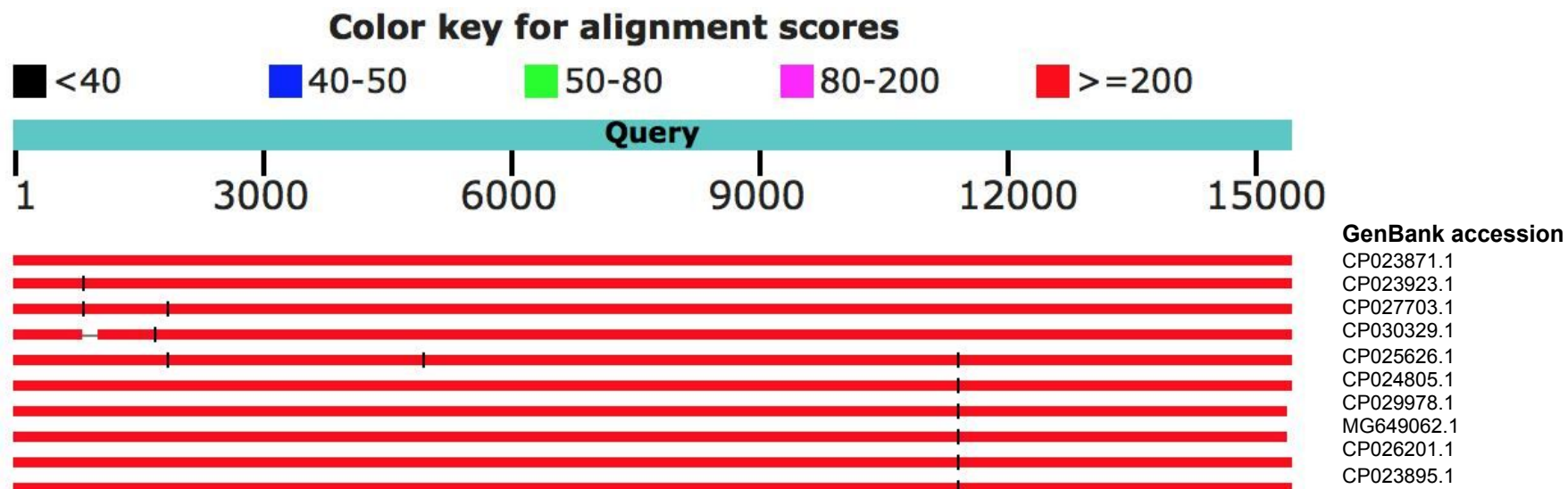
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Fig S1. Blastn alignments to multi-drug-resistance region of pCARB35-1 (last accessed: 10/Aug/2018) (This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*)



GenBank accession	Strain	Chromosome/Plasmid	Species	Country	Source
CP023871.1	FDAARGOS_434	Plasmid	<i>E. coli</i>		Unknown (unpublished)
CP023923.1	FDAARGOS_440	Plasmid	<i>K. pneumoniae</i>		Unknown (unpublished)
CP027703.1	675SK2	Plasmid	<i>E. coli</i>	Switzerland	Wastewater
CP030329.1	AR_452	Plasmid	<i>E. coli</i>		Unknown (unpublished)
CP025626.1	SCEC020007	Plasmid	<i>E. coli</i>		Unknown (unpublished)
CP024805.1	AMA1167	Plasmid	<i>E. coli</i>		Unknown (unpublished)
CP029978.1	51008369SK1	Plasmid	<i>E. coli</i>		Unknown (unpublished)
MG649062.1	Ec001	Plasmid	<i>E. coli</i>	Italy	Urine, human PMID: 29501819
CP026201.1	ECONIH6	Plasmid	<i>E. coli</i>	USA	Unknown (unpublished)
CP023895.1	FDAARGOS_433	Plasmid	<i>E. coli</i>		Unknown (unpublished)