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Dissemination of *bla*$_{NDM-5}$-carrying IncX3-type plasmid among non-clonal *Escherichia coli* strains colonising a dog with a skin infection caused by a carbapenem-resistant *Klebsiella pneumoniae*, United Kingdom

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ABSTRACT

A successful outcome of a post-surgical wound infection management by a carbapenem-resistant *Klebsiella pneumoniae* is described in a dog. Four multidrug-resistant and carbapenem-resistant *Escherichia coli* strains belonging to ST410 (n = 1) and ST648 (n = 3) were isolated from faecal samples and nasal swabs of this dog at admission to a veterinary hospital in the United Kingdom, and one month after discharge. Whole-genome sequencing analysis suggests dissemination of a 46,161-bp IncX3 *bla*$_{NDM-5}$-carrying plasmid among *E. coli* strains from the different lineages. In this study, the *E. coli* ST648 strains were virtually identical to each other (5 SNPs difference) indicating dissemination and persistence of this clone over time and across different anatomical sites in the same dog maybe due to the prolonged antimicrobial therapy. The carbapenemase carrying plasmid also showed homology with other publicly available plasmid sequences from Asian countries. These results suggests that plasmids may be a major vehicle in mediating the dissemination of carbapenem-resistance. Further studies investigating the selection and flow of plasmids carrying important resistance genes amongst companion animals are needed as it may further contaminate other environments posing a threat to public health.

Carbapenemase-producing Enterobacterales (CPE) represent a major public health issue as infections caused by these resistant bacteria have limited treatment choices [1]. In veterinary medicine no licensed carbapenem preparations are available for treatment of companion animals, however the management of multidrug-resistant (MDR) Gram-negative infections may encourage their exceptional use under the prescribing cascade [2], and the detection of CPE in animals has been increasing worldwide [1]. Horizontal gene transfer (HGT) plays an important role in the spread of resistance, as genes can be exchanged among different bacterial species through mobile genetic elements, such as plasmids, and transmitted between companion animals, the environment, and humans, and in this way spread to the community [1]. This study sought to assess CPE gastrointestinal and nasal carriage in dogs diagnosed with skin/soft tissue infections (SSTI) or urinary tract infection (UTI) attending the Royal Veterinary College (RVC), Hertfordshire, United Kingdom (UK).

Between 2018 and 2020, bacterial pathogenic strains causing SSTIs or UTIs were isolated at the Microbiology Clinical Laboratory of the RVC from 18 dogs with SSTI and four with UTI, following hospital admission to the RVC Queen Mother Hospital for Animals. Signed informed consent from the owners and ethical approval were taken (URN 2017 1750-3). Faecal samples and nasal swabs from these animals were obtained. Samples were inoculated on MacConkey agar plates (Oxoid, Basingstoke, UK) supplemented with 1.0 µg/mL meropenem (Sigma-Aldrich, Gillingham, UK) and 1.5 µg/mL cefotaxime (Cambridge Bioscience Ltd, Cambridge, UK) before and after pre-enrichment in peptone water (Oxoid). Susceptibility tests of colonising strains were performed by broth microdilution with MicroScan Neg MIC Panel Type 44 (Siemens) according to EUCAST

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Table 1
Resistance profiles of Enterobacterales strains isolated from faecal and nasal samples from a dog in the United Kingdom.

Antimicrobials tested	Minimum inhibitory concentration (mg/L)					
	***K. pneumoniae* T0 (UK111/0-D1F1XK1)**	***E. coli* T0 (UK111/0-D1F3E1)**	***E. coli* T0 (UK111/0-D1F3UE1)**	***E. coli* T2 (UK111/1-D1F1TE3)**	***E. coli* T2 (111/1-D1F4E1W)**	***E. coli* T2 (UK111/1-D1N3E1)**
Type of Sample	Faecal	Faecal	Faecal	Faecal	Faecal	Nasal
Amikacin	≤8	≤8	≤8	≤8	≤8	≤8
Amoxicillin/clavulanate	**16/8**	**> 16/8**	**> 16/8**	**> 16/8**	**> 16/8**	**> 16/8**
Ampicillin	**> 16**	**> 16**	**16**	**16**	**> 16**	**16**
Aztreonam	**> 16**	**16**	**> 16**	**> 16**	**> 16**	**> 16**
Ceftazidime	**> 16**	**> 16**	**> 16**	**> 16**	**> 16**	**> 16**
Cefepime	**> 16**	4	**> 16**	**> 16**	**> 16**	**> 16**
Cefotaxime	**> 32**	**> 32**	**> 32**	**> 32**	**> 32**	**> 32**
Cefoxitin	≤8	**> 16**	**> 16**	**> 16**	**> 16**	**> 16**
Ciprofloxacin	**> 2**	≤0.5	**> 2**	**> 2**	**> 2**	**> 2**
Chloramphenicol	≤8	≤8	**> 16**	**> 16**	≤8	**> 16**
Ertapenem	≤0.5	≤0.5	**> 1**	**> 1**	**> 1**	**> 1**
Gentamicin	**> 8**	≤2	**> 8**	**> 8**	**> 8**	**> 8**
Imipenem	≤1	≤1	**8**	**8**	**> 8**	**8**
Meropenem	≤1	≤1	**> 8**	**> 8**	**> 8**	**> 8**
Piperacillin/tazobactam	≤8	**64**	**> 64**	**> 64**	**> 64**	**> 64**
Tetracycline	**> 8**	≤4	**> 8**	**> 8**	**> 8**	**> 8**
Trimethoprim/ sulfamethoxazole	**> 4/76**	≤2/38	**> 4/76**	**> 4/76**	≤2/38	**> 4/76**

Bold text indicates strain classification as resistant according to clinical breakpoints set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2023 [3], except for amoxicillin/clavulanate and trimethoprim/sulfamethoxazole, for which criteria from the Clinical and Laboratory Standards Institute (CLSI) were used [4]. T0 concerns to sampling during admission; T2 was performed one month after T0.

and CLSI guidelines [3,4]. Isolates were identified by 16 S rRNA gene sequencing [5].

In 2019 in this cohort, a dog presented at the hospital for scrotal dermatitis following castration. Scrotal ablation was performed at the time of his recruiting to the study. The dog then re-presented for wound dehiscence and cellulitis extending into inguinal region and past hip joints laterally. A clinical carbapenem-resistant *Klebsiella pneumoniae* strain was diagnosed. Susceptibility testing of this clinical strain included resistance to amikacin, ampicillin, amoxicillin/clavulanate, carbenicillin, cefovecin, ceftazidime, cefuroxime, cephalexin, ciprofloxacin, enrofloxacin, imipenem, marbofloxacin and pradofloxacin. It was found to be susceptible to chloramphenicol, gentamicin, oxytetracycline, and trimethoprim in combination with sulfamethoxazole. Empiric therapy with amoxicillin/clavulanate 16 mg/kg BID and then pradofloxacin 3 mg/kg SID was added 2 days later. After open wound management for 8 days, and then surgical closure, the wound then healed unremarkably. Antimicrobial therapy was made for 20 days. Yet, no de-escalation of the antibiotics found resistant by the Lab was performed. The dog was studied for faecal and nasal colonisation and had two carbapenem-susceptible strains, one *K. pneumoniae* strain and one *Escherichia coli* strain and, a carbapenem-resistant *E. coli* (strain UK111/0-D1F3UE1) cultured from the faecal sample at hospital admission (before treatment). One month later, at the end of antimicrobial treatment, further carbapenem-resistant *E. coli* strains were recovered from faecal samples (strains UK111/1-D1F1TE3 and UK111/1-D1F4E1W) and nasal swabs (UK111/1-D1N3E1); two months after cessation of antimicrobials there was no bacterial growth on selective agar. All these carbapenem-resistant colonisation strains presented similar MDR phenotypes (Table 1).

Whole Genome Sequencing (WGS) analysis of carbapenem-resistant strains (n = 4) was performed using Illumina NovaSeq 6000 platform with 2 × 150 bp paired-end reads and assembled using spades v3.14.0. De novo genome assemblies presented an average L50 = 11, N50 = 1.5 × 10^5 and depth of 284x. These assemblies were used to assess antimicrobial resistance (AMR) genes, plasmids, virulence factors and MLST using the Center for Genomic Epidemiology tools [6]. Sequenced strains were deposited in the European Nucleotide Archive (ENA) repository, project number PRJEB55525. Alignment and visualisation of plasmids was performed with BRIG 0.95 [7].

Strain UK111/1-D1F4E1W belonged to sequence type (ST) 410 and all three others to ST648. These are two emerging clonal lineages with worldwide distribution, primarily linked to carbapenemase-producing *E. coli* strains causing infections in humans [8], but that have also been demonstrated within different hosts [5,9–12].

Sequencing analysis showed that all strains co-harboured $bla_{\text{NDM-5}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{OXA-1}}$ beta-lactam resistance genes, as well as, genes encoding resistance to aminoglycosides (*aac(3)-IIa, aac(6′)-Ib-cr*), macrolides (*mph*(A)), tetracyclines (*tet*(B)) and trimethoprim (*drfA17*). They also contained amino acid substitution associated with fluoroquinolone resistance in GyrA (S83L and D87N), ParC (S80I) and ParE (S458A). Additionally, *E. coli* strains from ST648 (UK111/0-D1F3UE1, UK111/1-D1F1TE3 and UK111/1-D1N3E1) also carried resistant genes for phenicols (*catA1*) and sulphonamides (*sul1*) (Supplementary Table A1).

In the UK, the $bla_{\text{NDM-5}}$ gene has been reported in an IncFII plasmid from a ST648 *E. coli* strain recovered from a human patient recently hospitalized in India [10] and in clinical ST410 *E. coli* strains of human origin [12]. In companion animals this resistance gene was only once reported in the UK, chromosomally integrated in a clinical canine *E. coli* strain [11]. Conversely, in all strains of the present study the $bla_{\text{NDM-5}}$ gene was located on IncX3 plasmids. These plasmids exhibiting broad similarity to other NDM-5-containing IncX3 plasmids in the NCBI database [13] (Fig. 1), sharing the same genetic context of IS3000-ΔISAba125-IS5-$bla_{\text{NDM-5}}$-ble_{MBL}-trpF-Δdct-IS26-ΔumuD, found in Enterobacterales of human, animal, and environmental origin in Asia (Fig. 1) [13]. The presence of identical putative plasmid pUK111DF across strains belonging to two different *E. coli* STs across two months in the same dog, support recent plasmid-based HGT. Furthermore, the similarity to plasmids from different sources identified worldwide highlight the importance of the IncX3 plasmid epidemiology in the spread of the $bla_{\text{NDM-5}}$ gene within a One Health context.

The genome of *E. coli* ST410 strain UK111/1-D1F4E1W exhibited a combination of virulence factors that classified it as an extra-intestinal pathogenic *E. coli* (ExPEC) [5] (Supplementary Table A1). The *E. coli* ST648 strains also carried UPEC virulence factors (*iutA,*

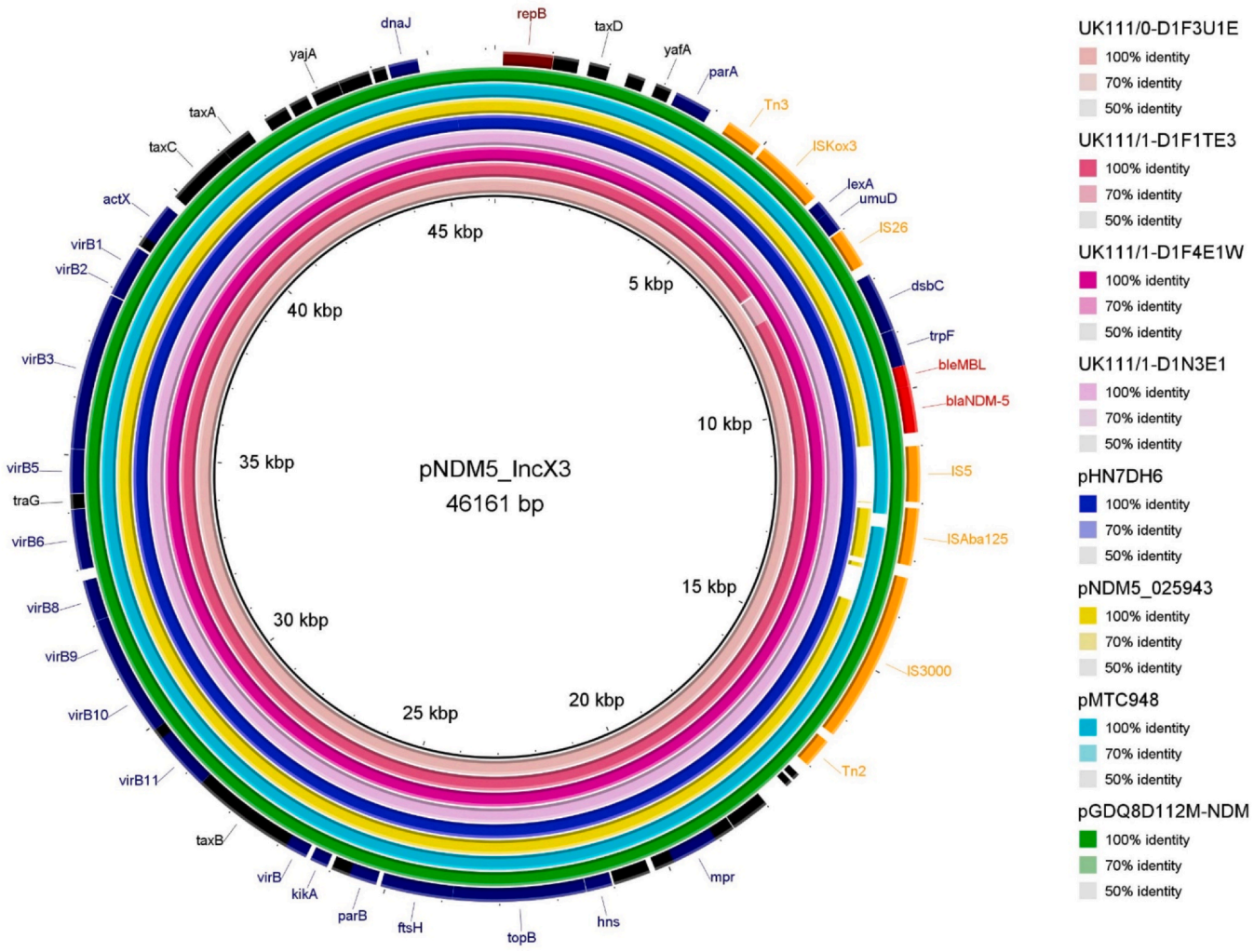

Fig. 1. Comparison of *bla*$_{NDM-5}$-carrying IncX3 plasmid from *Escherichia coli* strains colonising a dog, United Kingdom, with other reported plasmids. Rings are arranged from inside out starting with the backbone plasmid reference (black inner ring) pNDM5_IncX3 (GenBank Accession No. KU761328.1, human clinical isolate, China), pUK111D putative plasmids from a dog's isolates from this work (UK111/0-D1F3UE1, UK111/1-D1F1TE3, UK111/1-D1F4E1W and UK111/1-D1N3E1), pHN7DH6 (MN276080, dog faeces, China), pNDM5_025943 (CP027204, sewage, China), pMTC948 (MH349095, shrimp food sample, China) and pGDQ8D112M-NDM (MK628734, duck, China). Figure was drawn using BRIG 0.95 comparison tool [7]. BRIG considers sequences with 50–70 % similarity as identical. Genes are represented by coloured blocks in the outer circle: red, antibiotic resistance genes; orange, transposase genes, transposons, and insertion sequences; dark blue, genes associated with partition, modification, and stability systems; brown, replication genes; black, other genes.

iucC, sitA), alongside factors associated with avian pathogenic *E. coli* (APEC), such as outer membrane protein complement resistance (*traT*) and outer membrane protease (*ompT*) (Supplementary Table A1), highlighting their pathogenic potential.

In this study, the *E. coli* ST648 strains were virtually identical to each other (5 SNPs difference, Supplementary Table A2) indicating dissemination and persistence of this clone over time and across different anatomical sites in the same dog. The cross-selection of CPE strains may have occurred through the use of antibiotics to which the strains were resistant to. Yet, the successful outcome of the SSTI (wound) case in this dog may be explained by the open wound management and subsequent surgical closure. The potential for inadvertent selection of MDR-colonising bacteria should be considered in future antimicrobial stewardship strategies targeting rational antibiotic use.

The isolation of CPE in companion animals is an emerging one-health problem that should not be neglected and is a concern for both animal and human health and the environment. In this case, canine CPE successful colonisation could have been due to plasmid circulation inside the clinical veterinary setting, contact with owners (despite negative nasal swabs, their faeces were not tested, data not showed) or from contaminated feed or drinking water. There is an urgent need for improved clinical AMR monitoring programs, including integrated real-time data reporting, to support epidemiological tracking of key MDR-pathogens, especially as resistance to high and highest priority critical important antimicrobials increases in veterinary medicine.

Ethics approval

The study was conducted in accordance with the European Union Directive 2010/63/EU on the protection of animals used for scientific purposes and the Declaration of Helsinki. Ethical approval for collection of samples and data from humans, dogs and cats was obtained from the Royal Veterinary College Ethics and Welfare Committee (URN 2017 1750-3).

Consent to participate

Written informed consent was obtained prior to enrolment from each human participant included in the study for themselves and their companion animals.

Consent for publication

Informed consent was obtained from all individual participants included in the study, for themselves and their companion animals.

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CRediT authorship contribution statement

Study conception and design were performed by Anette Loeffler and Constança Pomba. All authors contributed to the material preparation and data collection. The first draft of the manuscript was written by Juliana Menezes and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not used any generative AI and AI-assisted technologies in the writing process.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2023.10.048.

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