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# The veterinary clinic backyard lawn used for relief walks of hospitalized dogs is a hotspot for OXA-type carbapenemases in multiple Enterobacterales

**Yvonne Spahr [1,2], Javier E. Fernandez [1], Andrea Endimiani [3], Simone Schuller [4], Helene Rohrbach [4] and Vincent Perreten [1]***

[1]*Division of Molecular Bacterial Epidemiology & Infectious Diseases, Institute of Veterinary Bacteriology, University of Bern, Bern, Switzerland;* [2]*Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern, Switzerland;* [3]*Institute for Infectious Diseases, University of Bern, Bern, Switzerland;* [4]*Department of Clinical Veterinary Medicine, University of Bern, Bern, Switzerland*

*Corresponding author. E-mail: vincent.perreten@unibe.ch

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**Objectives:** Carbapenemase-producing Enterobacterales (CPE) represent a serious health care concern and their spread in animals has become alarming. Following several cases of CPE infections in a companion animal clinic, the role of the clinic backyard lawn used for dog relief walks of hospitalized dogs as a CPE reservoir has been investigated over a 4-year period (2020–2023).

**Methods:** Soil surface samples were taken through application of sterile wipes. After enrichment in Mueller–Hinton broth and selection on CHROMID® (OXA-48, CARBA) agar plates, isolates were identified by MALDI-TOF MS. CPE isolates were submitted to microdilution antimicrobial susceptibility testing and sequenced with short (Illumina) and long (ONT) read technologies to obtain complete circular genomes to perform antimicrobial resistance gene screening, phylogenetic (cgMLST, cgSNPs) and plasmid analyses.

**Results:** A total of 32 CPE representing eight different species were isolated, with *E. coli* ($n=15$) and *E. hormaechei* ($n=7$) being predominant. Genome-wide typing identified 11 different subtypes of *E. coli* and three of *E. hormaechei*, highlighting bacterial diversity. Thirty CPE contained an identical 63 589-bp IncL plasmid only differing by inversion of the $bla_{OXA-48}$-containing transposon (Tn*1999.2*, invTn*1999.2*), and two CPE contained a 51 479-bp $bla_{OXA-181}$-containing IncX3 plasmid. Strains associated with infections or carriage in hospitalized pets were also present in the lawn.

**Conclusions:** The canine relief area of a veterinary clinic was identified as a long-term environmental reservoir for CPE, mainly due to the hyperepidemic $bla_{OXA-48}$-positive IncL plasmid spreading between various bacterial species, emphasizing the urgent need for an extended hygiene concept including the outdoor environment.

## Introduction

Carbapenemase-producing Enterobacterales (CPE) are a leading cause of human healthcare-associated infections with limited treatment options and are associated with high morbidity and mortality.[1] CPE can rapidly spread, challenging infection prevention and control (IPC) management and posing the risk of further disseminating within the community and environment.[2,3] So far, less frequently than in humans, CPE have also been detected in animals and veterinary health care settings, causing infections in companion animals, colonizing both companion animals and personnel, and contaminating the veterinary clinic environment. Once colonized at the gut level, animals may become silent spreaders of CPE to other animals and humans through close contact and to the environment during convenience walks.[4–6]

In Switzerland, several reports already mentioned the concerning presence of CPE in companion animal veterinary settings, with OXA-48 and OXA-181 being the most frequently detected carbapenemases.[7–9] Both enzymes belong to the OXA-48-like carbapenemases, one of the most reported groups worldwide.[10] OXA-48-like carbapenemases are class D serine β-lactamases, which hydrolyse penicillins and narrow-spectrum cephalosporins

well, but carbapenems only weakly.[11] Their successful dissemination is mainly mediated through highly transferable plasmids, and in some cases, their spread is associated with high-risk MDR clones.[11] The laboratory detection of OXA-48-like carbapenemase is complex, probably leaving them underreported and possibly spreading silently.[10,12,13]

The dissemination of OXA-48 is mainly mediated through promiscuous pOXA-48a-like IncL plasmids.[14] These plasmids contain variants of the $bla_{OXA-48}$-harbouring composite transposon Tn*1999*,[10] of which the flanking IS elements allow them to switch position within the genome.[15] The most frequently detected variant is Tn*1999.2*, also frequently forming an inverted composite transposon invTn*1999.2* within the same plasmid.[16,17] Inversion events of this transposon can occur and both forms can be present within a bacterial colony.[9] The OXA-181 carbapenemase was originally mobilized on ColE2 plasmids within Tn*2013*. This transposon was transferred to other plasmid types, including IncX3 plasmids, which contributed to the global spread of OXA-181.[10]

Following outbreaks with CPE producing OXA-48-like carbapenemases and cases of companion animal infections in a Swiss veterinary clinic in 2018, the indoor and outdoor clinical environments were identified as potential sources of CPE contamination.[8,18,19] While the indoor clinical environment could thoroughly and repeatedly be disinfected, the backyard lawn of the companion animal hospital, which is used as the designated canine relief area, was scrutinized over 4 years (2020–2023) to determine and evaluate presence and persistence of CPE. WGS was used to characterize the collected CPE and their antimicrobial resistance plasmids to determine their degree of relatedness and whether the lawn within the relief area represents a pool of clinically important, as well as new, emerging CPE.

## Methods

### *Sampling and bacterial identification*

The surface of a Swiss veterinary clinic's backyard lawn used as canine relief area was sampled in four areas of 2 $m^2$ using sterile wipes on six different time points between 2020 and 2023 (Figure 1). Each sampling wipe was placed into a 500 mL bottle containing 200 mL Mueller–Hinton broth (Becton Dickinson) and incubated overnight at 37°C under aerobic conditions without shaking. One loopful (10 µL) of the bacterial suspension was streaked onto selective agar plates CHROMID® OXA 48 and CHROMID® CARBA (bioMérieux). The resulting colonies were identified by MALDI-TOF MS and purified on trypticase soy agar containing 5% sheep blood (TSA-SB) (Becton Dickinson) at 37°C for 24 hours. Carbapenemase production was determined using the Blue-Carba test.[20] Isolates were kept in 30% glycerol stored at −80°C.

### *Antimicrobial susceptibility testing (AST)*

The MIC of 22 different antibiotics was determined by broth microdilution using Sensititre plates EUVSEC2 and EUVSEC3 according to the guidelines of EUCAST.[21] MICs were interpreted using the clinical breakpoints provided from EUCAST v.15.0 (https://www.eucast.org/clinical_breakpoints), except for cefoxitin, colistin, nalidixic acid, trimethoprim, sulfamethoxazole, chloramphenicol and tetracycline, for which the clinical breakpoints from CLSI were used.[22] No breakpoints were available for azithromycin and tigecycline (except for *Escherichia coli*). The MICs are presented in Table S1 (available as Supplementary data at *JAC* Online).

### *Whole genome sequencing (WGS)*

All strains were sequenced using both Illumina and Oxford Nanopore Technologies (ONT) sequencing methods. The DNA libraries for Illumina sequencing were obtained using the Nextera™ DNA Flex microbial colony extraction protocol and the Illumina DNA Prep (M) tagmentation library preparation kit (Illumina). The libraries were sequenced on a Novaseq 6000 at the Next Generation Sequencing platform, Institute of Genetics, University of Bern resulting in 2×150 bp paired-end reads. The reads were filtered with Trimmomatic v.0.39 (https://trimmomatic.com/). Genomic DNA for ONT sequencing was obtained using the DNeasy Blood and Tissue Kit (Qiagen) and sequenced on a MinION using native barcoding kit (SQK-NBD114) and FLO-FLG114 and FLO-MIN114 flow cells. The basecalling and demultiplexing were performed using Dorado v.0.6.4 (https://github.com/nanoporetech/dorado). Long reads were assembled using Autocycler v.0.4.0 (https://github.com/rrwick/Autocycler). The resulting assemblies were polished with Illumina short reads using Polypolish v.0.6.0 (https://github.com/rrwick/Polypolish). Genomes were annotated with Bakta v.1.11.3 (https://github.com/oschwengers/bakta) and used for genome analysis. On submission to GenBank, the genomes were annotated with the Prokaryotic Genome Annotation Pipeline (https://github.com/ncbi/pgap) at NCBI.

The complete genomes and all sequence reads are available at GenBank under BioProject PRJNA1308800 (Table S2).

### *WGS-based* in silico *analysis*

The genomes of each strain were submitted to the Type (Strain) Genome Server (https://tygs.dsmz.de/) for confirmation of species identification. MLST was performed at pubMLST (https://pubmlst.org/) for *E. coli*, *Enterobacter hormaechei* and *Citrobacter portucalensis* and at the Institute Pasteur (https://bigsdb.pasteur.fr/klebsiella/) for *Klebsiella* spp. Core genome MLST (cgMLST) was performed with Ridom SeqSphere+ v.11.0.2 on the seven *E. hormaechei* strains using the scheme for *E. hormaechei* resulting in complex types (CT). CgST of *E. coli* strains were obtained from EnteroBase (https://enterobase.warwick.ac.uk/species/index/ecoli). Antimicrobial resistance genes (ARGs) were identified with ResFinder v.4.7.2 available online at the Center for Genomic Epidemiology (https://genepi.dk/resfinder). Incompatibility (Inc) groups of plasmids were identified using ABRicate v.1.0.1 (https://github.com/tseemann/abricate) against the PlasmidFinder database. Clinker v.0.0.28 (https://github.com/gamcil/clinker) was used to create the gene cluster comparison figures of the plasmids. Sequence alignments and whole genome alignments were created using MAFFT from Geneious Prime v.2025.0.2 (Dotmatics). SNP were called using Snippy v.4.6.0 (https://github.com/tseemann/snippy) and recombination events were filtered with Gubbins v.3.4 (https://github.com/nickjcroucher/gubbins). The cgSNP-based trees were generated with FastTree v.2.1.11 and visualized in iTol v7 (https://itol.embl.de/). Genomes of strains isolated previously in the same companion animal hospital from hospitalized pets, obtained from either infection sites or gut colonization[9,23] and sharing the same sequence type (ST), were retrieved from GenBank. The closest related strains available from EnteroBase and GenBank were included for *E. coli* ST90, ST2973, ST297, ST602 and *C. portucalensis* ST1138, respectively, to carry out the cgSNP analysis. For each ST, one of the strains isolated in our study was used as the reference genome. The SNP distance matrices were created with snp-dists v.0.8.2 (https://github.com/tseemann/snp-dists).

## Results

### *Identification and characterization of CPE*

Over the 4-year period (2020–2023) of investigation, a total of 32 CPE strains belonging to eight different species were isolated from the lawn used for relief walks of hospitalized dogs. The


**Figure 1.** Timeline of the isolation and distribution of OXA-48-like producing CPE from the backyard lawn of a companion animal hospital and hospitalized pets in Switzerland and types of OXA-48-like plasmid. The figure presents the bacterial species, sequence types (ST) and carbapenemase plasmids that were found in the different sampling areas at each sampling time during the screening period. N/A, not available; red asterisk, clonal strains of a same ST differing by ≤35 cgSNPs; [a]Diverse STs: ST7427, ST88, ST973, ST58, ST405, ST6321, ST642, ST5073. Created in https://BioRender.com.

composition of the bacterial population varied over time, with only *E. coli* and *E. hormaechei* being almost consistently detected at all sampling times (Figure 1, Table S2).

As common genetic feature, all strains carried a 63 589-bp $bla_{OXA-48}$-positive IncL plasmid (OXA-48-IncL), except for two *L. amnigena*, which contained a 51 479-bp $bla_{OXA-181}$-positive

IncX3 plasmid (OXA-181-IncX3) (Figure S1). Among the OXA-48-producing CPE, the 15 *E. coli* strains were assigned to 11 different STs, including the newly identified ST17257. Only *E. coli* of ST90 ($n=3$), ST297 ($n=2$) and ST2973 ($n=2$) were detected more than once. While *E. coli* ST90 were all detected in October 2023, *E. coli* ST297 and ST2973 were present at different sampling times with *E. coli* ST297 isolated in May 2021 and October 2023, and *E. coli* ST2973 in January 2023 and March 2023 (Figure 1).

Genetic analysis of the *E. coli* strains of a same ST revealed that the strains of ST90 were identical, sharing the same cgST 356122 and differing by 0 SNPs. Strains of ST2973 slightly differed by cgMLST (cgST 356129 or 356130) due to an insertion, but not by SNPs (0 SNP). Strains of ST297 differed by both cgMLST (cgST 356127 and 356120) and by 435 SNPs, indicating non-clonality (Figures 2 and 3, Table S2). The *E. coli* of a same ST also contained identical ARGs, except *E. coli* ST297 and ST2973. One of the *E. coli* ST297 contained an additional IncF resistance plasmid. The two *E. coli* ST2973 clones differed from each other only by the presence of an additional *floR* gene on the IncY plasmid in one of them (Figure 2, Figure S2B).

The seven *E. hormaechei* strains belonged to ST114 ($n=6$) and ST418 ($n=1$). Five ST114 strains were detected in May 2021 and one in March 2023 (Figure 1). The cgMLST and cgSNP analyses confirmed the very close genetic relationship between the ST114 strains from 2021 (CT249, 6–14 SNPs) and a more distant relationship to the strain from 2023 (CT251, 160 SNPs) (Figures 2 and 3). The strain from 2023 also differed by the presence of a larger plasmid and additional ARGs, indicating that either it did not directly evolve from the same lineage as the ST114 from 2021 or evolved independently and acquired novel mobile genetic elements (MGEs) over time (Table S2).

The four *C. portucalensis* belonged to ST1138 and they all contained the same plasmids and ARGs. They were all identical (0–2 SNPs) and were isolated at two different sampling times (January and March 2023) (Figure 1). The two *L. amnigena* strains were also identical (8 SNPs) and share the same ARG profile and OXA-181-IncX3 plasmids (Figures 2–4).

K. *variicola* ST1270, *K. pneumoniae* ST8133, *Atlantibacter* sp. and *L. adecarboxylata* were detected one each. They all exhibited different ARG profiles and plasmid content, except for the common 63 589-bp OXA-48-IncL plasmid (Figures 2 and 4, Table S2).

## Genomic location of ARGs

In addition to the $bla_{\text{OXA-48}}$-like carbapenemase genes, almost half of the strains carried genes for resistance to aminoglycosides (19/32), quinolones (21/32), sulfonamides (18/32), tetracycline (19/32), trimethoprim (16/32) and phenicols (13/32). Genes associated with resistance to macrolides (3/32), rifampicin (1/32) and lincosamide (3/32) remained rare (Figure 2). The MICs of the strains are presented in Table S1. ARGs were detected on both the chromosome and plasmids, and their locations are described in detail next.

### *Plasmids carrying carbapenemase genes*

Both $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-181}}$ were identified on well-described epidemic plasmids and no other carbapenemase-encoding gene was identified throughout the study period. The same 63 589-bp OXA-48-IncL plasmid was detected in 30 strains, and the same 51 479-bp OXA-181-IncX3 plasmid in two strains (Figure 4). Eleven of the 30 OXA-48-IncL plasmids contained an inverted Tn*1999.2* (invTn*1999.2*) as unique difference (Figure S1).

The two 51 479-bp OXA-181-IncX3 plasmids, co-harbouring $bla_{\text{OXA-181}}$ and *qnrS1* on Tn*6163*, were detected in two *L. amnigena* strains. Remarkably, they were identical to pAN-OXA-181, which was identified in *E. coli* colonizing the gut of hospitalized companion animals in 2018 in the same clinic (Figure 4b).[7]

### *Additional ARG plasmids*

Nineteen strains [*E. hormaechei* ($n=6$), *C. portucalensis* ($n=4$), *E. coli* ($n=8$) and *L. adecarboxylata* ($n=1$)] harboured one additional ARG containing plasmid and one *E. hormaechei* strain (MEI61O-22) had two resistance plasmids (Table S2).

In *E. coli*, the additional resistance plasmids belonged to the IncF (IncFIA, IncFIB, IncFIC) and IncY plasmid families (Figure S2). Four different IncF plasmids of different sizes were identified in strains of four different STs (ST90, ST297, ST457, ST602). Only the three *E. coli* ST90 shared the same 131 216-bp IncF plasmid. Highly identical 138 568-bp and 142 852-bp IncY plasmids were detected in two *E. coli* of ST2973 (Figure S2). Each plasmid contained multiple ARGs, except the IncF plasmid of *E. coli* ST602, which only carried $bla_{\text{TEM-1B}}$ (Table S2).

The MDR plasmids of *E. hormaechei* belonged to the IncHI2, IncHI2A and pKPC-CAV1321 type in ST114. All strains isolated in 2021 shared highly similar ~248-kb plasmids except for strain MEI7BC-21, in which the plasmid was reduced in size to 214 563 bp due to a deletion, and strain MEI3B-1023, in which the plasmid was larger due to insertion of several ARGs. Two different IncF MDR plasmids (IncFIB, IncFII) of 143 779 bp and 80 070 bp were found in the single strain of *E. hormaechei* ST418 (Figure S3).

The four *C. portucalensis* strains harboured an identical 57 983-bp IncX3 MDR plasmid and the single *L. adecarboxylata* strain carried a 166 339-bp IncFII MDR plasmid (Table S2, Figure S4).

### *Chromosomal ARGs*

Chromosomally integrated ARGs were identified in eight strains of *E. hormaechei* ST114, one *E. hormaechei* ST418, four *C. portucalensis* ST1138, one *K. pneumoniae* ST8133 and one *K. variicola* ST1270. Amino-acid substitutions in the fluoroquinolone resistance determining region were detected in three strains of *E. coli* ST90, two *E. coli* ST2973 and one *L. adecarboxylata*. Substitutions in the OmpK porins were present in one *K. pneumoniae* ST8133 and one *K. variicola* ST1270. One *E. coli* ST297 contained a nucleotide substitution in the ampC promoter (Figure 2, Table S2).

## Relationship between strains from the lawn of the canine relief area and clinical strains

Two *E. coli* ST602 strains, one associated with gut colonization in a dog in 2020 and one from the relief lawn in 2020, were closely related by cgMLST and differed by one SNP (Figure 3), also sharing the same resistance plasmids. Two *E. coli* ST372 associated with carriage or bacteraemia in dogs in 2021 and 2022 were more distantly related to the relief lawn strain from 2023, indicated by

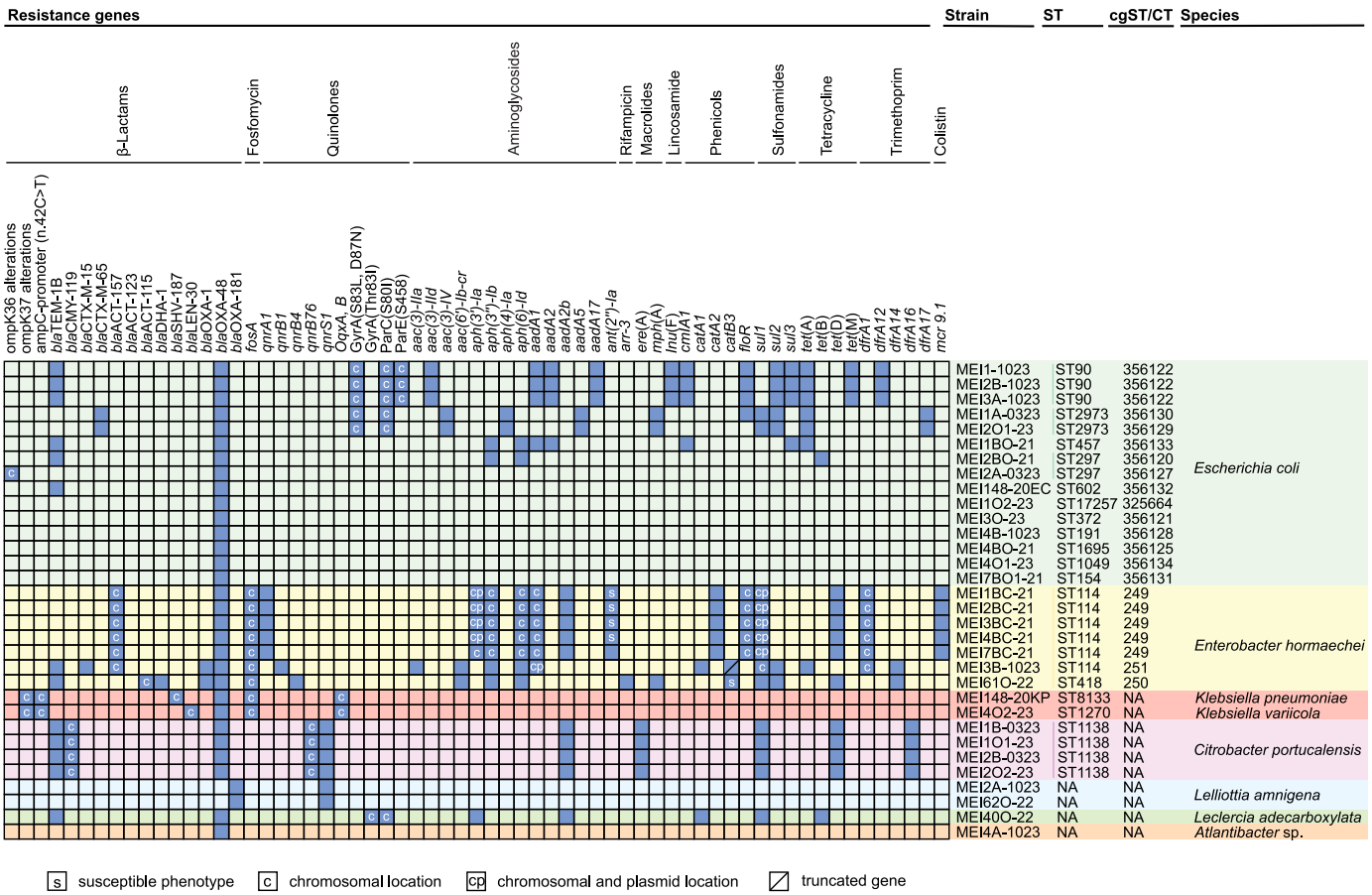


**Figure 2.** Genetic characteristics of CPE strains producing OXA-48-like variants isolated from the backyard lawn of a companion animal hospital in Switzerland. The illustration includes species identification, sequence type (ST), core genome ST (cgST) (*E. coli*) and complex type (CT) (*E. hormaechei*), as well as present antimicrobial resistance mechanisms. NA, not available. The illustration was created using Adobe Illustrator v.29.5.1.

different cgSTs (356121, 358495, 358497) and larger SNP distances (108–155 SNPs) (Figure 3).

*E. hormaechei* ST114 and ST418 isolated from different infection sites in companion animals between 2021 and 2022 were closely related to the strains detected in the relief area in 2021 and 2022, respectively. Strains from companion animals originated from different sources, including urine ($n=3$), abdominal effusion ($n=1$), abscess ($n=1$), wound swab ($n=2$) and hip joint swab ($n=1$). Six *E. hormaechei* ST114 from companion animals all belonged to the same CT as the relief lawn strains (CT249) and differed by 8–35 SNPs. They also carried highly similar ~248-kb IncHI2A resistance plasmids, except for one companion animal strain isolated in 2022 (22KM1314). This strain lacked the ~248-kb IncHI2A plasmid and instead harboured a 61 092-bp IncFII(pCoo) plasmid, which was also present in another companion animal strain (22KM0641), but absent from the relief lawn strains. Two *E. hormaechei* ST418 strains isolated from companion animals between 2021 and 2022 were closely related to the strain isolated from the relief lawn in 2022, as they shared the same CT (CT251), differed by 4–33 SNPs (Figure 3) and harboured identical resistance plasmids.

These CPEs from animals also carried an OXA-48-IncL plasmid identical to that found in the relief lawn strains. Furthermore, the exact same OXA-48-IncL plasmid was also detected in clinically relevant strains, which were not found in the relief lawn like *K. pneumoniae* ST11 and ST307, *E. hormaechei* ST78 and *E. coli* of different STs. Identical plasmids harbouring invTn*1999.2* were previously detected in *K. pneumoniae* (2018–2019), *E. coli* (2018–2020) and *E. hormaechei* (2021–2022) strains, and identical plasmids harbouring Tn*1999.2* were identified in strains of *E. coli* (2019–2022) and *E. hormaechei* (2021–2022) (Figure 4a).[8,9,23]

## Discussion

The backyard lawn of a companion animal clinic used as a relief area for hospitalized dogs was identified as a major source of multiple CPE species, highlighting environmental dissemination and IPC challenges in veterinary settings. Environments of veterinary hospitals in different countries have previously been reported as contaminated with CPE, mainly based on sporadic screening with only specific Enterobacterales such as *E. coli*, *Enterobacter* spp. and *Klebsiella* spp. identified.[5,18,24]

Our long-term observational and WGS-based study revealed diverse Enterobacterales, including species whose clinical and environmental impact remains undetermined. All CPE harboured

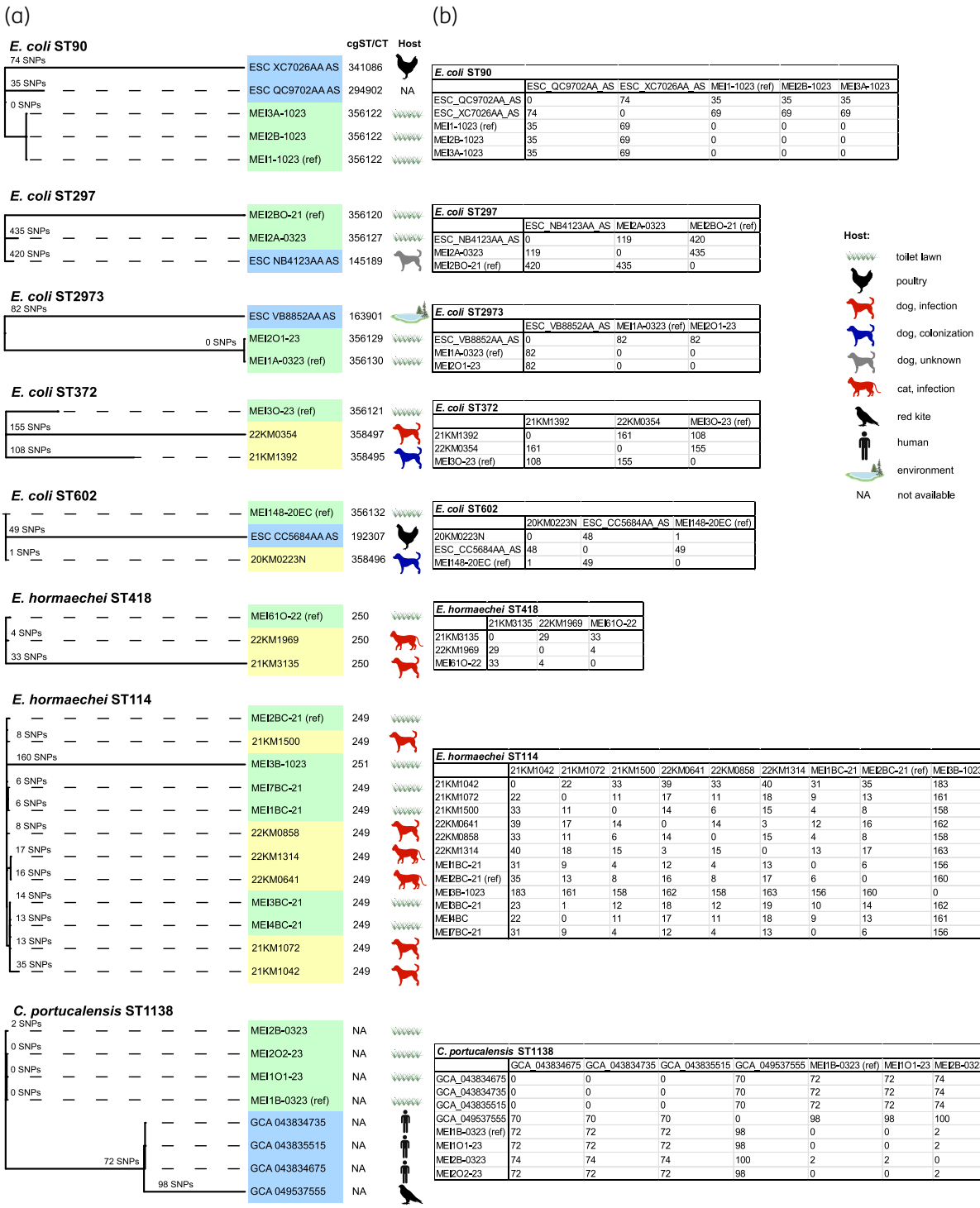


**Figure 3.** Core genome single nucleotide polypmorphism (cgSNP) analysis of different Enterobacterales of a same sequence type (ST) isolated from the lawn of the dog relief area within a Swiss veterinary clinic and from companion animals hospitalized in the same veterinary clinic. (a) cgSNP-based trees created per species and sequence type. The SNPs given in the illustration represent the distance to the reference genome. Strains highlighted in green were isolated from the relief lawn, the strains highlighted in blue were obtained from EnteroBase (*E. coli*) or GenBank (*C. portucalensis*), and strains highlighted in yellow were those associated with infections or carriage in companion animals hospitalized in the same veterinary clinic. (b) SNP distance matrices were created with snp-dists v.0.8.2. NA, not available; cgST, core genome ST; CT, complex type; ref, reference genome. The illustration was adapted in Adobe illustrator v.29.5.1.

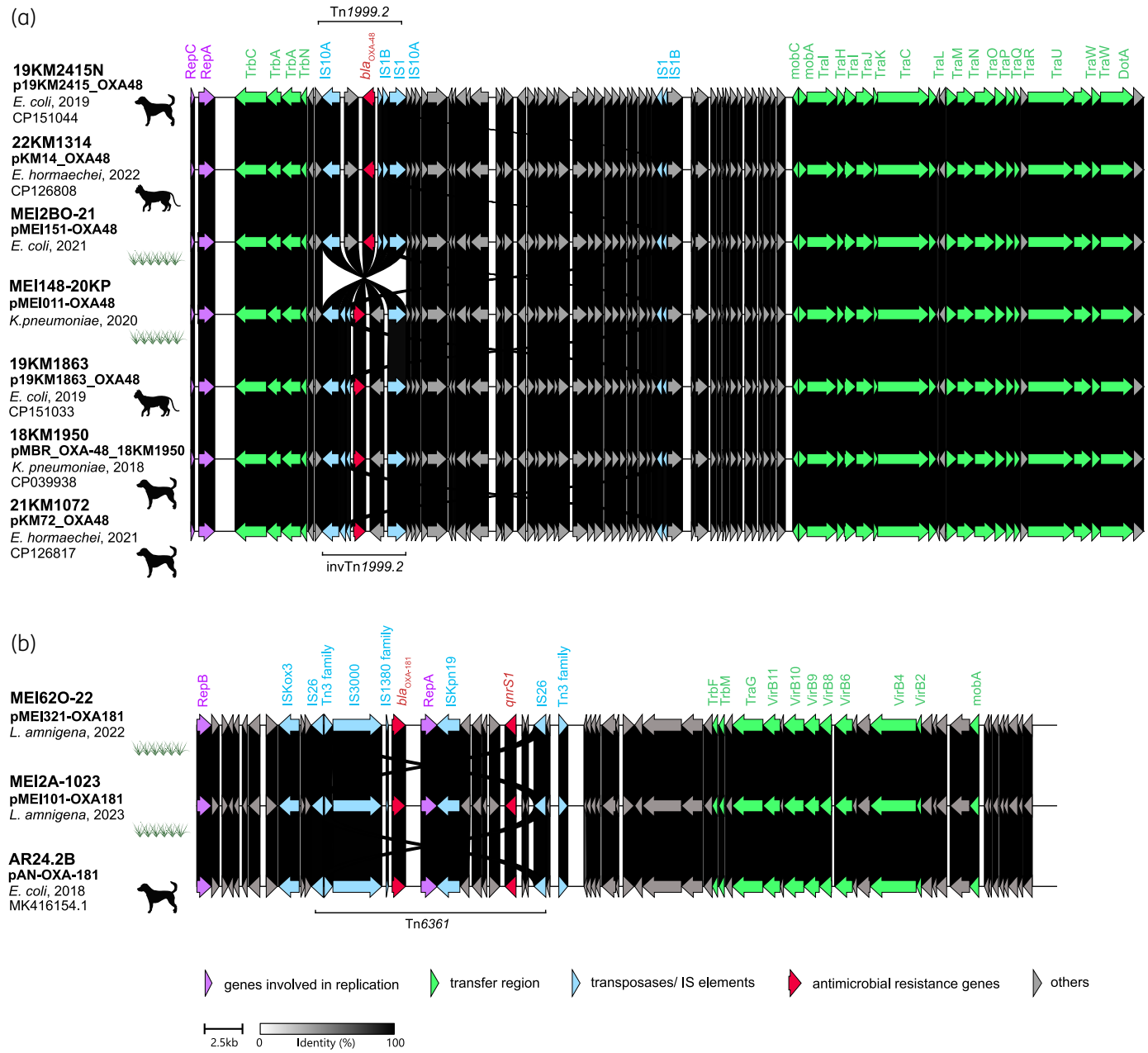


**Figure 4.** Comparison of the OXA-carbapenemase-carrying plasmids isolated from the backyard lawn used as canine relief area with representative plasmids isolated from companion animals in the same veterinary clinic in Switzerland. (a) Comparison of *bla*$_{OXA-48}$-positive IncL plasmids isolated from infection sites and the backyard lawn. Representative plasmids from this study and previous studies were chosen, covering both variants of the transposon (Tn*1999.2*, invTn*1999.2*). Representative strains of previous studies included: *E. coli* 19KM2415N and 19KM1863,[23] *E. hormaechei* 22KM1314 and 21KM1072,[9] and *K. pneumoniae* 18KM1950.[8] (b) Comparison of the *bla*$_{OXA-181}$-positive IncX3 plasmids isolated from companion animals and the clinic's backyard lawn used as dog relief area. AR24.2b was chosen as a representative strain of a previous study.[7] The GenBank accession number, the host species as well as the isolation source and year are given in the illustration. The illustration was created using clinker v.0.0.28 and adapted in Adobe illustrator v.29.5.1. The icons were retrieved from BioRender (https://BioRender.com).

the same OXA-48-IncL plasmid and to a lesser extent an OXA-181-IncX3 plasmid. Detection of identical plasmids across multiple species and lineages (e.g. 11 *E. coli* STs) highlights their dissemination potential. The high diversity of *E. coli* suggests multiple canine sources, each harbouring different *E. coli* strains in the gut, that independently acquired the same carbapenemase-encoding plasmid from nosocomial strains.

The companion animal clinic has experienced repeated cases of infection and carriage with OXA-48-producing *E. coli*, *E. hormaechei*, and *K. pneumoniae* since 2018.[7–9] These species, except for

*K. pneumoniae*, were also the most frequently isolated from the lawn, suggesting that they were released into the lawn by hospitalized dogs during their walks. In fact, some strains of *E. coli* and *E. hormaechei* isolated from the lawn were identical to those from hospitalized dogs that had used the area within the preceding 6 months. Clonally related *E. hormaechei* ST114, ST418 and *E. coli* ST602 were identified in the lawn and in hospitalized dogs and cats. These lineages seem to be able to persist in the hospital environment, to colonize animals and cause infections, with potential for further plasmid dissemination. In addition to OXA-48 plasmids, CPE may acquire further resistance determinants via MGEs.

The genetic relationship between these *E. hormaechei* and *E. coli* isolated from companion animals and from human patients in Switzerland showed no evidence of recent direct transmission.[9,23] However, cross-colonization between hospitalized animals and veterinarians has been reported,[6,25] and pet owners may develop CPE infections/colonization acquired from pets after veterinary hospitalization.[26,27] WGS-based analysis and metadata is therefore essential for epidemiological investigations.[4]

Detection of identical carbapenemase-encoding plasmids in bacteria (*C. portucalensis*, *L. amnigena*, *L. adecarboxylata*, *Atlantibacter* sp.) that are naturally present in water and in soil and not commonly associated with infections, suggests that the plasmid may have been acquired from clinical bacteria in the outdoor environment.[28–31] These species, which are also emerging human pathogens,[32–37] may contribute to maintenance of carbapenemase-carrying plasmids in the lawn and onward transfer to clinically relevant Enterobacterales.

Overall, plasmid exchange appears to play a central role in maintaining CPE in this setting, rather than clonal persistence, except for *E. hormaechei* ST114, which recurred over several years and seems well adapted to multiple hosts and environments.[4,9] IncL plasmids are the main vehicle for $bla_{OXA-48}$ dissemination and are genetically stable and highly transmissible between different bacterial species.[11,38–40] They are widely reported in humans and increasingly identified in animals and environmental sources,[11,41,42] including veterinary clinics in Switzerland and other countries.[24,42–45] This suggests that CPE in companion animal clinics is not unique and may already be endemic in dogs.[46] Detection of the $bla_{OXA-181}$-IncX3 plasmid in *L. amnigena* from the lawn in 2022 and 2023, identical to an outbreak plasmid detected in *E. coli* in 2018 is the same clinic,[7] further demonstrates long-term environmental persistence of carbapenemase-carrying plasmids in this niche. This epidemic plasmid was already reported in humans, companion animals and the food chain,[45,47–51] indicating that it may also rapidly spread further and establish in bacteria from different hosts and environments.

Use of penicillins in combination with β-lactamase inhibitors as well as early-generation cephalosporins in companion animals probably promotes selection of carbapenemase-carrying plasmids in the veterinary setting in Europe, since carbapenems are not being used. These β-lactams, especially penicillins, are well hydrolysed by OXA-48-like enzymes,[11] and are also the most frequently prescribed antibiotics in dogs and cats in Switzerland,[52] probably contributing to the selection and maintenance of OXA-48-producing bacteria in the veterinary settings.

Persistent CPE contamination of the relief area poses a risk of re-introduction into the clinic via dogs or veterinary staff, by contaminating paws and shoes.[53] The environmental indoor contamination in veterinary clinics with MDR bacteria, including CPE, was evaluated previously, revealing contamination of floors and instruments.[5,18,24,54] As Enterobacterales are shed in faeces, relief areas are particularly prone to contamination. One report from US identified $bla_{NDM-7}$–producing *E. hormaechei* in relief areas of companion animal hospitals,[5] indicating that the problem is not unique. Our findings suggest that relief areas may be critical but overlooked hotspots for environmental CPE contamination, especially in hospitals with a history of CPE outbreaks. Owing to this environmental persistence, it is very likely that uncolonized dogs and employees walking on a polluted toilet lawn may also become contaminated with CPEs, which could later lead to infections or colonization. Once discharged from the hospitals, colonized animals may introduce CPE into households, putting their owners at risk to become also carriers and develop severe infections as recently reported.[4,25]

The threat of CPE originating from veterinary clinics is increasingly recognized and requires immediate action to limit the proliferation of preventable CPE infections in animals and humans through adequate measures.[4] These findings highlight the need for reinforced IPC measures targeting both indoor and outdoor environments, and introduction of dog toilet systems that can be thoroughly cleaned and disinfected.[55]

### *Conclusions*

In conclusion, the lawn of the canine relief area was identified as a hotspot for long-term environmental contamination with multiple species of CPE in a veterinary hospital setting. This site appears to act as a niche where horizontal plasmid transfer may occur between different bacterial species. New emerging carbapenemase-producing pathogens may arise from veterinary clinics posing a threat to both animal and human health.

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## Transparency declarations

None to declare.

### *Author contributions*

Yvonne Spahr (Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing—original draft), Javier E. Fernandez (Data curation, Software, Writing—review & editing), Andrea Endimiani (Conceptualization, Validation, Writing—review & editing), Simone Schuller (Conceptualization, Methodology, Validation, Writing—review & editing), Helene Rohrbach (Conceptualization, Methodology, Resources, Validation, Writing—review & editing), and Vincent Perreten (Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing—review & editing)

## Data availability

The Illumina sequence reads, Oxford Nanopore sequence reads, and complete hybrid assemblies are deposited in GenBank under BioProject PRJNA1308800. The genomes are available under the following individual genome accession numbers: JBRWZN000000000, JBRWZO000000000, JBRWZP000000000, JBRWZQ000000000, JBRWZR000000000, JBRWZS000000000, JBRWZT000000000, JBRWZU000000000, JBRWZV000000000, JBRWZW000000000, JBRWZX000000000, JBRWZY000000000, JBRWZZ000000000, JBRXAA000000000, JBRXAB000000000, JBRXAC000000000, JBRXAD000000000, JBRXAE000000000, JBRXAF000000000, JBRXAG000000000, JBRXAH000000000, JBRXAI000000000, JBRXAJ000000000, JBRXAK000000000, JBRXAL000000000, JBRXAM000000000, JBRXAN000000000, JBRXAO000000000, JBRXAP000000000, JBRXAQ000000000, JBRXAR000000000, JBRXAS000000000, JBRXAT000000000, JBRXAU000000000 and JBRXAV000000000. Illumina reads of *E. coli* strains were additionally deposited in EnteroBase, available under the following Ueberstrains: ESC_PB6002AA, ESC_TB6021AA—ESC_TB6034AA.

## Supplementary data

Figures S1 to S4 and Tables S1 and S2 are available as Supplementary data at *JAC* Online.

## References


**1** Logan LK, Weinstein RA. The epidemiology of carbapenem-resistant Enterobacteriaceae: the impact and evolution of a global menace. *J Infect Dis* 2017; **215**: S28–36. https://doi.org/10.1093/infdis/jiw282

**2** World Health Organization. WHO Bacterial Priority Pathogens List, 2024: bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. World Health Organization, 2024. Licence: CC BY-NC-SA 3.0 IGO. https://iris.who.int/server/api/core/bitstreams/1a41ef7e-dd24-4ce6-a9a6-1573562e7f37/content

**3** World Health Organization. Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in health care facilities. World Health Organization, 2017. Licence: CC BY-NC-SA 3.0 IGO. https://iris.who.int/server/api/core/bitstreams/5c6f2512-2f33-4e47-9b91-9edd1d780f01/content

**4** Perreten V, Endimiani A. Carbapenemase-producing Enterobacterales in pets: a new threat from veterinary clinics. *Clin Infect Dis* 2025: ciaf544; https://doi.org/10.1093/cid/ciaf544

**5** Holzman RM, Hsieh E, Dietrich J *et al*. Veterinary hospital environments contain carbapenemase-producing Enterobacterales with clonal spread between facilities (Philadelphia, PA, 2022–2023). *Am J Vet Res* 2025; **86**: ajvr.25.01.0024. https://doi.org/10.2460/ajvr.25.01.0024

**6** Endimiani A, Brilhante M, Bernasconi OJ *et al*. Employees of Swiss veterinary clinics colonized with epidemic clones of carbapenemase-producing *Escherichia coli*. *J Antimicrob Chemother* 2020; **75**: 766–8. https://doi.org/10.1093/jac/dkz470

**7** Nigg A, Brilhante M, Dazio V *et al*. Shedding of OXA-181 carbapenemase-producing *Escherichia coli* from companion animals after hospitalisation in Switzerland: an outbreak in 2018. *Euro Surveill* 2019; **24**: 1900071. https://doi.org/10.2807/1560-7917.ES.2019.24.39.1900071

**8** Brilhante M, Gobeli Brawand S, Endimiani A *et al*. Two high-risk clones of carbapenemase-producing *Klebsiella pneumoniae* that cause infections in pets and are present in the environment of a veterinary referral hospital. *J Antimicrob Chemother* 2021; **76**: 1140–9. https://doi.org/10.1093/jac/dkab028

**9** Donà V, Nordmann P, Kittl S *et al*. Emergence of OXA-48-producing *Enterobacter hormaechei* in a Swiss companion animal clinic and their genetic relationship to clinical human isolates. *J Antimicrob Chemother* 2023; **78**: 2950–60. https://doi.org/10.1093/jac/dkad337

**10** Peirano G, Pitout JDD. Rapidly spreading Enterobacterales with OXA-48-like carbapenemases. *J Clin Microbiol* 2025; **63**: e01515-24. https://doi.org/10.1128/jcm.01515-24

**11** Pitout JDD, Peirano G, Kock MM *et al*. The global ascendency of OXA-48-type carbapenemases. *Clin Microbiol Rev* 2019; **33**: e00102-19. https://doi.org/10.1128/CMR.00102-19

**12** Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. *J Antimicrob Chemother* 2012; **67**: 1597–606. https://doi.org/10.1093/jac/dks121

**13** Simner PJ, Pitout JDD, Dingle TC. Laboratory detection of carbapenemases among gram-negative organisms. *Clin Microbiol Rev* 2024; **37**: e00054-22. https://doi.org/10.1128/cmr.00054-22

**14** Carattoli A, Seiffert SN, Schwendener S *et al*. Differentiation of IncL and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. *PLoS ONE* 2015; **10**: e0123063. https://doi.org/10.1371/journal.pone.0123063

**15** Partridge SR, Kwong SM, Firth N *et al*. Mobile genetic elements associated with antimicrobial resistance. *Clin Microbiol Rev* 2018; **31**: e00088-17. https://doi.org/10.1128/cmr.00088-17

**16** Sattler J, Tsvetkov T, Stelzer Y *et al*. Emergence of Tn*1999.7*, a new transposon in $bla_{OXA-48}$-harboring plasmids associated with increased plasmid stability. *Antimicrob Agents Chemother* 2022; **66**: e00787-22. https://doi.org/10.1128/aac.00787-22

**17** Beyrouthy R, Robin F, Delmas J *et al*. IS1R-Mediated plasticity of IncL/M plasmids leads to the insertion of $bla_{OXA-48}$ into the *Escherichia coli* chromosome. *Antimicrob Agents Chemother* 2014; **58**: 3785–90. https://doi.org/10.1128/aac.02669-14

**18** Schmidt JS, Kuster SP, Nigg A *et al*. Poor infection prevention and control standards are associated with environmental contamination with carbapenemase-producing Enterobacterales and other multidrug-resistant bacteria in Swiss companion animal clinics. *Antimicrob Resist Infect Control* 2020; **9**: 93. https://doi.org/10.1186/s13756-020-00742-5

**19** Dazio V, Nigg A, Schmidt JS *et al*. Acquisition and carriage of multidrug-resistant organisms in dogs and cats presented to small animal practices and clinics in Switzerland. *J Vet Intern Med* 2021; **35**: 970–9. https://doi.org/10.1111/jvim.16038

**20** Pires J, Novais Â, Peixe L. Blue-carba, an easy biochemical test for detection of diverse carbapenemase producers directly from bacterial cultures. *J Clin Microbiol* 2013; **51**: 4281–3. https://doi.org/10.1128/jcm.01634-13

**21** The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 15.0, 2025. https://www.eucast.org.

**22** CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Thirty-Fifth Edition: M100*. 2025.

**23** Donà V, Nordmann P, Fernandez JE *et al*. WGS-based characterization of trans-sectoral OXA-48-producing *Escherichia coli* in Switzerland. *One Health Adv* 2025; **3**: 18. https://doi.org/10.1186/s44280-025-00085-z

**24** Haenni M, Boulouis HJ, Lagrée AC *et al*. Enterobacterales high-risk clones and plasmids spreading $bla_{ESBL/AmpC}$ and $bla_{OXA-48}$ genes within and between hospitalized dogs and their environment. *J Antimicrob Chemother* 2022; **77**: 2754–62. https://doi.org/10.1093/jac/dkac268

**25** DeStefano IM, Fellman CL, Vagnone PMS *et al*. Human cases of carbapenemase-producing *Escherichia coli* linked to spread between

animals and the environment in a veterinary facility -Massachusetts, USA, 2023. *Clin Infect Dis* 2025: ciaf541; https://doi.org/10.1093/cid/ciaf541

**26** Wang MG, Fang C, Liu KD *et al*. Transmission and molecular characteristics of *bla*$_{NDM}$-producing *Escherichia coli* between companion animals and their healthcare providers in Guangzhou, China. *J Antimicrob Chemother* 2022; **77**: 351–5. https://doi.org/10.1093/jac/dkab382

**27** Grönthal T, Österblad M, Eklund M *et al*. Sharing more than friendship - transmission of NDM-5 ST167 and CTX-M-9 ST69 *Escherichia coli* between dogs and humans in a family, Finland, 2015. *Euro Surveill* 2018; **23**: 1700497. https://doi.org/10.2807/1560-7917.ES.2018.23.27.1700497

**28** Stock I, Burak S, Wiedemann B. Natural antimicrobial susceptibility patterns and biochemical profiles of *Leclercia adecarboxylata* strains. *Clin Microbiol Infect* 2004; **10**: 724–33. https://doi.org/10.1111/j.1469-0691.2004.00892.x

**29** Ribeiro TG, Gonçalves BR, da Silva MS *et al*. *Citrobacter portucalensis* sp. nov., isolated from an aquatic sample. *Int J Syst Evol Microbiol* 2017; **67**: 3513–7. https://doi.org/10.1099/ijsem.0.002154

**30** Birlutiu V, Birlutiu RM, Dobritoiu ES. *Lelliottia amnigena* and *Pseudomonas putida* coinfection associated with a critical SARS-CoV-2 infection: a case report. *Microorganisms* 2023; **11**: 2143. https://doi.org/10.3390/microorganisms11092143

**31** Brady C, Cleenwerck I, Venter S *et al*. Taxonomic evaluation of the genus *Enterobacter* based on multilocus sequence analysis (MLSA): proposal to reclassify *E. nimipressuralis* and *E. amnigenus* into *Lelliottia* gen. nov. as *Lelliottia nimipressuralis* comb. nov. and *Lelliottia amnigena* comb. nov., respectively, *E. gergoviae* and *E. pyrinus* into *Pluralibacter* gen. nov. as *Pluralibacter gergoviae* comb. nov. and *Pluralibacter pyrinus* comb. nov., respectively, *E. cowanii*, *E. radicincitans*, *E. oryza*e and *E. arachidis* into *Kosakonia* gen. nov. as *Kosakonia cowanii* comb. nov., *Kosakonia radicincitans* comb. nov., *Kosakonia oryzae* comb. nov. and *Kosakonia arachidis* comb. nov., respectively, and *E. turicensis*, *E. helveticus* and *E. pulveris* into *Cronobacter* as *Cronobacter zurichensis* nom. nov., *Cronobacter helveticus* comb. nov. and *Cronobacter pulveris* comb. nov., respectively, and emended description of the genera *Enterobacter* and *Cronobacter*. *Syst Appl Microbiol* 2013; **36**: 309–19. https://doi.org/10.1016/j.syapm.2013.03.005

**32** Guo K, Zhao Z, Yang Y *et al*. Emergence of an extensive drug resistant *Citrobacter portucalensis* clinical strain harboring *bla*$_{SFO-1}$, *bla*$_{KPC-2}$, and *bla*$_{NDM-1}$. *Infect Drug Resist* 2024; **17**: 2273–83. https://doi.org/10.2147/IDR.S461118

**33** Jiang G, Guo C, Kuai X *et al*. Foodborne diarrhea outbreaks linked to *Citrobacter* species in East China 2022–2023. *Food Microbiol* 2025; **132**: 104839. https://doi.org/10.1016/j.fm.2025.104839

**34** Raptis D, Aljareh A, Shah U *et al*. A case of *Lelliottia amnigena*-induced acute calculous cholecystitis and a literature review. *Cureus* 2025; **17**: e82743. https://doi.org/10.7759/cureus.82743

**35** Zayet S, Lang S, Garnier P *et al*. *Leclercia adecarboxylata* as emerging pathogen in human infections: clinical features and antimicrobial susceptibility testing. *Pathogens* 2021; **10**: 1399. https://doi.org/10.3390/pathogens10111399

**36** Corbellini S, Caccuri F, Caruso A *et al*. *Leclercia adecarboxylata*, an emerging pathogen: a narrative review. *J Infect Dis Ther* 2023; **11**: 1–8. https://doi.org/10.4172/2332-0877.1000573

**37** Hristova PM, Alexandrova AS, Lucanov M *et al*. Catheter-related bloodstream infection in hemodialysis patient due to *Atlantibacter hermannii*. *Case Rep Nephrol Dial* 2023; **13**: 142–7. https://doi.org/10.1159/000533581

**38** Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. *Antimicrob Agents Chemother* 2012; **56**: 559–62. https://doi.org/10.1128/aac.05289-11

**39** Potron A, Poirel L, Nordmann P. Derepressed transfer properties leading to the efficient spread of the plasmid encoding carbapenemase OXA-48. *Antimicrob Agents Chemother* 2014; **58**: 467–71. https://doi.org/10.1128/aac.01344-13

**40** Moser AI, Campos-Madueno EI, Perreten V *et al*. Genome stability during serial subculturing in hyperepidemic multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli*. *J Glob Antimicrob Resist* 2022; **31**: 152–61. https://doi.org/10.1016/j.jgar.2022.08.014

**41** Mairi A, Pantel A, Sotto A *et al*. OXA-48-like carbapenemases producing Enterobacteriaceae in different niches. *Eur J Clin Microbiol Infect Dis* 2018; **37**: 587–604. https://doi.org/10.1007/s10096-017-3112-7

**42** Pulss S, Stolle I, Stamm I *et al*. Multispecies and clonal dissemination of OXA-48 carbapenemase in Enterobacteriaceae from companion animals in Germany, 2009—2016. *Front Microbiol* 2018; **9**: 1265. https://doi.org/10.3389/fmicb.2018.01265

**43** Schmitt K, Biggel M, Stephan R *et al*. Massive spread of OXA-48 carbapenemase-producing Enterobacteriaceae in the environment of a Swiss companion animal clinic. *Antibiotics* 2022; **11**: 213. https://doi.org/10.3390/antibiotics11020213

**44** Haenni M, Métayer V, Lupo A *et al*. Spread of the *bla*$_{OXA-48}$/IncL plasmid within and between dogs in city parks, France. *Microbiol Spectr* 2022; **10**: e00403-22. https://doi.org/10.1128/spectrum.00403-22

**45** Moreira da Silva J, Menezes J, Fernandes L *et al*. Carbapenemase-producing Enterobacterales strains causing infections in companion animals—Portugal. *Microbiol Spectr* 2024; **12**: e03416-23. https://doi.org/10.1128/spectrum.03416-23

**46** Silva JMD, Menezes J, Marques C *et al*. Companion animals—an overlooked and misdiagnosed reservoir of carbapenem resistance. *Antibiotics* 2022; **11**: 533. https://doi.org/10.3390/antibiotics11040533

**47** Leelapsawas C, Sroithongkham P, Payungporn S *et al*. First report of *bla*$_{OXA-181}$-carrying IncX3 plasmids in multidrug-resistant *Enterobacter hormaechei* and *Serratia nevei* recovered from canine and feline opportunistic infections. *Microbiol Spectr* 2024; **12**: e03589-23. https://doi.org/10.1128/spectrum.03589-23

**48** Brilhante M, Menezes J, Belas A *et al*. OXA-181-producing extraintestinal pathogenic *Escherichia coli* sequence type 410 isolated from a dog in Portugal. *Antimicrob Agents Chemother* 2020; **64**: e02298-19. https://doi.org/10.1128/AAC.02298-19

**49** Zhang Z, Lei L, Zhang H *et al*. Molecular investigation of *Klebsiella pneumoniae* from clinical companion animals in Beijing, China, 2017-2019. *Pathogens* 2021; **10**: 271. https://doi.org/10.3390/pathogens10030271

**50** Spahr Y, Nordmann P, Fernandez JE *et al*. Genomic analyses of *Escherichia coli* ST410 harbouring the *bla*$_{OXA-181}$ carbapenemase gene from companion animals and humans in Switzerland. *J Appl Microbiol* 2025; **136**: lxaf181. https://doi.org/10.1093/jambio/lxaf181

**51** Zurfluh K, Poirel L, Nordmann P *et al*. First detection of *Klebsiella variicola* producing OXA-181 carbapenemase in fresh vegetable imported from Asia to Switzerland. *Antimicrob Resist Infect Control* 2015; **4**: 38. https://doi.org/10.1186/s13756-015-0080-5

**52** Bundesamt für Lebensmittelsicherheit und Veterinärwesen (BLV). Antibiotic consumption – veterinary medicines. https://www.dashboard.blv.admin.ch/animals/veterinary-medicines/antibiotic_consumption.

**53** Limper HM, Sier A, Warye K *et al*. A review of the evidence on the role of floors and shoes in the dissemination of pathogens in a healthcare setting. *Surg Infect* 2024; **25**: 46–55. https://doi.org/10.1089/sur.2023.194

**54** Kerek Á, Sterczer Á, Somogyi Z *et al*. Investigation of the environmental presence of multidrug-resistant bacteria at small animal hospitals in Hungary. *Acta Vet Hung* 2021; **68**: 387–92. https://doi.org/10.1556/004.2020.00055

**55** Anderson MEC, Wimmers M, Weese JS. *Infection Prevention and Control Best Practices for Small Animal Veterinary Clinics*, 2nd ed. Ontario Animal Health Network, 2019. https://www.amrvetcollective.com/assets/your-practice/resources/OAHN-IPC-Guide-SB-Final-Jan0820_All_tagged-SUR.pdf.