

00208

## Faecal colonisation with Carbapenemase and ESBL/AmpC-producing Enterobacterales in Portugal and the United Kingdom: A One Health perspective on the Human-Companion animal relationship

### 03. Bacterial susceptibility & resistance

3g. Spread of resistance incl. carriage, reservoirs, ecology, models (excl. nosocomial transmission)

#### Likely attendance

Onsite

*Juliana Menezes*<sup>1, 2</sup>, *Sian-Marie Frosini*<sup>3</sup>, *Joana Moreira Da Silva*<sup>1, 2</sup>, *Laura Fernandes*<sup>1, 2</sup>, *Adriana Belas*<sup>1, 2, 4</sup>, *Cátia Marques*<sup>1, 2, 4</sup>, *Anette Loeffler*<sup>3</sup>, *Andreia Amaral*<sup>1, 2, 5</sup>, *Constança Pomba*<sup>1, 2</sup>

<sup>1</sup>Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon - Lisbon (Portugal), <sup>2</sup>Associate Laboratory for Animal and Veterinary Sciences (AL4Animals), <sup>3</sup>Department of Clinical Sciences and Services, Royal Veterinary College, University of London - North Mymms, Hertfordshire (United Kingdom), <sup>4</sup>Faculty of Veterinary Medicine, Lusófona University - Lisbon (Portugal), <sup>5</sup>MED—Mediterranean Institute for Agriculture, Environment and Development and CHANGE – Global Change and Sustainability Institute, University of Évora, Polo da Mitra, Ap. 94, 7006-554, Évora, Portugal

### Background

Companion animals contribute to the maintenance and dissemination of clinically-important antimicrobial-resistant pathogens. The aim of this study was to evaluate the possible sharing of ESBL/AmpC- and carbapenemase-producing Enterobacterales between animals under antibiotic treatment and their human household members.

### Methods

A prospective longitudinal study was performed from 2018 to 2021 in Portugal and the UK. Faecal samples from dogs and cats with skin and soft tissue or urinary tract infections, and their cohabiting humans, were inoculated on MacConkey agar plates supplemented with 1.5 µg/mL cefotaxime and 1.0 µg/mL meropenem. Bacterial species and the presence of beta- lactamase genes were confirmed by PCR. Antimicrobial susceptibility was assessed by microdilution testing with MicroScan® Neg MIC Panel Type 44 (Siemens, US) and results interpreted according to EUCAST. Isolates' clonality was assessed by rep-PCR. WGS (Illumina NovaSeq), producing pair-end libraries (150 bp), was performed for animal/owner- derived paired strains with the same rep-PCR pattern to estimate genetic relatedness.

### Results

In Portuguese households, one dog (1/43, 2.3%) was colonised by a multidrug-resistant OXA-181-producing extraintestinal pathogenic *Escherichia coli*. Twenty-four companion animals (55.8%, n=43) and 28 humans (35.9%, n=78) harboured ESBL/AmpC-producing Enterobacterales in at least one timepoint.

In UK households, one dog (1/7, 14.3%) was colonised by a multidrug-resistant *E. coli* co-producing NDM-5 and CTX-M-15 beta-lactamases. ESBL/AmpC-Enterobacterales were isolated from five animals (71.4%, n=7) and three humans (37.5%, n=8).

In eight Portuguese households (18.6%, n=43) and two UK households (28.6%, n=7), at least one ESBL/AmpC-encoding gene was detected simultaneously in animal/owner paired strains (Figure 1). Of these, identical rep-PCR pattern was observed for animal/owner paired strains from six Portuguese (13.9%) and one UK household (14.3%). WGS SNPs analysis confirmed longitudinal sharing of *E. coli* strains for five Portuguese and one UK household (Figure 2). *Klebsiella pneumoniae* and *Enterobacter* spp. strains were also shared within two Portuguese households (Figure 3).

## Conclusions

The occurrence of ESBL/AmpC- and carbapenemase-producing Enterobacterales among companion animals and their simultaneous presence in human contacts, emphasizes the importance of incorporating dogs/cats in the assessment of antimicrobial resistance circulation in non-clinical settings. These results demonstrate the importance of the household as an epidemiological unit in any efforts to mitigate the spread of antimicrobial resistance.

## Co-carriage of ESBL/AmpC-encoding genes

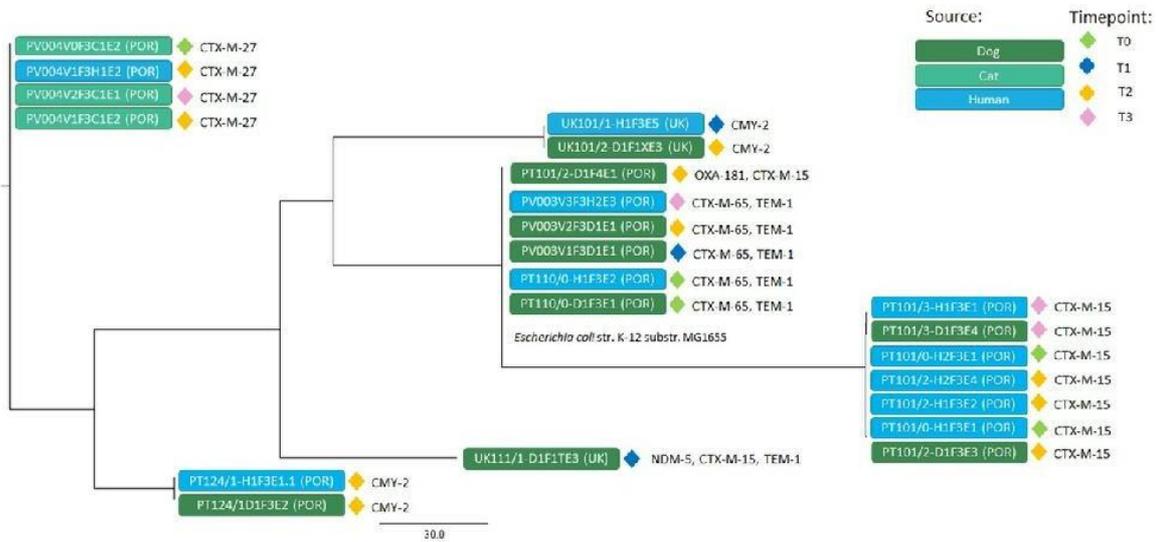
| Country        | Infection group | Household number | Household member | Timepoint <sup>1</sup> | Strain code <sup>2</sup> | Bacterial species            | REP-PCR group | Beta-lactames           | Resistance profile                               |
|----------------|-----------------|------------------|------------------|------------------------|--------------------------|------------------------------|---------------|-------------------------|--|
| United Kingdom | SSTI            | UK101            | Human 1          | T1                     | <b>UK101/1-H1F3E5</b>    | <i>Escherichia coli</i>      | BZ            | CMY-2                   | AMP, AMC, AZT, CAZ, CTX, FOX                     |
| United Kingdom | SSTI            | UK101            | Dog 1            | T2                     | <b>UK101/2-D1F1XE3</b>   | <i>Escherichia coli</i>      | R7            | CMY-2                   | AMP, AMC, AZT, C, CAZ, CTX, FOX, GEN             |
| United Kingdom | SSTI            | UK105            | Human 2          | T0                     | UK105/1-H1F3K1           | <i>Klebsiella pneumoniae</i> | GG            | CMY-2                   | AMP, AMC, AZT, C, CAZ, CTX, FOX                  |
| United Kingdom | SSTI            | UK105            | Human 2          | T0                     | UK105/1-H1F3K2           | <i>Klebsiella pneumoniae</i> | GG            | CMY-2                   | AMP, AMC, C, CAZ, CTX, FOX                       |
| United Kingdom | SSTI            | UK105            | Dog 2            | T1                     | UK105/0-D1F3LB.1         | <i>Escherichia coli</i>      | CE            | CMY-2                   | AMP, AZT, CAZ, CTX, FOX                          |
| Portugal       | SSTI            | PT101            | Dog 3            | T2                     | <b>PT101/2-D1F3E3</b>    | <i>Escherichia coli</i>      | AJ            | CTX-M-15                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | SSTI            | PT101            | Human 3          | T2                     | <b>PT101/2-H1F3E2</b>    | <i>Escherichia coli</i>      | AJ            | CTX-M-15                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | SSTI            | PT101            | Human 4          | T2                     | <b>PT101/2-H2F3E4</b>    | <i>Escherichia coli</i>      | AJ            | CTX-M-15                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | SSTI            | PT101            | Human 4          | T0                     | <b>PT101/0-H2F3E1</b>    | <i>Escherichia coli</i>      | AJ            | CTX-M-15                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | SSTI            | PT101            | Human 3          | T3                     | <b>PT101/3-H1F3E1</b>    | <i>Escherichia coli</i>      | AJ            | CTX-M-15                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | SSTI            | PT101            | Dog 3            | T3                     | <b>PT101/3-D1F3E4</b>    | <i>Escherichia coli</i>      | AJ            | CTX-M-15                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | SSTI            | PT101            | Human 3          | T0                     | <b>PT101/0-H1F3E1</b>    | <i>Escherichia coli</i>      | AJ            | CTX-M-15                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | SSTI            | PT101            | Dog 3            | T2                     | <b>PT101/2-D1F4E1</b>    | <i>Escherichia coli</i>      | AO            | OXA-181, CTX-M-15       | AMP, AMC, AZT, CAZ, CIP, CPM, CTX, FOX, ETP, TMS |
| Portugal       | SSTI            | PT110            | Human 5          | T0                     | <b>PT110/0-H1F3E2</b>    | <i>Escherichia coli</i>      | AX            | CTX-M-65, TEM-1         | AMP, AZT, C, CIP, CTX, TMS                       |
| Portugal       | SSTI            | PT110            | Dog 4            | T0                     | <b>PT110/0-D1F3E1</b>    | <i>Escherichia coli</i>      | AX            | CTX-M-65, TEM-1         | AMP, AZT, C, CIP, CPM, CTX, TMS                  |
| Portugal       | SSTI            | PT124            | Human 6          | T2                     | <b>PT124/1-H1F3E1.1</b>  | <i>Escherichia coli</i>      | BM            | CMY-2                   | AMP, AMC, AZT, CAZ, CPM, CTX, FOX                |
| Portugal       | SSTI            | PT124            | Dog 5            | T2                     | <b>PT124/2-D1F3E2</b>    | <i>Escherichia coli</i>      | BM            | CMY-2                   | AMP, AMC, AZT, CAZ, CIP, CTX, FOX                |
| Portugal       | SSTI            | PT125            | Dog 6            | T0                     | <b>PT125/0-D1F4E2U</b>   | <i>Enterobacter</i> spp.     | F             | ACT-24                  | AMP, AMC, AZT, CAZ, CIP, CTX, FOX                |
| Portugal       | SSTI            | PT125            | Human 7          | T2                     | <b>PT125/1-H2F3E1U</b>   | <i>Enterobacter</i> spp.     | F             | ACT-24                  | AMP, AMC, AZT, CAZ, CIP, CTX, FOX, ETP           |
| Portugal       | UTI             | PT218            | Human 8          | T3                     | PT218/1-H1F3E1           | <i>Escherichia coli</i>      | P             | CTX-M-1, TEM-1          | AMP, AZT, CPM, CTX, TMS                          |
| Portugal       | UTI             | PT218            | Dog 7            | T1                     | PT218/1-D1F3E1           | <i>Escherichia coli</i>      | Q             | CTX-M-1                 | AMP, AZT, CPM, CTX, TMS                          |
| Portugal       | UTI             | PT219            | Human 9          | T2                     | PT219/2-H2F3E1           | <i>Escherichia coli</i>      | V             | CTX-M-15                | AMP, AZT, CAZ, CIP, CPM, CTX                     |
| Portugal       | UTI             | PT219            | Cat 1            | T0                     | PT219/0-C1F3KE1          | <i>Escherichia coli</i>      | X             | CTX-M-15, TEM-1         | AMP, AMC, AZT, CAZ, CIP, CTX, TMS                |
| Portugal       | UTI             | PT219            | Human 10         | T3                     | PT219/3-H1F3E1           | <i>Escherichia coli</i>      | T             | CTX-M-15                | AMP, AMC, AZT, CAZ, CIP, CPM, CTX, GEN           |
| Portugal       | UTI             | PT219            | Cat 1            | T0                     | PT219/0-C1F4K1           | <i>Klebsiella pneumoniae</i> | HI            | CTX-M-15, TEM-1         | AMP, AZT, CIP, CPM, CTX, TMS                     |
| Portugal       | UTI             | PT219            | Cat 1            | T1                     | PT219/1-C1F3K1           | <i>Klebsiella pneumoniae</i> | HI            | CTX-M-15, TEM-1         | AMP, AMC, AZT, CAZ, CIP, CPM, FOX, TMS           |
| Portugal       | UTI             | PV003            | Dog 8            | T1                     | <b>PV003V1F3D1F1</b>     | <i>Escherichia coli</i>      | AB            | CTX-M-65, TEM-1         | AMP, AMC, AZT, CAZ, CIP, CPM, CTX, TMS           |
| Portugal       | UTI             | PV003            | Dog 8            | T2                     | <b>PV003V2F3D1E1</b>     | <i>Escherichia coli</i>      | AB            | CTX-M-65, TEM-1         | AMP, AMC, AZT, CIP, CTX, TMS                     |
| Portugal       | UTI             | PV003            | Human 11         | T3                     | <b>PV003V3F3H2E3</b>     | <i>Escherichia coli</i>      | AB            | CTX-M-65, TEM-1         | AMP, AZT, C, CIP, CPM, CTX, TMS                  |
| Portugal       | UTI             | PV003            | Human 12         | T1                     | <b>PV003V1F3H1K4</b>     | <i>Klebsiella pneumoniae</i> | R             | CTX-M-15, SHV-11, TEM-1 | AMP, AZT, CAZ, CPM, CTX, TMS                     |
| Portugal       | UTI             | PV003            | Human 11         | T1                     | <b>PV003V1F3H2K1</b>     | <i>Klebsiella pneumoniae</i> | R             | CTX-M-15, SHV-11, TEM-1 | AMP, AZT, CAZ, CPM, CTX, TMS                     |
| Portugal       | UTI             | PV003            | Dog 8            | T1                     | <b>PV003V1F3D1K2</b>     | <i>Klebsiella pneumoniae</i> | R             | CTX-M-15, SHV-11, TEM-1 | AMP, AZT, CAZ, CPM, CTX, TMS                     |
| Portugal       | UTI             | PV003            | Human 12         | T2                     | <b>PV003V2F3H1K2</b>     | <i>Klebsiella pneumoniae</i> | R             | CTX-M-15, SHV-11, TEM-1 | AMP, AZT, CAZ, CPM, CTX, TMS                     |
| Portugal       | UTI             | PV003            | Dog 8            | T3                     | <b>PV003V3F3D1K4</b>     | <i>Klebsiella pneumoniae</i> | R             | CTX-M-15, SHV-11, TEM-1 | AMP, AZT, CAZ, CPM, CTX, TMS                     |
| Portugal       | UTI             | PV004            | Cat 2            | T0                     | <b>PV004V0F3C1E2</b>     | <i>Escherichia coli</i>      | AG            | CTX-M-27                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | UTI             | PV004            | Human 13         | T2                     | <b>PV004V1F3H1E2</b>     | <i>Escherichia coli</i>      | AG            | CTX-M-27                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | UTI             | PV004            | Cat 2            | T2                     | <b>PV004V1F3C1E2</b>     | <i>Escherichia coli</i>      | AG            | CTX-M-27                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |
| Portugal       | UTI             | PV004            | Cat 2            | T3                     | <b>PV004V2F3C1E1</b>     | <i>Escherichia coli</i>      | AG            | CTX-M-27                | AMP, AZT, CAZ, CIP, CPM, CTX, TMS                |

Legend: AMC: amoxicillin/clavulanate; AMP: ampicillin; AZT: Aztreonam; C: chloramphenicol; CAZ: ceftazidime; CIP: ciprofloxacin; CPM: cefepime; CTX: cefotaxime; FOX: cefoxitin; ETP: ertapenem; GEN: gentamicin; SSTI: Skin and soft tissue infection; TMS: sulfamethoxazole/trimethoprim; UTI: Urinary tract infection.

<sup>1</sup> T0 concerns the sampling before antimicrobial intake; T1 was done taken one week after antimicrobial treatment started; T2 was taken one month after antimicrobial treatment started; T3 was achieved taken two months after antimicrobial treatment started.

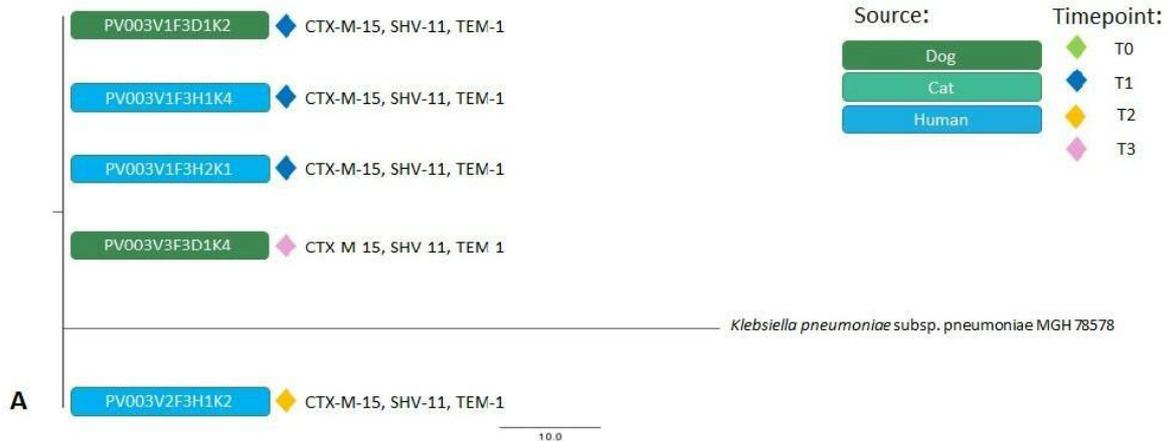
<sup>2</sup> Strain code in bold refers to strains selected for WGS sequencing.

## Phylogeny of beta-lactamase producing *E. coli*



Phylogenetic tree of ESBL/AmpC- and carbapenemase producing *Escherichia coli* strains from companion animals and owners based on whole genome single nucleotide polymorphism. Analysis was inferred by CSI Phylogeny 1.4 (<https://cge.cbs.dtu.dk/services/CSIPhylogeny/>) with the standard parameters, using *Escherichia coli* K-12 MG1655 as reference genome. The first five algorithms on the strains' identification represents the household code number.

## Correlation between dogs/owners bacterial strains



**B**

| SNP differences                | <i>K. pneumoniae</i> MGH 78578 | PV003V3F3D1K4 | PV003V1F3H2K1 | PV003V1F3D1K2 | PV003V1F3H1K4 | PV003V2F3H1K2 |
|--------------------------------|--------------------------------|---------------|---------------|---------------|---------------|---------------|
| <i>K. pneumoniae</i> MGH 78578 | 0                              | 30892         | 30895         | 30894         | 30893         | 30890         |
| PV003V3F3D1K4                  | 30892                          | 0             | 7             | 6             | 5             | 6             |
| PV003V1F3H2K1                  | 30895                          | 7             | 0             | 5             | 4             | 5             |
| PV003V1F3D1K2                  | 30894                          | 6             | 5             | 0             | 3             | 4             |
| PV003V1F3H1K4                  | 30893                          | 5             | 4             | 3             | 0             | 3             |
| PV003V2F3H1K2                  | 30890                          | 6             | 5             | 4             | 3             | 0             |

**C**

| SNP differences | NCTC 9394 | PT125/0-D1F4E2U | PT125/1-H2F3E1U |
|-----------------|-----------|-----------------|-----------------|
| NCTC 9394       | 0         | 151507          | 151509          |
| PT125/0-D1F4E2U | 151507    | 0               | 16              |
| PT125/1-H2F3E1U | 151509    | 16              | 0               |

**A.** Phylogenetic tree of ESBL/AmpC-producing *Klebsiella pneumoniae* strains from companion animals and owners based on whole genome single nucleotide polymorphism. Analysis was inferred by CSI Phylogeny 1.4 (<https://cge.cbs.dtu.dk/services/CSIPhylogeny/>) with the standard parameters, using *K. pneumoniae* MGH 78578 as reference genome; **B.** Pairwise SNP comparison between *K. pneumoniae* strains; **C.** Pairwise SNP comparison between *Enterobacter* spp. strains using complete chromosome sequence of *Enterobacter hormaechei* NCTC 9394 as reference genome. The first five algorithms on the strains' identification represents the household code number.

**Keyword 1**  
Antimicrobial resistance (AMR)

**Keyword 2**

Zoonoses, vector-borne and One Health

**Keyword 3**

ESBL-sharing

**Acknowledgement of grants and fundings, word count: 30 words**

Funding by JPIAMR/0002/2016 Project—PET-Risk Consortium, CIISA and AL4AnimalS through FCT—Fundação para a Ciência e Tecnologia (UIDB/00276/2020;LA/P/0059/2020); JM and JMS were supported by a PhD fellowship (2020.07562.BD; 2020.06540.BD).

*Conflicts of interest*

**Do you have any conflicts of interest to declare?**

No

# CERTIFICATE OF ATTENDANCE

This is to certify that

Juliana Menezes

menezesjulianacruz@gmail.com

Participated onsite in the

33rd European Congress of Clinical  
Microbiology & Infectious Diseases

that took place in Copenhagen, Denmark  
& online from

15 – 18 April 2023

Yours sincerely,



Jacob Moran-Gilad  
ECCMID Programme Director

ESCMID European Society of Clinical Microbiology and Infectious Diseases

**ESCMID Executive Committee:** A. Zinkernagel, President, Zurich, Switzerland; M. Sanguinetti, Immediate Past-President and Guidelines Officer, Rome, Italy; R. Skov, President-elect and Secretary General, Copenhagen, Denmark; A. Friedrich, Treasurer and Financial Support Officer, Münster, Germany; E. Cambau, Education Officer, Paris, France; J. S. Friedland, Scientific Affairs Officer, London, United Kingdom; J. R. Paño-Pardo, Publication, Communication & Guidelines Officer, Zaragoza, Spain; A. Kantele, Professional Affairs Officer, Helsinki, Finland  
**Ad hoc Members:** J. Moran-Gilad, ECCMID Programme Director, Beer Sheva, Israel; L. Leibovici, CMI Editor-in-Chief, Petah-Tiqva, Israel; L. Scudeller, ESCMID Guidelines Director, Pavia, Italy; C. Giske, EUCAST Chairperson, Stockholm, Sweden; EUCIC Chairperson: E. Tacconelli, Verona Italy; M. Akova, ESCMID Membership Counsellor, Ankara, Turkey;

