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Short Communication

Multiple clonal transmissions of clinically relevant extended-spectrum beta-lactamase–producing *Escherichia coli* among livestock, dogs, and wildlife in Chile

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ABSTRACT

Objectives: Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) are a main cause of human deaths associated with antimicrobial resistance (AMR). Despite hundreds of reports of the faecal carriage of ESBL-*E. coli* in domestic and wild animals, the dynamics of its circulation remains poorly understood.

Methods: We used whole genome sequencing of 19 ESBL-*E. coli* previously isolated in the same local setting from dogs, livestock, and a wild rodent in Central Chile to assess potential cross-species transmission of ESBL-*E. coli*.

Results: Isolates harboured a large number of AMR (n = 95) and virulence (n = 45) genes, plasmids replicons (n = 24), and *E. coli* sequence types including top extraintestinal pathogenic *E. coli* ST410, ST58, ST88, and ST617. Almost identical clones (<50 single nucleotide polymorphisms difference, same antibiotic and heavy metal resistance genes, virulence genes, and plasmids) were found in faeces of dogs, cattle, or sheep from the same farm, and in a dog and a wild rodent living in proximity.

Conclusions: To our knowledge, this is the first report of multiple clonal cross-species transmission of ESBL-*E. coli* in domestic and potentially wild animals of Latin America. Our results suggest that relatively rare spread of AMR across animal species can still occur by both clonal and plasmid dissemination. Our study highlights the need for establishing preventive measures to limit the circulation of these bacteria among animals in agricultural settings, particularly given the highly pathogenic profile of several *E. coli* strains detected in these animals.

1. Introduction

Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) represent a major cause of human deaths associated with antimicrobial resistance (AMR) worldwide [1]. ESBL-*E.*

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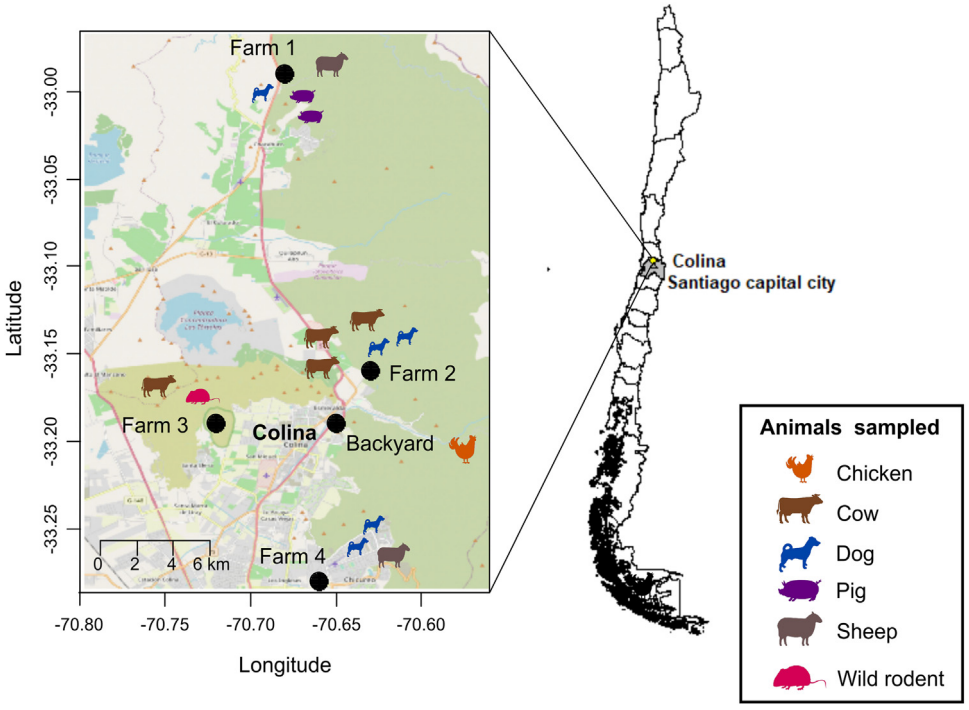

Fig. 1. Map of sampled farms and animal sources of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) in Central Chile. Animals carrying ESBL-*E. coli* are shown for each farm.

coli also circulate among domestic animals [2,3] and wildlife [2,4], but the dynamics of the circulation in farming settings remains poorly understood. For example, most studies on ESBL-*E. coli* at this interface have sampled either one population (domestic or wildlife) or compared populations at large spatial scales [5]. Thus, One Health approaches with local and simultaneous sampling of multiple species are required to better understand and prevent AMR circulation in both agricultural and natural environments.

In Latin America, the circulation of ESBL-*E. coli* across domestic and wild animals at the local scale is still poorly understood [2,4,6]. Genomic studies of *E. coli* at the domestic–wild animal interface combined with epidemiological meaningful study designs (e.g., same location and time) can largely contribute to better understanding the dynamics of ESBL-*E. coli* within this interface [7]. For example, a genomic study showed that the same *E. coli* sequence type (ST) 744 containing the same CTX-M-15 gene was detected in a pig and a vampire bat feeding on that population in Peru [4]. The aim of this study was to use whole genome sequencing to better understand the circulation of ESBL-*E. coli* previously isolated from livestock, dogs, and wild mammals of small-scale farms in Central Chile [2].

ESBL-*E. coli* infections are an important threat to Chile's public health, and the widespread misuse of antibiotics in the veterinary sector could lead to the dissemination of ESBL-*E. coli* among livestock, dogs, and wildlife [6,8]. However, little is known on AMR circulation among domestic and wild animals in Chile. ESBL-*E. coli* have been detected in dogs, cattle, pigs, and sheep [2], but to our knowledge, no study has characterised full-length genomes of ESBL-*E. coli* isolates of domestic and wild animals leaving in the same area [2]. Therefore, the aims of this study were to (i) identify the *E. coli* lineage, AMR and virulence genes, and plasmid replicons from genomes of ESBL-*E. coli* previously isolated from faecal samples of livestock, dogs, and a wild mammal living in the same agricultural setting of Central Chile; (ii) use phylogenomics to assess potential ESBL-*E. coli* interspecies transmission within and across farms; and (iii) identify *E. coli* clones of public health concern.

2. Methods

2.1. Sample preparation and DNA extraction

Sampling and microbiological analyses for these samples have been previously described [2]. Briefly, fresh faecal samples were collected in 2019 around 13 farming localities in the peri-urban area of the Santiago Capital City (Fig. 1). Samples were part of a study aiming to estimate ESBL-*E. coli* prevalence on 332 livestock, 82 dogs, and 173 wild animals. These isolates were previously analysed in terms of antimicrobial susceptibility and presence of CTX-M genotypes. From the 47 confirmed ESBL-*E. coli* in our previous study [2], and given logistical limitations, we randomly sequenced 19 isolates including at least one isolate from each livestock species samples (four isolates from different cattle, three isolates from two different pigs, two isolates from different sheep, and two isolates from the same chicken), seven isolates from dogs (including two dogs with two isolates), and the unique isolate recovered from a wild rodent.

2.2. Sequencing

Whole genome sequencing was performed using the same methodology described in Benavides et al. 2022 [4]. The libraries were sequenced on a HiSeq platform (Illumina).

2.3. Bioinformatics analyses

The Illumina raw reads of the 19 isolates were submitted to a Nextflow pipeline (https://github.com/jhayer/baargin) to perform the following in silico analyses. All steps are described on the github repository of the 'baargin' workflow, which used Resistance Gene Identifier CARD and AMRFinderPlus (95% identity and 80% of coverage of the reference length), multilocus sequence typing with the PubMLST typing scheme, VirulenceFinder (http://cge.cbs.dtu.dk/services/VirulenceFinder/) (90% threshold, 60% coverage), Plas-

Table 1
Sample information and assembly metrics for raw and decontaminated contigs

Sample ID	Source animal	Farm	Assembly size (Mbp)		N50 (kbp)		L50		Number of scaffolds >500 bp		BUSCO score (%)	Avg. cov. depth
			R	D	R	D	R	D	R	D	R and D	
56036_V2071C	Cow	2	5.06	5.04	233.55	233.55	8	8	56	44	99.8	107
56037_Po151C	Pig	1	5.34	5.18	406.19	406.19	6	6	76	57	100.0	110
56038_P241C	Dog	2	5.05	5.03	206.01	206.01	9	9	77	58	99.8	43
56039_P52C	Dog	4	4.86	4.85	279.63	279.63	6	6	54	44	100.0	63
56040_Po62C	Pig	1	5.16	5.06	109.01	111.40	16	15	131	100	99.6	42
56041_P71C	Dog	1	5.23	5.20	213.92	213.92	8	8	85	69	100.0	63
56042_V2031C	Cow	2	5.05	5.01	191.27	191.27	10	10	83	61	99.8	56
56043_P42AC	Dog	4	4.71	4.69	131.62	131.62	10	10	73	60	100.0	115
56044_P351C	Dog	2	5.06	5.04	211.98	211.98	9	9	70	56	99.8	112
56045_V1982C	Cow	2	5.40	5.29	191.27	191.27	11	11	385	268	99.8	45
56046_P43AC	Dog	4	4.89	4.87	341.17	341.17	6	6	51	37	100.0	70
56047_P72C	Dog	1	5.23	5.20	208.77	208.77	9	9	94	74	100.0	63
56048_A71C	Chicken	BY	4.74	4.73	143.37	143.37	11	11	69	57	100.0	76
56049_Sh282C	Sheep	1	5.05	4.98	145.19	165.07	11	10	116	78	99.6	82
56050_A73C	Chicken	BY	4.98	4.95	87.98	87.98	18	18	134	117	100.0	86
56051_V1672C	Cow	3	4.94	4.91	115.38	115.38	15	15	120	105	100.0	196
56052_Po61C	Pig	1	5.02	4.87	126.06	126.44	13	12	113	87	97.5	51
56053_R352C	Rodent	3	5.30	5.26	263.46	263.46	8	8	96	73	100.0	97
56054_Sh41C	Sheep	4	4.86	4.84	214.48	214.48	7	7	54	45	100.0	65

BY, backyard; D, decontaminated; R, raw.

midFinder 2.1.6 and Platon 1.6. Contigs with CTX-m genes detected as plasmids were clustered using CD-hit (90% identity threshold).

2.3.1. Phylogenomic analyses of E. coli

With the pangenome analysis, a multiple alignment of the core genes (i.e., genes common to all 19 isolates) was produced using MAFFT. This alignment was used to build a phylogenetic tree with PhyML [9] using a generalised time-reversible model. The single nucleotide polymorphisms (SNPs)–based phylogeny on the decontaminated contigs was calculated using CSIPhylogeny online (https://cge.food.dtu.dk/services/CSIPhylogeny/), using the decontaminated contigs of the isolate from the sample 56045_V1982C as a reference.

2.3.2. Global analyses of the ESBL-E. coli ST2973 isolated from a wild rodent and a dog

We conducted a query on Enterobase [10] regarding ST2973, retrieving a list of sequence read archive accession numbers. Forty-two data sets were retrieved and analysed in the same way as the 19 data sets from our study. A phylogenetic tree was built based on the pangenome analysis including the three isolates from our study. The core genome alignment was used to build a phylogenetic tree using PhyML, and an additional SNP tree was inferred using CGE's CSIPhylogeny online tool.

3. Results

3.1. De novo assembly of the 19 ESBL-E. coli

ESBL-*E. coli* were isolated from four farms and a backyard in Central Chile located less than 20 km from each other (Fig. 1). The raw reads of the 19 data sets are deposited to the European Nucleotide Archive under project accession No. PRJEB62462. All 19 data sets produced near-complete assemblies based on the presence of 440 housekeeping genes in the order *Enterobacterales* detected using BUSCO (Table 1). The assembly sizes were 4.71 to 5.40 Mbp (raw) and 4.69 to 5.29 Mbp (decontaminated).

3.2. E. coli sequence types and pangenome analyses

The multilocus sequence typing analyses identified ten different STs in the studied isolates (Fig. 2). The ST410 was assigned to five isolates: ST2973 to 3 isolates and STs 58, 2541, and 6438 to2 isolates. STs 88, 617, 744, 1011, and 8553 were assigned to one isolate each. ST410 was isolated from three cows and two dogs from the same farm (farm 2). ST2973 was isolated from two dogs of farm 1 and from a wild rodent (*Octodon degus*) overlapping territory with farm 3, which was located less than 15 km from farm 1 (Fig. 1).

E. coli isolates belonging to the same ST clustered together in the phylogenetic tree based on the core genome alignment (Fig. 2). The SNP-based tree produced a similar topology, and the SNP matrix showed that the minimum difference between two isolates ranged from 4 to 33 577 SNPs. ST410 *E. coli* isolated in dogs and cattle from the same farm 2 (Peldehue) differed by 4 to 12 SNPs. The two *E. coli* ST2973 isolated from the same dog in farm 1 differed by nine SNPs, whereas they each differed from the rodent isolate found at a different farm (farm 3) by 18 and 27 SNPs. Two isolates of ST2541 from a dog and a sheep in farm 4 (Chicureo) differed by only seven SNPs. Two ST58 *E. coli* isolated from the same farm (farm 1 in Tupaco) differed by 1921 SNPs, whereas two isolates of ST6438 differed by only five SNPs but were not isolated from the same farm: One was isolated from a chicken in a backyard and the other from a cow in farm 3, about 7 km apart.

3.3. Antimicrobial resistance

3.3.1. AMR genes

Both methods of AMR gene detection (CARD RGI and NCBI AMRFinderPlus) detected mostly the same genes, so we present the combined detection of each gene by at least one method. Several genes coding for beta-lactamase enzymes were identified, including six CTX-M genes. *E. coli* isolates belonging to the same ST had very similar AMR gene profiles (Fig. 2). Most isolates harboured $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-55}}$ genes, including the five ST410 carrying $bla_{\text{CTX-M-15}}$, as well as ST6438 and one ST58. The gene $bla_{\text{CTX-M-55}}$ was carried by all ST2973 and by ST1011 and ST58 isolated from a pig and a sheep in the same farm (farm 1). Only two ST2541 isolated from animals of the same farm did not carry any $bla_{\text{CTX-M}}$ gene, but they both carried $bla_{\text{CMY-2}}$, also detected in the 5 ST410. The gene $bla_{\text{OXA-1}}$ was exclusively detected in ST410. The $bla_{\text{TEM-1}}$ gene was identified in the three isolates from livestock of farm 1 (Tupaco). The sulfonamide-resistant gene *sul2* was present in ST410 and ST2973, whereas *sul1* was found in all ST410 isolated at farm 2.

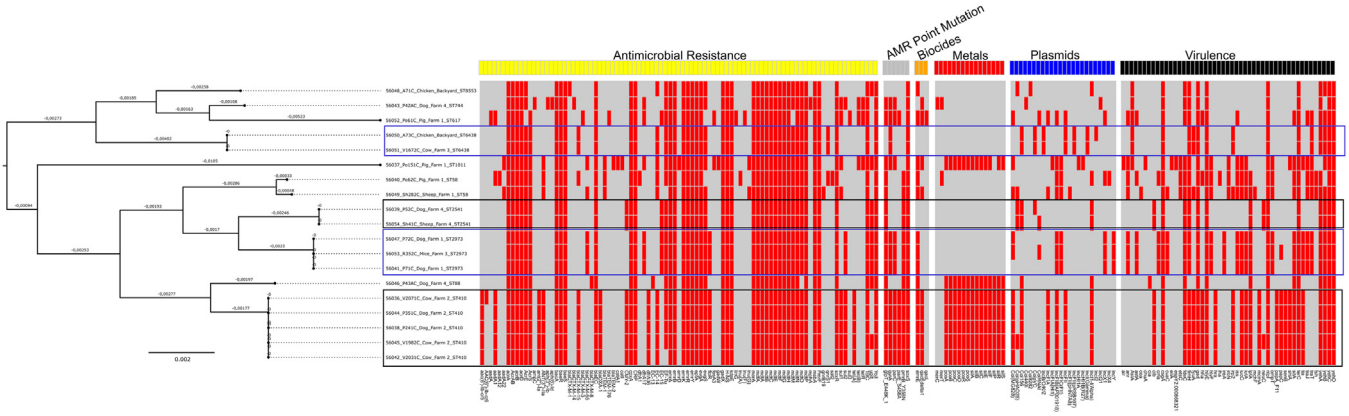

Fig. 2. Phylogenetic tree of the 19 extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) isolates with their sequence type (ST), antimicrobial resistance (AMR) gene profiles, virulence factors genes, and plasmids Inc types. Red represents the presence of a gene, and grey represents the absence of a gene (or plasmid). *E. coli* isolates that appear to be clonal are highlighted, with black frames suggesting intrafarm transmission and blue frames suggesting interfarm transmission. The phylogeny was reconstructed by PhyML using the generalised time-reversible model. AMR genes' presence/absence was detected with both AMRFinderPlus and CARD RGI, and virulence genes with VirulenceFinder (CGE).

3.3.2. Antibiotics efflux and point mutations

All 19 isolates presented several genes coding for antibiotic efflux systems including *Acr, mdt*, and *emr* genes (Fig. 2). The point mutation *soxS_A12S* conferring resistance to ampicillin, chloramphenicol, quinolone, rifampin, and tetracycline was detected in all 19 isolates. The *glpT_E448K* mutation, conferring resistance to Fosfomycin, was found in most isolates. Two mutations involved in the resistance to quinolones *gyrA_D87N* and *gyrA_S83L* were identified in most isolates except for ST58, ST88, ST2541, and ST8553. The mutation *pmrB_Y358N*, involved in the resistance to colistin, was found in 13 isolates from STs 410, 2973, 58, 88, and 2541.

3.4. Resistance to metals and biocides

A total of 16 genes conferring resistance to metals were detected, particularly in all isolates from farm 2 (ST410), two isolates from a pig of farm 1 (ST1011), and a dog of farm 4 (ST88). Resistance to biocides was also identified in almost all isolates, including the *qacEdelta1* gene coding for a quaternary ammonium compound efflux SMR transporter QacE delta 1 present in nine isolates, and the gene *emrE*, coding for the multidrug efflux SMR transporter EmrE present in 14 isolates.

3.5. Plasmids

Similar plasmids replicons were identified in all the ST410 isolates including IncI1-I(Alpha), IncFII, IncFII, IncFIB(AP001918), IncFIA, and Col156. Replicons IncY, IncX1, IncFIB(AP001918), and IncFIC(FII) were detected in all ST2973 isolates (Fig. 2). In almost all isolates, the beta-lactamase genes from the $bla_{\text{CTX-M}}$ family, but also $bla_{\text{CMY-2}}$ and $bla_{\text{OXA-1}}$ genes, were carried on contigs that are recognised as plasmids either by Platon or PlasmidFinder. Contig cluster analyses showed a similar plasmid contigs carrying $bla_{\text{CTX-M-15}}$ in ST410 from a dog and a cow of the same farm, carrying $bla_{\text{oxa-1}}$ in ST410 from a dog and two cows of that same farm, and carrying $bla_{\text{CTX-M-55}}$ in ST2973 from a dog and the wild rodent from farms.

3.6. Virulence genes

A total of 49 virulence genes were detected by VirulenceFinder, and most isolates harboured a high number of virulence genes (mean: 21, median: 26, range: 10–28). Virulence profiles were similar among isolates that clustered together in the phylogenetic tree (Fig. 2). Virulence genes detected in all 19 isolates included *yehC* and *yehD* (YHD fimbriael cluster), *terC* (Tellurium ion resistance protein), *nlpI* (lipoprotein NlpI precursor), and *hylE* (coding for the Avian *E. coli* haemolysin) (Fig. 2).

3.7. Global analyses of ST2973

ST2973 isolates were recovered from 2013 to 2021 in 12 countries and included isolates from domestic animals, humans, and the environment (Fig. 3A). The wild rodent isolate of this study was the only one from wildlife in the data set. The pangenomic analysis of 45 isolates resulted in 3856 genes making up the core genome and 8816 accessories genes. The inferred phylogenetic tree showed that genomes differed by zero to 2913 SNPs and did not clearly cluster by source or continent, although most isolates from the same country clustered together if collected in a similar time period (Fig. 3B). Chilean isolates of this study clustered together and were most like a human isolate from the United States, and one isolate from poultry in China.

4. Discussion

The dynamics of circulation of ESBL-*E. coli* within and between domestic and wild animals in rural environments remains poorly understood. In this study, genomic analyses of ESBL-*E. coli* collected in closely located farms showed a high diversity of *E. coli* ST (10 different out of 19 isolates), ESBL genes, and virulence genes despite a low prevalence of ESBL detected in a previous study in this population (<3% [2]). Several clinically relevant STs (e.g., ST410) with a very low number of SNP difference and with the same or similar AMR genes profiles, plasmid replicons, and virulence genes were isolated among dogs and cattle of the same farm, strongly suggesting clonal interspecies transmission between dogs and livestock. The ST2973 found in a dog and a wild rodent sampled about 15 km apart also suggested interspecies transmission of clonal ESBL-*E. coli* between domestic and wild species found in geographical proximity.

Cross-species transmission of ESBL-*E. coli* between livestock and dogs has been suggested worldwide, but rarely proven [8,11]. Our study revealed that clonal transmission of ESBL-*E. coli* can occur between dogs and livestock within and between farms. In fact, farmers in this area assemble cattle across farms for both parasite control and summer migration to high altitude pastures. Our study supports that AMR can also be transmitted clonally in addition to the horizontal gene transfer of plasmids commonly reported and suggested in our study. To our knowledge, this is the first evidence

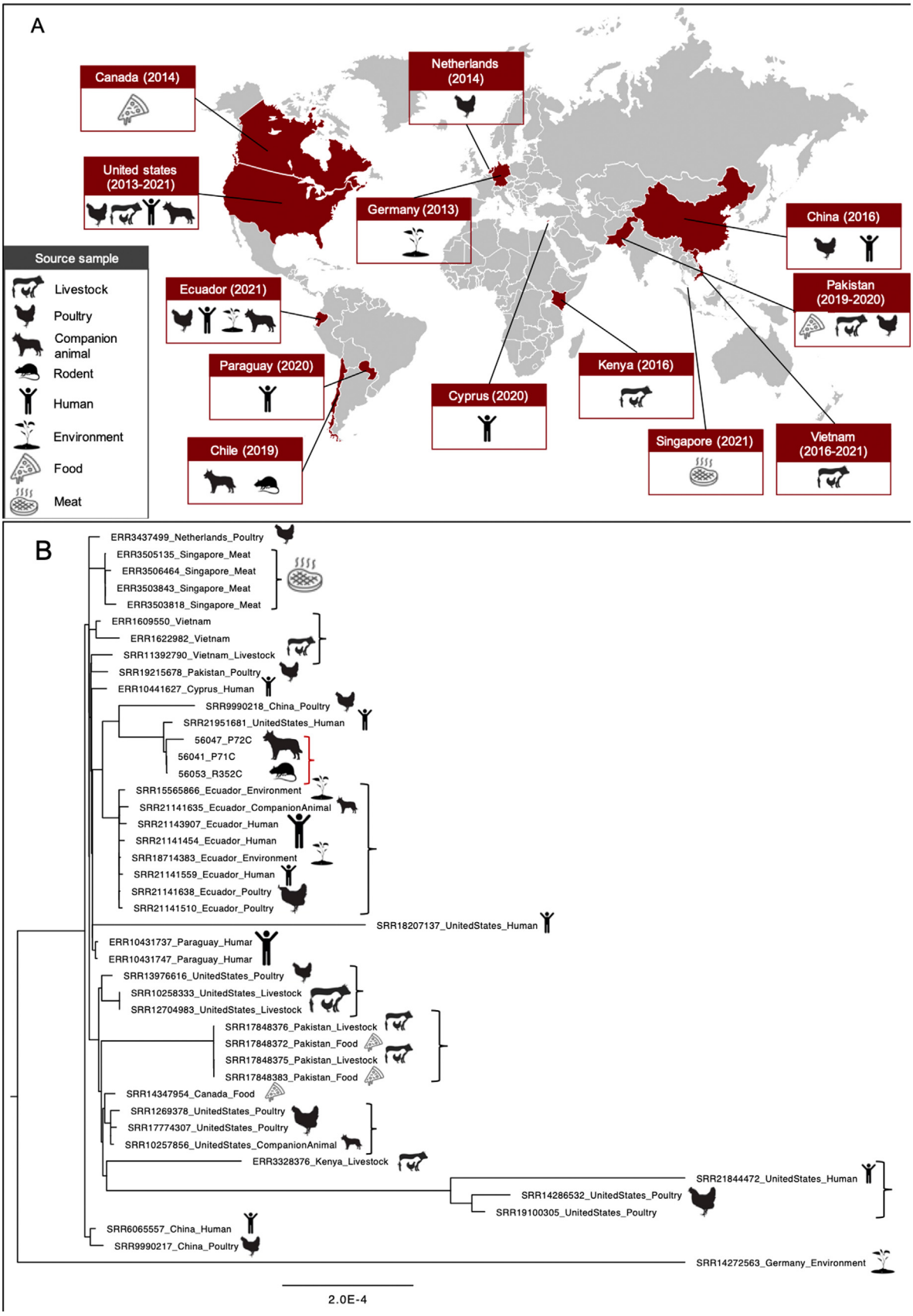

Fig. 3. Global location and phylogenomics of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) ST2973 found in a dog and a wild rodent in this study. (A) Map showing the country and source type of the 42 isolates retrieved from the European Nucleotide Archive using Enterobase to obtain accession numbers of ST2973 isolates, and three isolates from our study. (B) Phylogenetic tree of the 45 *E. coli* isolates of ST2973 reconstructed using PhyML with the generalised time-reversible model.

of cross-species clonal transmission of ESBL-*E. coli* among animals of Latin America. Future research should understand its drivers to limit the spread of AMR in countries like Chile with a high density of dogs in rural areas [12].

Our study also suggests the potential for rare but existing clonal AMR transmission between a dog and a wild rodent that were sampled a few kilometres apart. Movements of dogs and rodents across these areas are not unusual. A larger sampling of rodents within farms would contribute to test the existence and frequency of this interspecies transmission. To our knowledge, our study is the first report of *E. coli* ST2973 in a free-range wild animal. As ST2973 carrying $bla_{\text{CTX-M-55}}$ has been associated with urinary and bloodstream infections in humans [13,14], future studies should evaluate the public health implication of this *E. coli* clone for the human population of Chile.

ESBL-*E. coli* are among the leading pathogens causing human deaths associated with AMR worldwide [1]. We recovered isolates from animals without visible signs of illness, but several isolated *E. coli* have pathogenic potential for humans, including ST410, ST58, ST88, and S617, all considered among the top 20 extra-intestinal pathogenic *E. coli* [15]. ST410 has been recently classified as a pandemic lineage after being identified as circulating among humans and domestic animals worldwide [16]. In addition to their pathogenic potential, isolates also showed high potential to resist heavy metals and biocides, increasing their likelihood to remain in the environment. Future studies should also pay particular attention to resistance to quaternary ammonium—one of the most-used disinfectants in veterinary expected to kill 99.9% of bacteria including *E. coli* [17].

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Competing interests: None declared.

Ethical approval: This study was approved by the Ethical Committee of the Universidad Andrés Bello (permit number: 018/2018). Capture and sampling of rodents were also approved by Servicio Agricola Ganadero (permit number: 2118/2019). Informed consent was obtained from all farmers for the inclusion of their dogs and/or livestock.

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