Science of the Total Environment 916 (2024) 170139

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Application of phylodynamics to identify spread of antimicrobial-resistant *Escherichia coli* between humans and canines in an urban environment

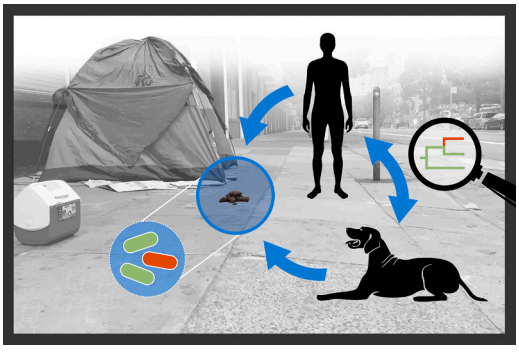
Nikolina Walas [a,*], Nicola F. Müller [b], Emily Parker [a], Abigail Henderson [a], Drew Capone [c], Joe Brown [c], Troy Barker [c], Jay P. Graham [a]

[a] University of California, Berkeley, CA, USA
[b] Fred Hutch, Seattle, WA, USA
[c] The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

HIGHLIGHTS

- Feces on the sidewalks of San Francisco contain multi-drug resistant bacteria
- The majority of fecal samples lacked the human mtDNA marker, suggesting canine origin
- Genomic sequencing of *E. coli* reveals recent putative canine-human transmission events
- Environmental surveillance of street fecal contamination provides insights on spillover of antimicrobial resistance

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Kyle Bibby

Keywords:
ESBL
Phylodynamics
Genomic epidemiology
Antimicrobial resistance
Canines
Environment

ABSTRACT

The transmission of antimicrobial resistant bacteria in the urban environment is poorly understood. We utilized genomic sequencing and phylogenetics to characterize the transmission dynamics of antimicrobial resistant *Escherichia coli* (AMR-Ec) cultured from putative canine ($canine_p$) and human feces present on urban sidewalks in San Francisco, California. We isolated a total of fifty-six AMR-Ec isolates from human (n = 20) and $canine_p$ (n = 36) fecal samples from the Tenderloin and South of Market (SoMa) neighborhoods of San Francisco. We then analyzed phenotypic and genotypic antimicrobial resistance (AMR) of the isolates, as well as clonal relationships based on cgMLST and single nucleotide polymorphisms (SNPs) of the core genomes. Using Bayesian inference, we reconstructed the transmission dynamics between humans and $canines_p$ from multiple local outbreak clusters using the marginal structured coalescent approximation (MASCOT). Our results provide evidence for multiple sharing events of AMR-Ec between humans and $canines_p$. In particular, we found one instance of likely transmission from $canines_p$ to humans as well as an additional local outbreak cluster consisting of one $canine_p$ and one human sample. Based on this analysis, it appears that non-human feces act as an important reservoir of clinically relevant AMR-Ec within the urban environment for this study population. This work showcases the utility of genomic epidemiology to reconstruct potential pathways by which antimicrobial resistance spreads.

* Corresponding author at: 2121 Berkeley Way West, Berkeley, CA 94720-7358, USA.
E-mail address: nwalas@berkeley.edu (N. Walas).

https://doi.org/10.1016/j.scitotenv.2024.170139
Received 29 August 2023; Received in revised form 10 January 2024; Accepted 11 January 2024
Available online 17 January 2024

1. Introduction

Antimicrobial resistance (AMR) is a global health crisis with more than 28 million antimicrobial-resistant infections occurring in the US each year (Centers for Disease Control and Prevention (U.S.), 2019). In 2019, *E. coli* was estimated to be the top contributor to deaths attributable to bacterial AMR (Murray et al., 2022). Despite the increasing rates of AMR bacterial infections, these pathogenic species targeted by antibiotics constitute a small proportion of the gut microbiome. Nonetheless, resistance to common antimicrobials can pass between this population and the normal gut flora or surrounding environmental bacteria (Woolhouse et al., 2015). Studies have shown *E. coli,* a key species associated with AMR, to be a member of the gut microbiome in about 90 % of individuals (AbuOun et al., 2020; Tenaillon et al., 2010). In 2020, the Global Antimicrobial Resistance and Use Surveillance System (GLASS) estimated the global prevalence of ciprofloxacin-resistance in *E. coli* from environmental and clinical settings ranged from 8.4 % to 92.9 % (Wang et al., 2023; World Health Organization, 2020). Models predict over half of *E. coli* invasive species may become 3rd generation cephalosporin resistant by 2030 (Alvarez-Uria et al., 2018). Increasing rates of resistance in such populations have been attributed to the circulation of specific resistant bacterial clonal species as well as transmission of antimicrobial resistance genes (ARGs), often mediated by mobile genetic elements such as integrons, transposons, and plasmids (Carattoli, 2009; Zhang et al., 2020).

The global dissemination of antimicrobial resistant bacteria (ARB) such as *E. coli* is best described using the One Health approach where humans, animals, and the environment act as overlapping domains of transmission (Zhang et al., 2020; Robinson et al., 2016). Despite this, research has focused heavily on clinical interventions to reduce AMR transmission in human pathogens. Studies have suggested animals and the environment play equally important roles as reservoirs (Musoke et al., 2021), but the extent of their contribution to the AMR transmission cycle is still not well understood (Robinson et al., 2016). It is known, however, that uncollected fecal waste and runoff contaminated with ARGs from human and livestock waste exacerbates the natural exchange of resistance genes from environmental bacteria to animal and human pathogens (Reinthaler et al., 2003).

Domesticated animals have long been established as a reservoir for AMR but there is still controversy regarding the exact role canines play in transmission to humans and environmental contamination. Canines have been shown to act as vectors for clinically relevant extended spectrum β-lactamase (ESBL) producing bacteria and genes in urban settings (Damborg et al., 2015). With regard to humans, various cohabitation studies have shown a wide range of overlap in the resistance profile of domesticated canines and their respective owners (Marchetti et al., 2021; Zhao et al., 2022). For example, Johnson et al. showed that a strain of uropathogenic *E. coli* appeared to move between a canine and humans in the same household (Johnson et al., 2003). These relationships were not found in different settings, most likely due to limitations in sampling time and the transient nature of microbiota in the gut (Røken et al., 2022).

Another challenge in studying the burden of non-human reservoirs on AMR transmission to humans is the lack of computational tools to robustly infer transmission directionality. The majority of studies investigating the role of animals, for example, use the co-occurrence of resistance as an indicator of spread or rely on methods that are insufficient to conclude directionality (Muloi et al., 2018). For example, vertical evolution of shared bacterial species has traditionally been investigated using phylogenetic modeling. However, these analyses are usually constrained to the core genome of bacteria due to the high degree of conservation (Keller and Ankenbrand, 2021) and largely ignore metadata during model generation (Ingle et al., 2021). Phylodynamics, which uses genetic data to infer epidemiological dynamics, is an increasingly popular framework that can be used to infer transmission events. It has been widely used to study viral transmission networks (Ingle et al., 2021), but its application to bacterial pathogens (Guinat et al., 2021) has lagged due to the often more complex evolutionary mechanisms (*e.g.*, mobile genetic elements). Applying phylodynamics to understand the transmission dynamics of AMR-Ec between species has the potential to help inform disease control strategies and public health interventions.

Most studies investigating the role of non-human hosts in AMR have been conducted in low- and middle-income countries (LMICs) that are known to bear the greatest burden of AMR due to a range of political, economic, and infrastructure factors (Robinson et al., 2016; Pokharel et al., 2019). Few studies (Damborg et al., 2015) have detailed the resistance profiles of canines with regards to its contribution to environmental contamination of ARGs which is imperative to understanding the risk of transmission and ultimate burden on human infections in surrounding settings. Therefore, the aim of this study was to characterize the prevalence of AMR-Ec in human and putative canine ($canine_p$) fecal samples found on the sidewalks of San Francisco, CA, USA where the density of unhoused individuals is high. We used a novel phylodynamic model, the marginal approximation of the structured coalescent (MASCOT), which has been previously used to investigate transmission directionality in respiratory viruses and bacterial pathogens (Bedford et al., 2020; Canini et al., 2023; Perets et al., 2022; Müller et al., 2021). To our knowledge, this is the first application of such an approach to interhost transmission of multidrug resistant bacteria isolated from the environment. Results from this study can be used to better understand the public health risk of antimicrobial resistance from environmental fecal contamination.

2. Materials and methods

2.1. Sample collection and DNA isolation

Fecal samples were located based on open defecation hotspots that were determined by San Francisco's 311 municipal system that allows citizens to report issues to the city's department of public works. Samples were collected from a perimeter of 20 blocks in the Tenderloin and SOMA neighborhoods and included sidewalks on either side of the street. Biospecimens were collected on Wednesday mornings before daily street cleaning in September and October of 2020, and were presumed to be less than 24 h old based on observations. Samples were placed into one-liter biohazard bags (Millipore Sigma, Darmstadt, Germany) and stored in a cooler with ice packs for a maximum of 4 h before being transferred and stored in 1.5 mL cryotubes at −20 °C.

The QIAamp 96 Virus QIAcube HT Kit was used to purify DNA. DNA was first extracted from 100 mg of stool using a bead beating tube and 1 mL Qiagen Buffer ASL. As a positive control for DNA extraction, G-block from IDT matching sequence for phocine herpesvirus was spiked in. The bead beating tubes were vortexed for 5 min, incubated at room temperature for 15 min, and centrifuged at 14,000 rpm for 2 min, in alignment with previous study methods (Knee et al., 2021). Extraction was completed using the QIAamp 96 Virus 281 QIAcube HT Kit and done on the QIAcube. The extracted nucleic acids were stored at 4 °C for less than 24 h before being stored at −80 °C.

Human and non-human fecal samples were determined using a previously validated dPCR method to detect human mitochondrial DNA (mtDNA) with a sensitivity and specificity of 100 % and 97 %, respectively (Zhu et al., 2020). This assay was not formally validated on canine fecal detection, but canine consensus sequences showed a similar number of mismatches as pig samples during assay development and design. The assay was able to distinguish between human and pig samples using orders of magnitude during validation. Therefore, cross reactivity between the human mtDNA and canine sequences was not determined to be a major concern. Additional visual inspection of the feces suggested all originated from large mammals, which in downtown San Francisco is limited to humans and canines, such as domesticated dogs and the rare coyote. A QIAGEN QIAcuity Four machine was used to

run 40 μL reactions on QIAcuity Nanoplate 26K 24-wll plates. Reactions included 2 μL template, 27.2 μL nucleotide-free H20, 1× CIAcuity PCR MasterMix, 400 nM sun probe, and 800 nM of forward and reverse primers. Cycles consisted of 2 min at 95 °C, 40 cycles of 15 s at 95 °C, and 30 s at 59 °C. Positive and negative controls were run each day of analysis. Qubit dsDNA results were used to normalize gene copy estimates with a positive cut-off log-adjusted value of 1.

2.2. Isolate selection and phenotypic analysis

In order to identify resistant isolates, fecal samples were first streaked with a sterile rayon swab on three plates containing MacConkey, MacConkey and Ampicillin (32 μg/mL), and MacConkey and Ceftriaxone (1 μg/mL) mediums. Plates were inverted and incubated for 18–24 h at 37 °C and up to six random lactose positive colonies were selected from each plate and inoculated in LB broth. If no lactose positive colonies were identified, a lactose negative sample was selected. Broth cultures were incubated for 18–24 h at 37 °C then stored in a 15 % glycerol solution at −80 °C. Individual colonies were confirmed to be *E. coli* through indole production verification using pre-specified methods (MacWilliams, 2012). In short, isolates were grown on trypticase soy agar and incubated for 18–24 h at 37 °C. A 1 μL inoculation loop was used to place a small amount of sample on filter paper and Indole DMACA Spot solution was applied.

Indole positive, *E. coli* isolates were then grown on Mueller Hinton II agar for 18–24 h at 37 °C. Three to five isolates were then selected from the growth plate and placed into Mueller Hinton II cation adjusted broth which was adjusted until the optical density (OD) was between 0.10 and 0.12. The sample was then streaked onto a Mueller Hinton II agar for disc diffusion susceptibility testing. We used a disc diffusion panel of ten antimicrobials in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, n.d.): ampicillin (AMP; 10 μg disc), ertapenem (ETP; 10 μg disc), ciprofloxacin (CIP; 5 μg disc), trimethoprim-sulfamethoxazole (SXT; 25 μg, disc, 1:19), nitrofurantoin (NIT; 300 μg, disc), cefepime (FEP; 30 μg, disc), piperacillin-tazobactam (TZP; 110 μg, disc, 10.5:1), cefazolin (CFZ; 30 μg, disc), cefotaxime (CTX; 30 μg, disc) and ceftazidime (CAZ; 30 μg, disc). *E. coli* ATCC® 25922™ from the American Type Culture Collection was used as a reference strain against all samples tested. Isolates resistant to at least one antimicrobial and with a unique resistance profile were selected for whole genome sequencing. If more than one bacterial isolate showed identical resistance profiles and originated from the same fecal sample, one isolate was randomly chosen as a representative for testing.

2.3. Whole genome sequencing, assembly, and analysis

DNA for whole genome sequencing was purified from AMR isolates using the Qiagen DNeasy Blood and Tissue extraction kit. Whole-genome sequencing data was generated using an Illumina NovaSeq 6000 platform with a paired-end protocol (Nextera XT library; Illumina). Quality of reads was assessed using FastQC v0.11. *De novo* assemblies of the paired short reads were generated using Unicycler v.0.3.0b which acts as a SPAdes optimizer (Wick et al., 2017). Contigs below 500 bp were excluded from the final draft assemblies. Quality of assembled sequences was assessed using QUAST v5.0 (Mikheenko et al., 2018).

Antimicrobial resistance genes, plasmid types, and virulence genes were identified using the ABRicate tool (version 1.0) (Seemann, n.d.-a). The ResFinder database was used to detect resistance genes with a 90 % minimum match and 80 % minimum length. The PlasmidFinder database was used to detect the plasmid replicons in isolates with an 80 % minimum coverage and identity. The VFDB and Ecoli_VF databases were used to detect virulence genes with an 80 % minimum coverage and identity. Pathotypes of diarrheagenic *E. coli* were screened according to the presence of previously defined virulence genes (Vidal et al., 2005). Plasmid content of the isolates was determined by assigning contigs of draft genomes as plasmid or chromosomal using mlplasmids (Arredondo-Alonso et al., 2018) and MOB-suite (Robertson and Nash, 2018). Contigs smaller than 1000 base pairs were filtered out of the analysis. The *E. coli* reference database was used for MOB-suite analysis with a stringent cut-off of a Mash distance of 0.05 (Matlock et al., 2021; Robertson and Nash, 2018). MOB-suite results were used in place of mlplasmids for contig calls with a minimum posterior probability below 75 % from mlplasmids. Contigs with discrepant results from the two programs were not included in the final analysis.

MLST version 2.19 (Seemann, n.d.-b) was used to perform *in silico* multilocus sequence typing (MLST), based on seven housekeeping genes (adk, fumC, gyrB, icd, mdh, purA, and recA). cgMLSTFinder version 1.1 (Jolley and Maiden, 2010; Clausen et al., 2018) accessed through the Center for Genomic Epidemiology was used to assign the cgMLST to each *E. coli* isolate. Isolates were considered epidemiologically linked with a genetic distance, calculated by the number of allele differences divided by the number of alleles shared between two isolates, below 0.0105 according to previous methods (Kluytmans-van den Bergh et al., 2016). Phylogroup assignment was determined using the *in silico* Clermont 2013 PCR typing method tool EzClermont (Waters and Pritchard, n.d.).

2.4. Phylogenetic and MASCOT analysis

Assembled draft genomes were annotated using Prokka version 1.12 (Seemann, 2014). Pan-genome analysis was completed using ROARY version 3.13 (Page et al., 2015) with a core gene defined as being present in over 99 % of the isolates. Core genome alignment comprised of 2905 core genes was generated using the MAFFT setting in ROARY. SNP-sites (Page et al., 2016) was used to extract 200,473 single-nucleotide polymorphisms (SNPs) from the core genome alignment. A maximum-likelihood tree was then calculated using RAxML version 8.2.12 (Stamatakis, 2014) with the general time-reversible model (GTRCAT) and 100 bootstrap replicates. Recombination events detected by ClonalFrameML version 1.12 (Didelot and Wilson, 2015) were masked to produce a recombination free phylogenetic tree. The tree was then visualized in iTol version 6.5.8 (Letunic and Bork, 2007).

To infer the transmission dynamics between canines$_p$ and humans, we first split the dataset into local outbreak clusters according to previous methods (Müller et al., 2020). We defined a local outbreak cluster as any set of sequences that are at most 200 SNPs apart in alignment with previous literature (Clausen et al., 2018; Kluytmans-van den Bergh et al., 2016). MASCOT was then used jointly on all outbreak clusters inferring the effective population size of *E. coli* in humans and canines$_p$, the rates of transmission between them, and the rate of introduction of *E. coli* into either compartment. We assumed a rate of evolution of 4 SNP's per year, based on the rate estimate for *Shigella sonnei* from previous literature (Müller et al., 2022). As a site model, we used a GTR + Γ_4 with estimated rates. Additionally, we reconstructed the host type of internal nodes in the local outbreak clusters, as well as the posterior distribution of host transfers, as the number of edges for which parent and child node are inferred to be in different hosts. Tree output was visualized using densitree (Bouckaert, 2010) and ggtree (Yu et al., 2017).

2.5. Statistical analysis and data visualization

The quantitative output of the number of ARGs in a species was recoded as a binary, presence or absence, categorical variable in the statistical tests. Contingency tables based on ARG presence or absence in the species were then generated and analyzed using the non-parametric two-tailed Fischer's Exact test due to the small sample size. The same methodology was used to analyze virulence gene presence. The significance value was set at 5 %.

All data manipulation and analyses were done using R Studio Software version 1.4.1073 in tandem with the following R packages: ggplot (Wickham, 2016), dplyr (Wickham et al., 2021), stringr (Wickham,

2019), tidyr (Wickham, 2020), and ggsankey (Sjoberg, 2022). Spatial distribution of sample sites was visualized using QGIS (QGIS Association, 2022) with a pseudo-Mercator projection.

3. Results

3.1. Putative canine and human fecal sample characteristics

We sampled sixty fecal samples over the course of five weeks from September to October 2020 in San Francisco, CA, USA that were suspected to be human or $canine_p$ based on visual discrimination by field staff. The range of sample sites covered one mile and spanned from the Tenderloin district to south of Market Street (Fig. 1). Source attribution by mtDNA analysis was possible on 59 of the 60 fecal samples and determined that 20 % (12/59) originated from humans and 80 % (47/59) originated from non-humans (Zhu et al., 2020; Barker et al., 2023). Due to the large size and visual appearance, all non-human fecal samples were assumed to be putative canine ($canine_p$) feces; canines were commonly observed in the area during sampling. In total, 205 *E. coli* isolates were collected across the fecal samples, 125 originated from MacConkey plates, 72 from MacConkey and Ampicillin plates, and 7 from MacConkey and Ceftriaxone plates. The 205 isolates originated from fifty-six (human =20; $canine_p$ = 36) unique fecal samples. All isolates were phenotypically resistant to at least one antimicrobial as determined by disc diffusion methods and were sent for whole genome sequencing (Supplemental Table 3). Between two and three phenotypically unique AMR-Ec were isolated from 58 % (7/12) of human fecal samples and 19 % (9/47) of $canine_p$ samples. The final set of antimicrobial resistant *E. coli* isolates (AMR-Ec) originated from 33 of the 60 fecal samples: nine (75 %) from confirmed human fecal samples and 24 (51 %) from $canine_p$ fecal samples.

3.2. Resistance, plasmid carriage, and pathotypes in human and canines

The resistant isolates from both sources showed a similar distribution in phenotypic resistance (Fig. 2A). Close to 100 % of the isolates from both sources were phenotypically resistant to the penicillin, ampicillin, and the first-generation cephalosporin, cefazolin. A larger proportion of human isolates compared to $canine_p$ isolates were phenotypically resistant to the trimethoprim-sulfamethoxazole (75 %, 52 %) and ciprofloxacin (40 %, 8 %) respectively. Phenotypic resistance to the third and fourth generations of cephalosporin drugs cefotaxime, ceftazidime, and cefepime was higher in humans (20 %, 20 %, 15 %) compared to $canines_p$ (5 %, 14 %, 3 %). Despite being phenotypically resistant to cefazolin in almost 100 % of the isolates, none of the isolates carried cefazolin specific resistance genes.

With regards to antimicrobial resistance genes (ARGs), we identified a total of 28 ARGs, with 5 ARGs found only in humans and 9 ARGs found only in $canines_p$. Although only 50 % of ARGS were shared by both species, there was no significant difference in the presence of resistance genes between two species (Table 1). The mean number of ARGs per isolate was 7.20 in human and 5.11 in $canines_p$. The most common resistance gene was the broad spectrum *mdf(A)* gene, present in all isolates, followed by the β-lactamase gene $bla_{TEM\text{-}1B}$ present in 65 % (n = 13) and 70 % (n = 25) of isolates in humans and $canines_p$, respectively. The class A β-lactamase bla_{TEM104} was only present in one human isolate. There was no overlap in the carriage of the $bla_{CTX\text{-}M}$ extended-spectrum β-lactamase (ESBL) genes between the two species (Fig. 2B). The only ESBL gene detected in $canine_p$ isolates was $bla_{CTX\text{-}M\text{-}27}$ while human isolates carried $bla_{CTX\text{-}M\text{-}55}$ and $bla_{CTX\text{-}M\text{-}15}$.

We detected twenty-seven plasmid replicons, six of which were unique to $canines_p$ and ten to humans (supplemental table 1). Col156 was the most prevalent replicon in human isolates (n = 15) and second most prevalent in $canine_p$ isolates (n = 15). IncFIB was the most prevalent in $canine_p$ isolates (n = 20) and third most prevalent in human isolates (n = 13). In terms of ARG localization, a greater proportion of ARGs in human isolates were plasmid bound compared to $canine_p$ isolates (Fig. 3). Only one of the detectable β-lactamase genes, $bla_{CTX\text{-}M\text{-}15}$, in human isolates was chromosomal. Seven of $bla_{OXA\text{-}1}$ (n = 1) and $bla_{TEM\text{-}1B}$ (n = 6) β-lactamase genes were chromosomal in $canines_p$. The ESBL genes $bla_{CTX\text{-}M\text{-}27}$ (n = 1) and $bla_{CTX\text{-}M\text{-}55}$ (n = 2) in $canine_p$ and human isolates, respectively, were detected on plasmid-called contigs.

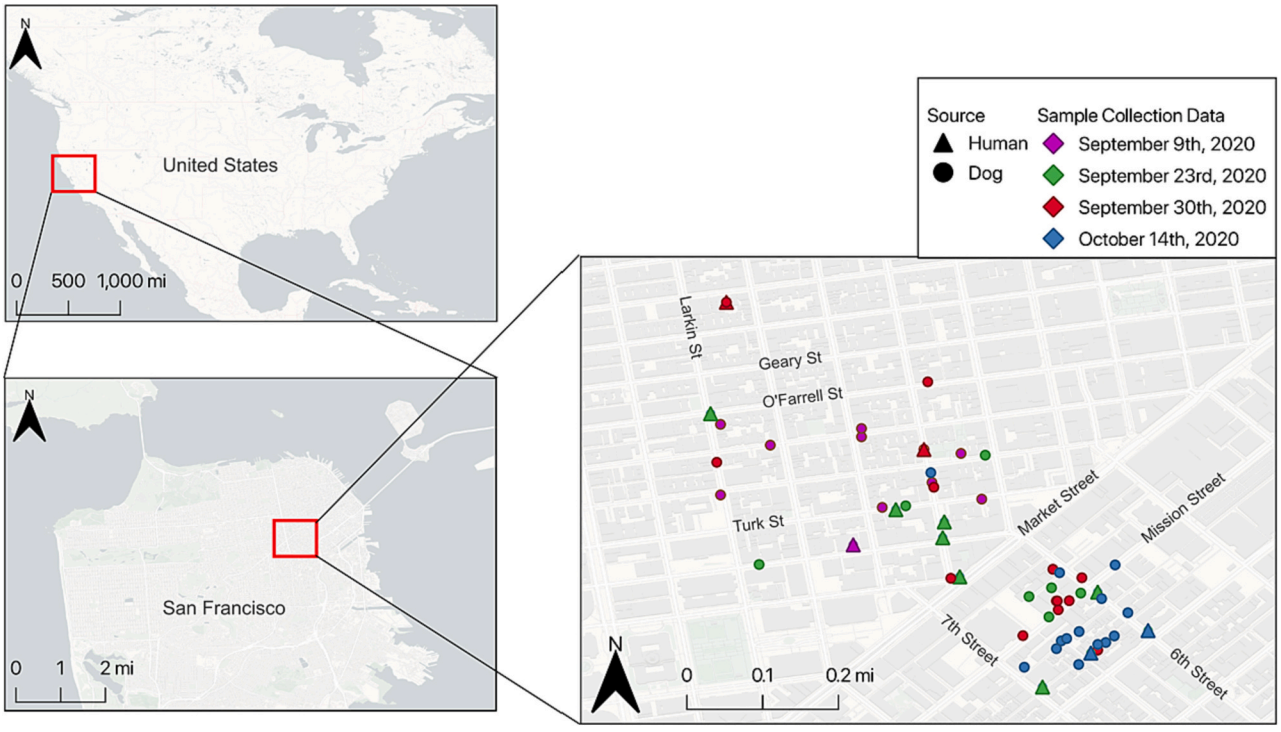


Fig. 1. Sixty fecal samples were collected over a one-mile range in the Tenderloin and SoMa neighborhoods of San Francisco, CA, USA. Samples were collected over one month period on four collection dates. Spatial distribution of sample sites was visualized using QGIS in a Pseudo-Mercator projection.

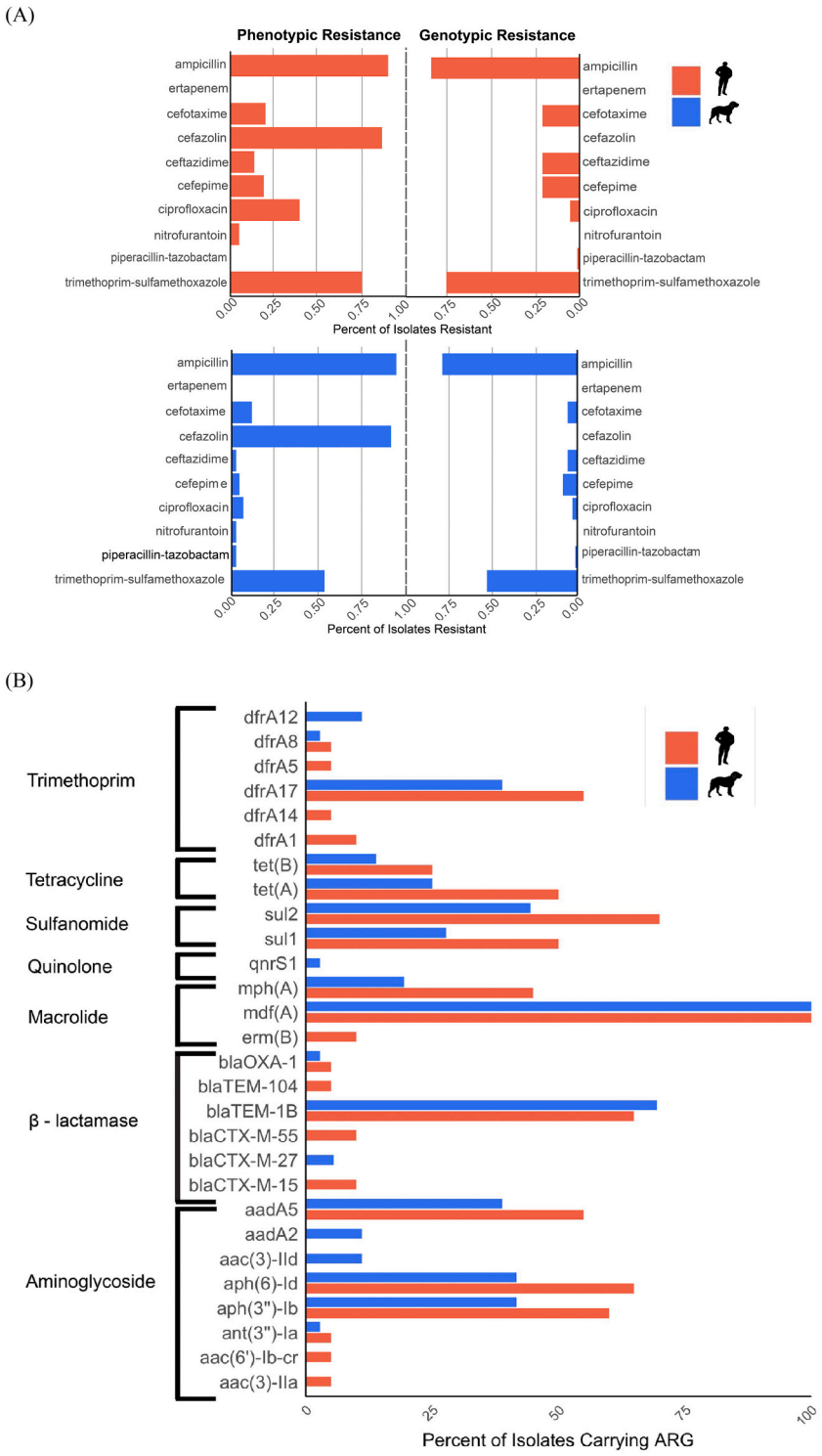


Fig. 2. Isolates from humans and dogs were selectively plated on MacConkey, MacConkey and Ampicillin, and, MacConkey and Ceftriaxone, and assessed for phenotypic resistance (A). Sequences of isolates determined by whole genome sequencing were analyzed using the ResFinder database with ABRicate (B).

Plasmid replicon type could not be identified for the majority of resistance genes carrying plasmid contigs. Human fecal samples carried an IncK2/Z plasmid contig carrying sulfonamide, trimethoprim, and aminoglycoside genes. Three of the seven detected plasmid replicons were shared between species. We found plasmid contigs carrying *bla*$_{\text{TEM-1B}}$ and identified as belonging to IncFIA, IncFIA/IncFIC and IncFIA/IncFII replicons in human (n = 1, n = 2, n = 1) and canine$_p$ (n = 3, n = 4, n = 1) isolates (Table 2).

Pre-defined virulence genes used to identify the six diarrheagenic *E. coli* pathotypes determined that one canine$_p$ isolate belonging to ST131 carried the *daaE* gene indicative of diffuse adherent *E. coli*. About 25 % of human and canine$_p$ isolates carried the virulence gene *eae*. Virulence gene carriage was not significantly different between the two species (Supplemental table 2).

3.3. Clonal and phylogenetic relationships spanned species

Twenty-eight sequence types were detected, including the well-

Table 1
Distribution of ARGs between Human and Canine Isolates.

Resistance group	Gene	Human (%, n = 20)	Canine (%, n = 36)	*p*-value[a]
Aminoglycoside	*aac (3)-IIa*	5.0	0.0	0.357
	aac(6′)-Ib-cr	5.0	0.0	1.000
	ant(3″)-Ia	5.0	2.78	1.000
	aph(3″)-Ib	60.0	41.7	0.266
	aph(6)-Id	65.0	41.7	0.163
	aac(3)-IId	0.0	11.1	0.285
	aadA2	0.0	11.1	0.285
	aadA5	55.0	38.9	0.428
β-lactamase	$bla_{\text{CTX-M-15}}$	10.0	0.0	0.123
	$bla_{\text{CTX-M-27}}$	0.0	5.6	0.533
	$bla_{\text{CTX-M-55}}$	10.0	0.0	0.123
	$bla_{\text{OXA-1}}$	5.0	2.8	1.000
	$bla_{\text{TEM-1B}}$	65.0	69.4	0.772
	$bla_{\text{TEM-104}}$	5.0	0.0	0.357
Macrolide	*erm(B)*	10.0	0.0	0.123
	mdf(A)	100.0	100.0	1.000
	mph(A)	45.0	19.4	0.064
Quinolone	*qnrS1*	0.0	2.8	1.000
Sulfonamide	*sul1*	50.0	27.8	0.146
	sul2	70.0	44.4	0.095
Tetracycline	*tet(A)*	50.0	25.0	0.080
	tet(B)	25.0	13.9	0.468
Trimethoprim	*dfrA1*	10.0	0.0	0.123
	dfrA5	5.0	0.0	0.357
	dfrA8	5.0	2.8	1.000
	dfrA12	0.0	11.1	0.285
	dfrA14	5.0	0.0	0.357
	dfrA17	55.0	38.9	0.275

[a] Alpha value of 0.05.

known pandemic lineages 131, 69 and 1193 (Fig. 4). Human isolates spanned 11 STs and canine_{p} isolates spanned 17 STs. The most common phylogroup was A (n = 17) followed by B2 (n = 15) and B1 (n = 13). Phylogroups B2 contained human isolates carrying the ESBL genes $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-15}}$ and belonging to the pandemic lineages ST1193 and ST131, respectfully. Canine_{p} isolates containing ESBL gene $bla_{\text{CTX-M-27}}$ belonged to phylogroup F (Fig. 4).

Core genome MLST revealed six epidemiologically linked clonal groups (Table 3), using previously defined thresholds (Kluytmans-van den Bergh et al., 2016). All clonal groups contained isolates carrying the β-lactamase gene, $bla_{\text{TEM-1B}}$. Five of the clonal groups contained two isolate pairs that originated from canine_{p} fecal samples. Three of the clonal groups belonged to the pandemic lineages ST131, ST69 and ST10. Clonal group 2 belonging to ST69 contained isolates sampled on different days. The largest clonal group was group 6 with isolates originating from both human (n = 1) and canine_{p} (n = 5) fecal samples and spanning three sampling dates.

By performing Bayesian phylogenetic inference from local outbreak clusters using MASCOT, we found support for at least two host transfers of *E. coli* between humans and $\text{canines}_{\text{p}}$ (Fig. 5A). In particular, one of the local outbreak clusters shows that human samples are nested in a clade of canine_{p} sequences (Fig. 5B). Additionally, we found a second outbreak cluster with sequences from humans and $\text{canines}_{\text{p}}$, which indicates a second host transfer. In this case, however, the root of the local outbreak clusters lies several years in the past, and as such, may have taken different routes of transmission that are not accounted for in the model. The different *E. coli* isolates contained largely consistent plasmid profiles within the same local outbreak clusters (Fig. 5C).

4. Discussion

Our study suggests that non-human sources of fecal contamination may play an important role in the spread of AMR in urban settings within high-income countries. Because the global prevalence of resistant infections is projected to increase and therapeutic alternatives are limited, it is important to understand interspecies transmission and evolutionary dynamics to develop effective mitigation practices. Our results demonstrated that on average humans presented a 0.5 times higher proportion of antimicrobial resistant *E. coli* (AMR-Ec) isolates compared to $\text{canines}_{\text{p}}$, but the ARGs present in the isolates were similar between the two species. We were surprised to find that despite the high-profile reports of human fecal waste in San Francisco heavily featured in the media (Amato et al., 2022), the majority of our samples belonged to non-human sources.

Our results show that carriage of drug-resistant pathotypes of *E. coli* was similar between species, in accordance with overall pathogen carriage from previous research (Barker et al., 2023). Aside from one isolate in $\text{canines}_{\text{p}}$ determined to be diffuse adherent *E. coli,* neither species carried any of the six diarrheagenic *E. coli* pathotypes based on previously defined definitions. A comparable percentage of *E. coli* in both species did carry the virulence gene *eae,* which is known to enhance virulence of STEC infections (Hua et al., 2020), but did not carry the additional *stx1/stx2* or *bfp* genes of STEC and typical enteropathogenic *E. coli*, respectively. Both human and canine_{p} *E. coli* showed high rates of ampicillin and trimethoprim-sulfamethoxazole phenotypic resistance, suggesting the need for drug monitoring and appropriate administration for community-acquired bacterial infections. The lack of cefazolin specific resistance genes in both species was surprising considering the high rate of phenotypic resistance to the drug. Cefazolin resistance has been shown to be mediated by *ampC* β-lactamases (Kawamura et al., 2022), which were not detected in this study group. The high counts of $bla_{\text{TEM-1B}}$, known to confer resistance to structurally similar first-generation cephalosporin, cephalothin (Wick and Preston, 1972), in addition to the presence of other β-lactamase genes are most likely are conferring resistance to cefazolin, but further investigation is needed to confirm this. It is also possible the observed phenotype counts are due to genetic mechanisms that were not explored in this study such as efflux pumps and porins which are known to confer cross-resistance to multiple antibiotics (Ishii et al., 2021; Bratu et al., 2008).

In alignment with previous studies (Salinas et al., 2021; Zhao et al., 2022; Musoke et al., 2021) our results also show there was some overlap in the resistance genes and plasmids found in human and canine_{p} samples. The most frequent ARG in both species was the broad-spectrum macrolide gene *mdf(A)*, followed by the non-ESBL gene, $bla_{\text{TEM-1B}}$. Only 50 % of the ARGs were present in both species but their presence was not significantly different. The limited sample size may explain why some resistance genes were only detected in one species and why no significant difference in gene presence was found. Nevertheless, the similarity in the distribution and prevalence of shared ARGs suggests a similar composition of resistance between the two species despite recent findings challenging this notion (Røken et al., 2022). This discrepancy with the literature may be explained by multiple factors such as environmental setting and sampling window, as *E. coli* presence in the gut is known to be highly dynamic due to selection pressure and clonal competition (Loayza et al., 2020).

Both species had a high prevalence of the Col156 and IncFIB plasmid replicons in addition to various other replicon types unique to the species. The ESBL genes, $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-27}}$ were exclusively found on plasmid-called contigs in humans and $\text{canines}_{\text{p}}$, respectively. The missing replicon assignment to the majority of ARG carrying plasmid fragments is most likely due to a lack of plasmid markers on the short-read contigs (Juraschek et al., 2021). Nevertheless, three IncF-type plasmids harboring the non-ESBL $bla_{\text{TEM-1B}}$ gene were found in both species. IncF plasmids are well known to disseminate a range of resistance genes including $bla_{\text{CTX-M-15}}$ (Kim et al., 2011) while being stably maintained without antimicrobial pressure (Lucas et al., 2018). This suggests plasmid mediated ARG exchange and persistence between the two species may be occurring regardless of antimicrobial usage in the community.

This potential for ARG exchange is further supported by the overlap in clonal species between the two sources. The maximum-likelihood

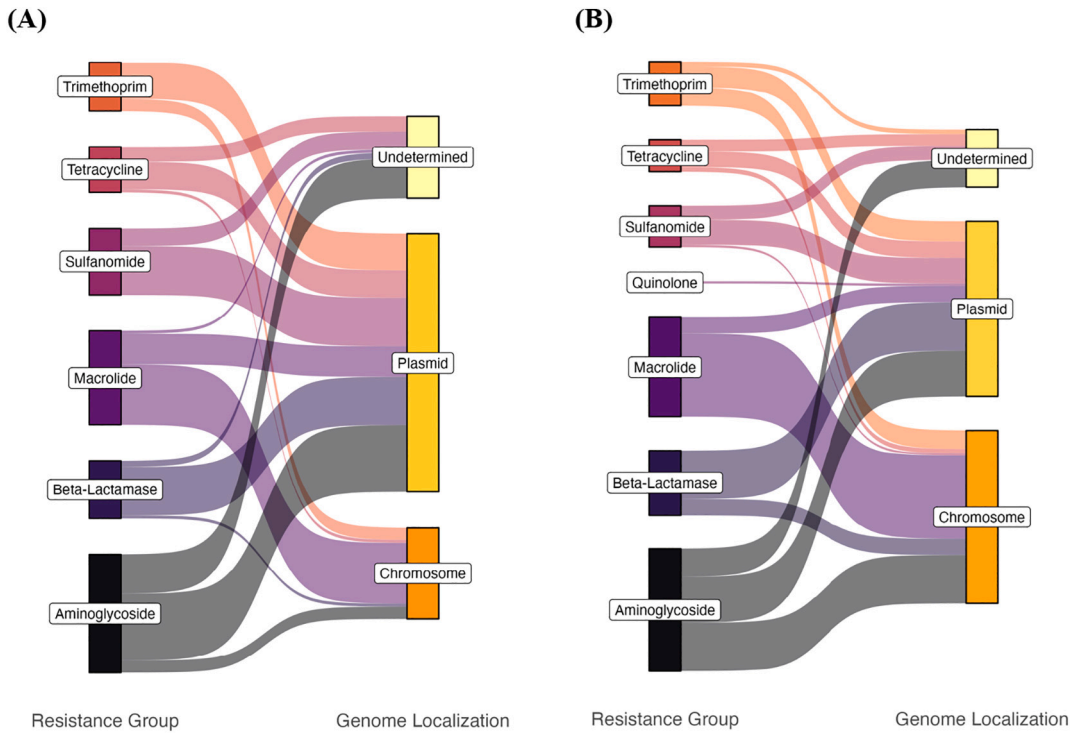


Fig. 3. Contigs containing ARGs determined by ResFinder were assigned as plasmid or chromosomal according to mlplasmids and MOB-suite in human (A) and canine (B) isolates. Distribution of localization was visualized using ggsankey in R studio.

phylogenetic tree showed no clustering by species, with canine$_p$ and human isolates equally distributed across sequence types and phylogroups. Core genome MLST found six epidemiologically related clonal groups across canine$_p$ fecal samples, three of which belonged to pandemic linages ST131, ST69, and ST10. The clonal group ST2541 contained epidemiologically linked isolates from both human and canines$_p$ over multiple sample dates, suggesting recent clonal dissemination across the two species. Past studies in Brazil (Melo et al., 2018) and Norway (Toombs-Ruane et al., 2020) have found ST2541 to be prevalent in stray and domestic canines, but to our knowledge this is the first instance of it occurring in both humans and canines of the same study base.

Our phylodynamic analyses demonstrate that there is potential to utilize genomic sequencing to reconstruct host transfer events on a local scale. Our analyses also suggest that there is cross-species sharing of *E. coli* between canines$_p$ and humans, with the samples in the same outbreak clusters having the same or very similar plasmid profiles. Similar plasmid profiles between local outbreak clusters inferred from chromosomal DNA suggest that at the time scales we are investigating, plasmids are largely maintained even during cross-species transmission. Using more isolates or explicitly tracking the movement of plasmids between different bacterial lineages could shine additional light into the cross-species transmission of *E. coli* (Müller et al., 2022). With relatively few host transfers captured by the genomic data, quantifying the rates of host transfers is complex. This could be enabled by larger datasets and could be used to, for example, estimate the number of cases in each species directly caused by host transfers (Clausen et al., 2018; Page et al., 2015). For this analysis, we were unable to find geographically matched, clonally related *E. coli* isolates in public databases to supplement this analysis. Knowing these rates could potentially be used to parameterize epidemiological models to predict the impact of interventions to reduce the burden of ARB gene carrying *E. coli*.

This paper makes no statements suggesting a direct transmission route from canines to humans in the urban built environment, but our findings suggest recent clonal sharing of resistant bacterial clones between human and non-human species. One possible explanation for the high similarity and transmission of the AMR-*E. coli* in humans and canines$_p$ is cohabitation between the two species such as canine ownership, which has been shown to contribute to *E. coli* sharing and long-term colonization (Johnson et al., 2016; Toombs-Ruane et al., 2020; Habib et al., 2023). Alternatively, the similarity could be due to shared exposure sources such as intermediate hosts unaccounted for in

Table 2
Distribution of ARG carrying plasmid replicons between human and canine isolates.

Plasmid replicon type	Resistance genes	Number of isolates (n = 20)	Sequence types (n = 36)
IncFIA	bla_{TEM-1B}	Human (1) Canine (3)	131 (1), 29 (1), 69 (2)
IncFIA/IncFIB/IncFIC	bla_{TEM-1B}	Human (1)	58 (1)
IncFIA/IncFIC	bla_{TEM-1B}	Human (2) Canine (3)	29 (2), 569 (1), 10 (2)
	bla_{TEM-1B}, *aac(3″)-IIb*	Canine (1)	162(1)
IncFIA/IncFII	bla_{TEM-1B}	Canine (1)	69
	bla_{TEM-1B}, *aph(3″)-Ib, aph(6)-Id, sul2*	Human (1)	10
IncFIB/IncI-gamma/K1	bla_{TEM-1B}	Canine (1)	8857 (1)
IncFIC	bla_{TEM-1B}	Canine (2)	969 (2)
IncK2/Z	*aph(6)-Id, dfrA14, sul2*	Human (1)	131 (1)

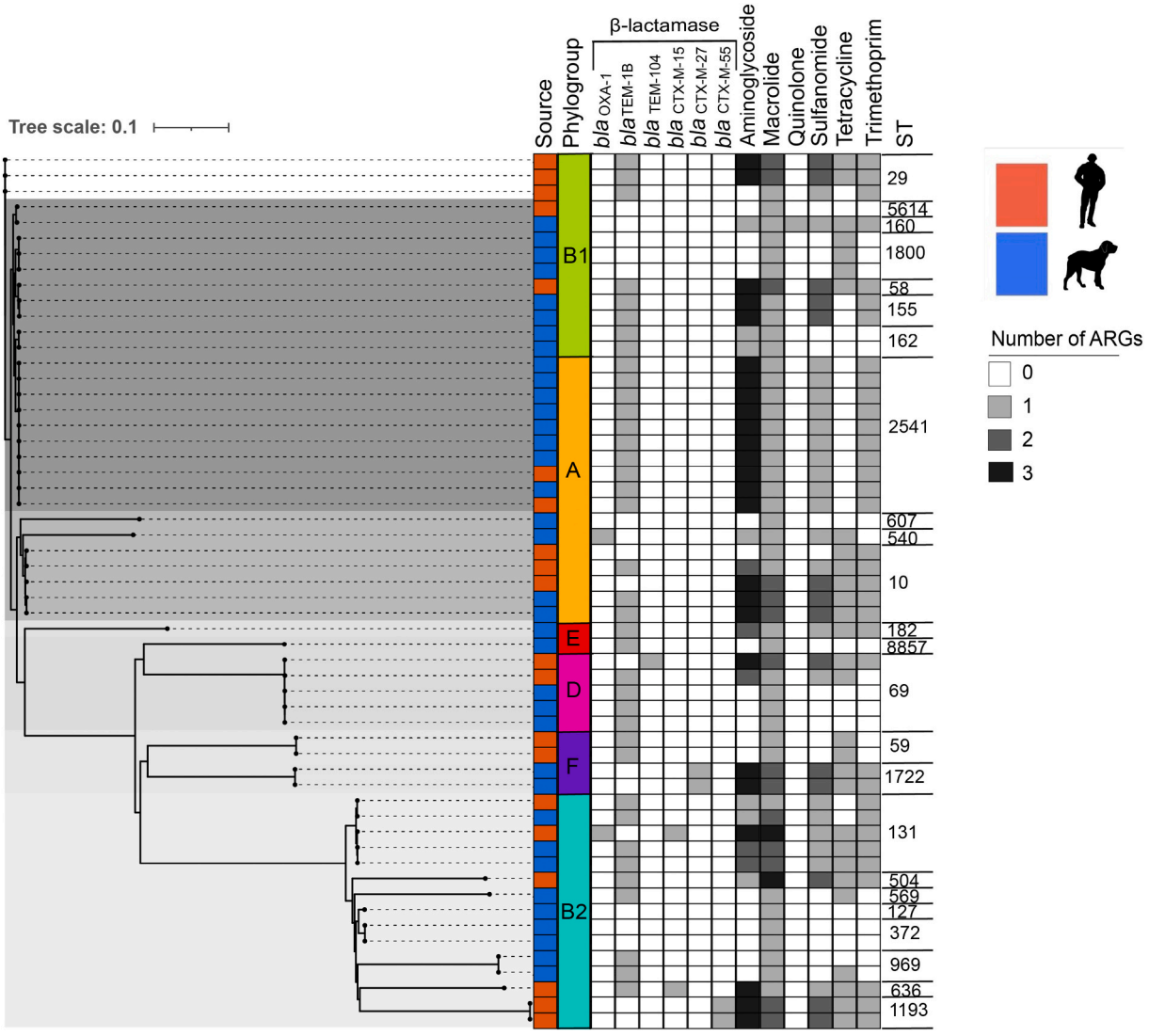


Fig. 4. Maximum likelihood phylogenetic analysis. Phylogram depicting the best estimate of the phylogenetic relationships was computed with RAxML using 200,473 SNP sites among the core genome of *E. coli* isolates with bootstrapping of 100 replicates. ClonalFrameML was used to correct the branch lengths of the tree to account for recombination. Fecal sample source, phylogroups, sequence type and ARG carriage of each isolate is indicated. Tree clades are indicated by gray shading.

Table 3
Epidemiologically linked clonal groups of isolates.

Clonal group	Number of isolates	ST	cgST	Avg allele difference	Phenotypic resistance profile	Genotypic resistance profile	Sample mtDNA (n)	Sampling date
1	2	131	136,431	0	AMP, KZ, SXT	*aac(3)-IId_1, aadA2, bla*$_{TEM-1B}$*, dfrA12, mdfA, mphA, sul1_5, tetA*	Canine	9/30/20
2	2	69	133,094	1	AMP, KZ	*bla*$_{TEM-1B}$*, mdfA*	Canine	9/23/20, 9/30/20
3	2	969	40,914	0	AMP, KZ	*bla*$_{TEM-1B}$*, mdfA, tetA*	Canine	9/23/20
						bla$_{TEM-1B}$*, mdfA*		
4	2	155	62,601	2	AMP, KZ, SXT	*aadA2, aph(3″)-Ib, aph(6)-Id, bla*$_{TEM-1B}$*, dfrA12, mdfA, sul1_5, sul2_3,*	Canine	9/30/20
5	2	10	78,278	1	AMP, SXT	*aadA5, aph(3″)-Ib, aph(6)-Id, bla*$_{TEM-1B}$*, dfrA17, mdfA, mphA, sul1_5, sul2_2, tetA,*	Canine	10/14/20
6	6	2541	130,412	2	AMP, KZ, SXT	*aadA5, aph(3″)-Ib, aph(6)-Id, bla*$_{TEM-1B}$*, dfrA17, mdfA, sul2_14*	Human (1)	9/23/20, 9/30/20,
					AMP, CTX, KZ, SXT	*aadA5, aph(3″)-Ib, aph(6)-Id, bla*$_{TEM-1B}$*, dfrA17, mdfA, sul2_21*	Canine (5)	10/14/20

our sampling scheme, shared food waste consumption, or contact with communal contaminated wastewater run-off. A wider sampling approach with more environmental samples would help refine insight into proposed transmission pathways. Regardless, the human fecal samples likely originated from open defecation due to lack of access to sanitation facilities for the unhoused population in San Francisco (Amato et al., 2022). Individuals experiencing homelessness have been shown to be at greater risk for exposure to infectious diseases due to

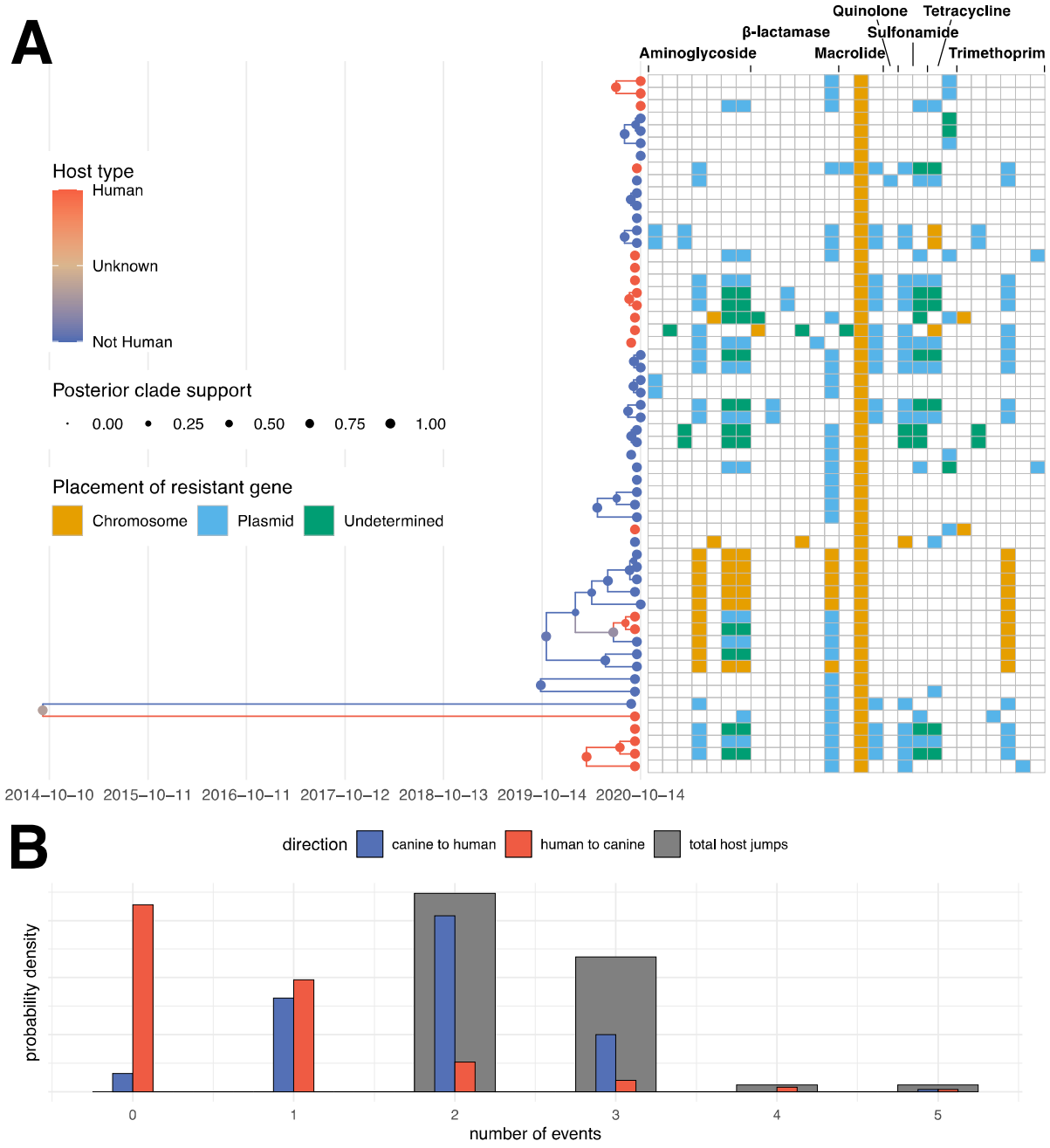


Fig. 5. Transmission of *E. coli* between canine and humans. (A) Maximum clade credibility tree of the different outbreak clusters inferring using MASCOT. The color of each edge denotes the inferred location of the child node of that edge. The color scale denotes the certainty of the node state reconstruction. The size of each node denotes the posterior support for a clade. For each tip in the tree, we show the antibiotic resistance profile based on the location of the resistance genes on either a plasmid or the chromosome (or an undetermined location). Any sequences that are linked by edges are in the same outbreak cluster, while unlinked sequences are in different outbreak clusters. (B) Inferred number of host jumps. Host jumps are computed as the number of edges for which parent and child nodes are in different states. The total number denotes the posterior distribution of host jump events in either direction.

compounding factors (Fazel et al., 2014; Liu et al., 2020). Residence in areas where fecal contamination is prevalent, such as sidewalks, can lead to increased exposure to AMR-*E. coli* resulting in spillover, gene exchange and *E. coli* colonization from canine feces through the environment.

Our study has several limitations. Firstly, we were not able to gather individual data for fecal samples, so it is possible some fecal samples originated from the same human or canine$_p$. It is possible the non-human fecal samples originated from animals other than canines, though this is less likely due to the limited wildlife ecology of downtown San Francisco and the appearance of the feces. Secondly, we were not able to establish the extent of contact between the individual hosts contributing the fecal samples, which would help support inference on direct transmission events as opposed to alternative pathways that may be mediating clonal sharing between the two hosts. Due to the lack of funding, we were not able to sample other potential reservoirs that would help us identify the likely transmission pathways. Our limited sample size restricts inferences we can make from our study group to the larger San Francisco community.

In conclusion, our study found a high degree of similarity in AMR-*E. coli* in human and canine$_p$ fecal samples on the sidewalks of San Francisco as well as one recent host transfer event from canines$_p$ to

humans. Our results support the wider use of phylodynamic methods in bacterial surveillance to refine insights on cross-species AMR sharing that may warrant further investigation. We discovered a wide variety of resistance genes including a high prevalence of macrolide and β-lactamase genes in canines$_p$. The prevalence of canine$_p$ phenotypic and genotypic resistance suggests domesticated and stray canines play an important role as a reservoir and vector for environmental contamination of ARGs. Canines are required to be on leash when off personal property, and owners are required to remove and dispose properly of any feces. Despite these ordinances there remains a high frequency of canine feces on San Francisco sidewalks. This study supports public health efforts to report and remove both human and non-human feces on city streets, such as San Francisco's municipal 311 program, as well as increased signage and enforcement of ordinances. Public health measures should be continued in order to reduce the spread of AMR in the environment and reduce the risk of AMR transmission to vulnerable communities.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.170139.

Abbreviations

AMR	Antimicrobial resistance
AMR-Ec	Antimicrobial resistant *Escherichia coli*
ARG	Antimicrobial resistance genes
ESBL	Extended spectrum β-lactamases
MDR	Multidrug resistant
Canine$_p$	putative canine

Funding

N.F.M. is supported by NIH NIGMS R35 GM119774.
J.P.G. is supported on NIH NIAID R01AI167989.

CRediT authorship contribution statement

Nikolina Walas: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Nicola F. Müller:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis. **Emily Parker:** Methodology, Investigation, Data curation. **Abigail Henderson:** Methodology, Investigation, Data curation. **Drew Capone:** Methodology, Investigation. **Joe Brown:** Investigation, Formal analysis. **Troy Barker:** Methodology, Investigation. **Jay P. Graham:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Conceptualization.

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.

Data availability

The draft assembled genomes for this project have been deposited in a DDBJ/ENA/GenBank Bioproject under the accession PRJNA910158. The version described in this paper is version 1. Individual genome sequences can be found with the following link: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA910158

Acknowledgments

We express gratitude to Tony Sherg for assistance in graphic design.
This research used the Savio computational cluster resource provided by the Berkeley Research Computing program at the University of California, Berkeley (supported by the UC Berkeley Chancellor, Vice Chancellor for Research, and Chief Information Officer).

References

AbuOun, M., O'Connor, H.M., Stubberfield, E.J., Nunez-Garcia, J., Sayers, E., Crook, D. W., et al., 2020. Characterizing antimicrobial resistant Escherichia coli and associated risk factors in a cross-sectional study of pig farms in Great Britain. Front. Microbiol. 25 (11), 861. May.

Alvarez-Uria, G., Gandra, S., Mandal, S., Laxminarayan, R., 2018. Global forecast of antimicrobial resistance in invasive isolates of Escherichia coli and Klebsiella pneumoniae. Int. J. Infect. Dis. 68, 50–53. Mar.

Amato, H.K., Martin, D., Hoover, C.M., Graham, J.P., 2022. Somewhere to go: assessing the impact of public restroom interventions on reports of open defecation in San Francisco, California from 2014 to 2020. BMC Public Health 22 (1), 1673. Sep 4.

Arredondo-Alonso S, Rogers MRC, Braat JC, Verschuuren TD, Top J, Corander J, et al. mlplasmids: a user-friendly tool to predict plasmid- and chromosome-derived sequences for single species. Microbial Genomics [Internet]. 2018 Nov 1 [cited 2021 Sep 3];4(11). Available from: https://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.000224.

Barker, T., Capone, D., Amato, H.K., Clark, R., Henderson, A., Holcomb, D.A., 2023. Public toilets have reduced enteric pathogen hazards in San Francisco. PLOS Water 2 (8), e0000152.

Bedford, T., Greninger, A.L., Roychoudhury, P., Starita, L.M., Famulare, M., Huang, M.L., et al., 2020. Cryptic transmission of SARS-CoV-2 in Washington state. Science 370 (6516), 571–575. Oct 30.

Bouckaert, R.R., 2010. DensiTree: making sense of sets of phylogenetic trees. Bioinformatics 26 (10), 1372–1373. May 15.

Bratu, S., Landman, D., Martin, D.A., Georgescu, C., Quale, J., 2008. Correlation of antimicrobial resistance with β-lactamases, the OmpA-like porin, and efflux pumps in clinical isolates of *Acinetobacter baumannii* endemic to New York City. Antimicrob. Agents Chemother. 52 (9), 2999–3005. Sep.

Canini L, Modenesi G, Courcoul A, Boschiroli M, Durand B, Michelet L. Deciphering the role of host species for two *Mycobacterium bovis* genotypes from the European 3 clonal complex circulation within a cattle-badger-wild boar multihost system. MicrobiologyOpen [Internet]. 2023 Feb [cited 2023 Aug 10];12(1). Available from: https://onlinelibrary.wiley.com/doi/10.1002/mbo3.1331.

Carattoli, A., 2009. Resistance Plasmid Families in Enterobacteriaceae. Antimicrob. Agents Chemother. 53 (6), 2227–2238. Jun.

Centers for Disease Control and Prevention (U.S.), 2019. Antibiotic Resistance Threats in the United States. Centers for Disease Control and Prevention (U.S.) [Internet]. 2019 Nov [cited 2022 Dec 30]. Available from: https://stacks.cdc.gov/view/cdc/82532.

Clausen, P.T.L.C., Aarestrup, F.M., Lund, O., 2018. Rapid and precise alignment of raw reads against redundant databases with KMA. BMC Bioinformatics 19 (1), 307. Dec.

CLSI M100- Performance Standards for Antimicrobial Suceptibility Testing.

Damborg, P., Morsing, M.K., Petersen, T., Bortolaia, V., Guardabassi, L., 2015. CTX-M-1 and CTX-M-15-producing *Escherichia coli* in dog faeces from public gardens. Acta Vet. Scand. 57 (1), 83. Dec.

Didelot X, Wilson DJ. ClonalFrameML: efficient inference of recombination in whole bacterial genomes. Prlic A, editor. PLoS Comput Biol. 2015 Feb 12;11(2):e1004041.

Fazel, S., Geddes, J.R., Kushel, M., 2014. The health of homeless people in high-income countries: descriptive epidemiology, health consequences, and clinical and policy recommendations. Lancet 384 (9953), 1529–1540. Oct.

Guinat, C., Vergne, T., Kocher, A., Chakraborty, D., Paul, M.C., Ducatez, M., et al., 2021. What can phylodynamics bring to animal health research? Trends Ecol. Evol. 36 (9), 837–847. Sep.

Habib, I., Mohteshamuddin, K., Mohamed, M.Y.I., Lakshmi, G.B., Abdalla, A., Alkaabi, Bakhit Ali, A., 2023. Domestic pets in the United Arab Emirates as reservoirs for antibiotic-resistant bacteria: a comprehensive analysis of extended-spectrum beta-lactamase producing Escherichia coli prevalence and risk factors. Animals 9;13(10):1587. May.

Hua, Y., Bai, X., Zhang, J., Jernberg, C., Chromek, M., Hansson, S., et al., 2020. Molecular characteristics of *eae* -positive clinical Shiga toxin-producing *Escherichia coli* in Sweden. Emerg. Microbes Infect. 9 (1), 2562–2570. Jan 1.

Ingle, D.J., Howden, B.P., Duchene, S., 2021. Development of phylodynamic methods for bacterial pathogens. Trends Microbiol. 29 (9), 788–797. Sep.

Ishii, A., Shigemura, K., Kitagawa, K., Harada, M., Kan, Y., Hayashi, F., et al., 2021. Cross-resistance and the mechanisms of cephalosporin-resistant bacteria in urinary tract infections isolated in Indonesia. Curr. Microbiol. 78 (5), 1771–1777. May.

Johnson, J.R., Kaster, N., Kuskowski, M.A., Ling, G.V., 2003. Identification of urovirulence traits in *Escherichia coli* by comparison of urinary and rectal *E. coli* isolates from dogs with urinary tract infection. J. Clin. Microbiol. 41 (1), 337–345. Jan.

Johnson, J.R., Davis, G., Clabots, C., Johnston, B.D., Porter, S., DebRoy, C., et al., 2016. Household clustering of Escherichia coli sequence type 131 clinical and fecal isolates according to whole genome sequence analysis. Open Forum Infect. Dis. 1;3(3): ofw129. May.

Jolley, K.A., Maiden, M.C., 2010. BIGSdb: scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11 (1), 595. Dec.

Juraschek, K., Borowiak, M., Tausch, S.H., Malorny, B., Käsbohrer, A., Otani, S., et al., 2021. Outcome of different sequencing and assembly approaches on the detection of plasmids and localization of antimicrobial resistance genes in commensal Escherichia coli. Microorganisms 9 (3), 598. Mar 14.

Kawamura, M., Ito, R., Tamura, Y., Takahashi, M., Umenai, M., Chiba, Y., et al., 2022 Jun 29. Overproduction of chromosomal *ampC* β-lactamase gene maintains resistance to

cefazolin in Escherichia coli isolates. Wang H, editor. Microbiol Spectr. 10 (3) e00058-22.
Keller, A., Ankenbrand, M.J., 2021. Inferring core genome phylogenies for bacteria. In: Mengoni, A., Bacci, G., Fondi, M. (Eds.), Bacterial Pangenomics [Internet]. Springer US, New York, NY [cited 2023 May 2]. p. 59–68. (Methods in Molecular Biology; vol. 2242). Available from: https://link.springer.com/10.1007/978-1-0716-1099-2_4.
Kim, J., Bae, I.K., Jeong, S.H., Chang, C.L., Lee, C.H., Lee, K., 2011. Characterization of IncF plasmids carrying the blaCTX-M-14 gene in clinical isolates of Escherichia coli from Korea. J. Antimicrob. Chemother. 66 (6), 1263–1268. Jun 1.
Kluytmans-van den Bergh, M.F.Q., Rossen, J.W.A., Bruijning-Verhagen, P.C.J., Bonten, M.J.M., Friedrich, A.W., Vandenbroucke-Grauls, C.M.J.E., et al., 2016 Dec. Whole-genome multilocus sequence typing of extended-spectrum-beta-lactamase-producing enterobacteriaceae. Ledeboer NA, editor. J. Clin. Microbiol. 54 (12), 2919–2927.
Knee, J., Sumner, T., Adriano, Z., Anderson, C., Bush, F., Capone, D., et al., 2021. Effects of an urban sanitation intervention on childhood enteric infection and diarrhea in Maputo, Mozambique: a controlled before-and-after trial. eLife 10. Apr 9. e62278.
Letunic, I., Bork, P., 2007. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23 (1), 127–128. Jan 1.
Liu, C.Y., Chai, S.J., Watt, J.P., 2020. Communicable disease among people experiencing homelessness in California. Epidemiol. Infect. 148, e85.
Loayza F, Graham JP, Trueba G. Factors obscuring the role of *E. coli* from domestic animals in the global antimicrobial resistance crisis: an evidence-based review. IJERPH. 2020 Apr 28;17(9):3061.
Lucas, P., Jouy, E., Le Devendec, L., de Boisséson, C., Perrin-Guyomard, A., Jové, T., et al., 2018. Characterization of plasmids harboring blaCTX-M genes in Escherichia coli from French pigs. Vet. Microbiol. 224, 100–106. Oct.
MacWilliams, M.P., 2012. Indole test protocol. American Society for Microbiology, Washington, DC.
Marchetti, L., Buldain, D., Gortari Castillo, L., Buchamer, A., Chirino-Trejo, M., Mestorino, N., 2021. Pet and Stray Dogs as Reservoirs of Antimicrobial-Resistant Escherichia coli. Prasad S, editor. Int. J. Microbiol. 2021 Jan 25, 1–8.
Matlock, W., Chau, K.K., AbuOun, M., Stubberfield, E., Barker, L., Kavanagh, J., et al., 2021. Genomic network analysis of environmental and livestock F-type plasmid populations. ISME J. 15 (8), 2322–2335. Aug.
Melo, L.C., Oresco, C., Leigue, L., Netto, H.M., Melville, P.A., Benites, N.R., et al., 2018. Prevalence and molecular features of ESBL/pAmpC-producing Enterobacteriaceae in healthy and diseased companion animals in Brazil. Vet. Microbiol. 221, 59–66. Jul.
Mikheenko, A., Prjibelski, A., Saveliev, V., Antipov, D., Gurevich, A., 2018. Versatile genome assembly evaluation with QUAST-LG. Bioinformatics 34 (13), i142–i150. Jul 1.
Müller, N.F., Wüthrich, D., Goldman, N., Sailer, N., Saalfrank, C., Brunner, M., et al., 2020. Characterising the epidemic spread of influenza A/H3N2 within a city through phylogenetics. In: Lauring, AS (Ed.), PLoS Pathog. 16 (11), e1008984 (Nov 19).
Müller, N.F., Wagner, C., Frazar, C.D., Roychoudhury, P., Lee, J., Moncla, L.H., et al., 2021. Viral genomes reveal patterns of the SARS-CoV-2 outbreak in Washington State. Sci. Transl. Med. 13 (595), eabf0202 (May 26).
Müller, N.F., Duchêne, S., Williamson, D.A., Bedford, T., Howden, B.P., Ingle, DJ., 2022. Tracking the horizontal transfer of plasmids in *Shigella sonnei* and *Shigella flexneri* using phylogenetics [Internet]. Evol. Biol. Oct [cited 2023 May 3]. Available from: http://biorxiv.org/lookup/doi/10.1101/2022.10.27.514108.
Muloi, D., Ward, M.J., Pedersen, A.B., Fèvre, E.M., Woolhouse, M.E.J., van Bunnik, B.A. D., 2018. Are food animals responsible for transfer of antimicrobial-resistant *Escherichia coli* or their resistance determinants to human populations? A systematic review. Foodborne Pathog. Dis. 15 (8), 467–474. Aug.
Murray, C.J., Ikuta, K.S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., et al., 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet 399 (10325), 629–655. Feb.
Musoke, D., Namata, C., Lubega, G.B., Niyongabo, F., Gonza, J., Chidziwisano, K., et al., 2021. The role of environmental health in preventing antimicrobial resistance in low- and middle-income countries. Environ. Health Prev. Med. 26 (1), 100. Dec.
Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., et al., 2015. Roary: rapid large-scale prokaryote pan genome analysis. Bioinformatics 31 (22), 3691–3693. Nov 15.
Page, A.J., Taylor, B., Delaney, A.J., Soares, J., Seemann, T., Keane, J.A., et al., 2016. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microbial Genomics [Internet] 2 (4), e000056. Apr 29 [cited 2022 Jul 14];2(4). Available from: https://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.000056.
Perets, V., Allen, A., Crispell, J., Cassidy, S., O'Connor, A., Farrell, D., et al., 2022. Evidence for local and international spread of Mycobacterium avium subspecies paratuberculosis through whole genome sequencing of isolates from the island of Ireland. Vet. Microbiol. 268. May. 109416.
Pokharel, S., Raut, S., Adhikari, B., 2019. Tackling antimicrobial resistance in low-income and middle-income countries. BMJ Glob. Health 4 (6). Nov. e002104.
QGIS Association, 2022. QGIS Geographic Information System [Internet]. Available from: http://www.qgis.org.
Reinthaler, F.F., Posch, J., Feierl, G., Wüst, G., Haas, D., Ruckenbauer, G., et al., 2003 Apr. Antibiotic resistance of E. coli in sewage and sludge. Water Res. 37 (8), 1685–1690.
Robertson, J., Nash, J.H.E., 2018. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microbial Genomics [Internet]. 4 (8). Aug 1 [cited 2022 May 11]. Available from: https://www.microbiologyresearch.org/content/journal/mgen/10.1099/mgen.0.000206.
Robinson, T.P., Bu, D.P., Carrique-Mas, J., Fèvre, E.M., Gilbert, M., Grace, D., et al., 2016. Antibiotic resistance is the quintessential one health issue. Trans. R. Soc. Trop. Med. Hyg. 110 (7), 377–380. Jul.
Røken, M., Forfang, K., Wasteson, Y., Haaland, A.H., Eiken, H.G., Hagen, S.B., et al., 2022. Antimicrobial resistance—do we share more than companionship with our dogs? J. Appl. Microbiol. 133 (2), 1027–1039 (May 29;jam.15629).
Salinas, L., Loayza, F., Cárdenas, P., Saraiva, C., Johnson, T.J., Amato, H., et al., 2021. Environmental spread of extended Spectrum beta-lactamase (ESBL) producing *Escherichia coli* and ESBL genes among children and domestic animals in Ecuador. Environ. Health Perspect. 129 (2). Feb. 027007.
Seemann, T., 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30 (14), 2068–2069. Jul 15.
Seemann T. Abricate [internet]. Github; Available from: https://github.com/tseemann/abricate.
Seemann T. mlst [Internet]. Github; Available from: https://github.com/tseemann/mlst.
Sjoberg, David. ggsankey: Sankey, Alluvial and Sankey Bump Plots. 2022.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30 (9), 1312–1313. May 1.
Tenaillon, O., Skurnik, D., Picard, B., Denamur, E., 2010. The population genetics of commensal Escherichia coli. Nat. Rev. Microbiol. 8 (3), 207–217. Mar.
Toombs-Ruane, L.J., Benschop, J., French, N.P., Biggs, P.J., Midwinter, A.C., Marshall, J. C., et al., 2020 Nov 24. Carriage of extended-spectrum-beta-lactamase- and AmpC beta-lactamase-producing Escherichia coli strains from humans and pets in the same households. Dozois CM, editor. Appl. Environ. Microbiol. 86 (24) e01613-20.
Vidal, M., Kruger, E., Durán, C., Lagos, R., Levine, M., Prado, V., et al., 2005. Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic *Escherichia coli* associated with enteric infections. J. Clin. Microbiol. 43 (10), 5362–5365. Oct.
Wang S, Zhao S, Zhou Y, Jin S, Ye T, Pan X. Antibiotic resistance spectrum of *E. coli* strains from different samples and age-grouped patients: a 10-year retrospective study. BMJ Open. 2023 Apr;13(4):e067490.
Waters, Nick, Pritchard, Leighton. EzClermont [Internet]. Available from: https://github.com/nickp60/EzClermont.
Wick, W.E., Preston, D.A., 1972. Biological properties of three 3-heterocyclic-thiomethyl cephalosporin antibiotics. Antimicrob. Agents Chemother. 1 (3), 221–234. Mar.
Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. Phillippy AM, editor. PLoS Comput Biol. 2017 Jun 8;13(6):e1005595.
Wickham H. ggplot2: Elegant Graphics for Data Analysis [Internet]. Springer-Verlag New York; 2016. Available from: https://ggplot2.tidyverse.org.
Wickham H. stringr: Simple Consistent Wrappers for Common String Operations [Internet]. Vol. R package version 1.4.0. 2019. Available from: https://CRAN.R-project.org/package=stringr.
Wickham H. tidyr: Tidy Messy Data [Internet]. Vol. R package version 1.1.2. 2020. Available from: https://CRAN.R-project.org/package=tidyr.
Wickham, H., Francois, R., Henry, L., Müller, K., 2021. dplyr: A Grammar of Data Manipulation [Internet]. R package version 1.0.3. Available from: https://CRAN.R-project.org/package=dplyr.
Woolhouse, M., Ward, M., van Bunnik, B., Farrar, J., 2015. Antimicrobial resistance in humans, livestock and the wider environment. Philos. Trans. R. Soc. B 370 (1670). Jun 5. 20140083.
World Health Organization. Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2020.: 2020.
Yu, G., Smith, D.K., Zhu, H., Guan, Y., Lam, T.T., 2017 Jan. GGTREE: an R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. McInerny G, editor. Methods Ecol. Evol. 8 (1), 28–36.
Zhang, S., Abbas, M., Rehman, M.U., Huang, Y., Zhou, R., Gong, S., et al., 2020. Dissemination of antibiotic resistance genes (ARGs) via integrons in Escherichia coli: a risk to human health. Environ. Pollut. 266. Nov. 115260.
Zhao R, Hao J, Yang J, Tong C, Xie L, Xiao D, et al. The co-occurrence of antibiotic resistance genes between dogs and their owners in families. iMeta [Internet]. 2022 Jun [cited 2022 Jul 10];1(2). Available from: https://onlinelibrary.wiley.com/doi/10.1002/imt2.21.
Zhu, K., Suttner, B., Pickering, A., Konstantinidis, K.T., Brown, J., 2020. A novel droplet digital PCR human mtDNA assay for fecal source tracking. Water Res. 183. Sep. 116085.