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Long-term maintenance of multidrug-resistant *Escherichia coli* carried by vampire bats and shared with livestock in Peru

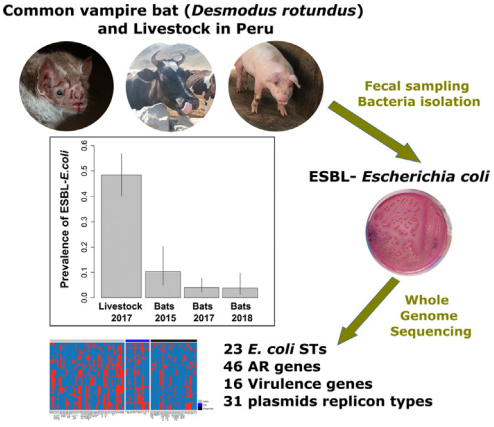
Julio A. Benavides [a,b,c,h,*], Sylvain Godreuil [d,e,f], Andrés Opazo-Capurro [g,h], Oumar O. Mahamat [e,f,i], Nestor Falcon [j], Katarina Oravcova [b], Daniel G. Streicker [b,k], Carlos Shiva [j]

[a] Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile
[b] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical Veterinary and Life Sciences, University of Glasgow, Graham Kerr Building, Glasgow, Scotland, UK
[c] Centro de Investigación para la Sustentabilidad, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile
[d] Laboratoire de Bactériologie, Centre Hospitalier Universitaire de Montpellier, Montpellier, France
[e] MIVEGEC, IRD, CNRS, Université de Montpellier, Montpellier, France
[f] Laboratoire Mixte International, DRISA, IRD, Montpellier, France
[g] Laboratorio de Investigación en Agentes Antibacterianos, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile
[h] Millennium Nucleus for Collaborative Research on Bacterial Resistance, MICROB-R, Santiago, Chile
[i] Service de laboratoire Hôpital de la Mère et de l'Enfant, N'Djaména, Chad
[j] Facultad de Medicina Veterinaria y Zootecnia de la Universidad Peruana Cayetano Heredia, Lima, Peru
[k] MRC–University of Glasgow Centre for Virus Research, Glasgow, UK

HIGHLIGHTS

- Longitudinal sampling and whole genome sequencing can track AMR circulation.
- The role of bats in the transmission of AMR with livestock is poorly understood.
- Multidrug-resistant ESBL-*E. coli* can persist in vampire bats over several years.
- ESBL-*E. coli* carried 46 AMR genes, 16 virulence genes and 31 plasmid replicons.
- ESBL-*E. coli* are shared between vampire bats and livestock in Peru.

GRAPHICAL ABSTRACT



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ABSTRACT

Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) have been reported in wildlife worldwide. Whether wildlife is a transient host of ESBL-*E. coli* or comprises an independently maintained reservoir is unknown. We investigated this question by longitudinally monitoring ESBL-*E. coli* in common vampire bats and nearby livestock in Peru. Among 388 bats from five vampire bat colonies collected over three years, ESBL-*E. coli* were detected at a low prevalence (10% in 2015, 4% in 2017 and 2018) compared to a high prevalence (48%) from 134 livestock sampled in 2017. All ESBL-*E. coli* were multidrug-resistant, and whole genome sequencing of 33 randomly selected ESBL-*E. coli* isolates (18 recovered from bats) detected 46 genes conferring resistance to antibiotics including third-generation cephalosporins (e.g., $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}65}$, $bla_{CTX\text{-}M\text{-}3}$, $bla_{CTX\text{-}M\text{-}14}$), aminoglycosides,

* Corresponding author at: Departamento de Ecología y Biodiversidad, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, Chile.
E-mail address: benavidesjulio@yahoo.fr (J.A. Benavides).

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fluoroquinolones, and colistin (*mcr-1*). The *mcr-1* gene is reported for the first time on a wild bat in Latin America. ESBL-*E. coli* also carried 31 plasmid replicon types and 16 virulence genes. Twenty-three *E. coli* sequence types (STs) were detected, including STs involved in clinical infections worldwide (e.g., ST 167, ST 117, ST 10, ST 156 and ST 648). ESBL-*E. coli* with identical cgMLST (ST 167) were detected in the same bat roost in 2015 and 2017, and several ESBL-*E. coli* from different bat roosts clustered together in the cgMLST reconstruction, suggesting long-term maintenance of ESBL-*E. coli* within bats. Most antibiotic resistance and virulence genes were detected in *E. coli* from both host populations, while ESBL-*E. coli* ST 744 was found in a bat and a pig from the same locality, suggesting possible cross-species exchanges of genetic material and/or bacteria between bats and livestock. This study suggests that wild mammals can maintain multidrug-resistant bacteria and share them with livestock.

1. Introduction

The increase of antimicrobial resistance (AMR) is a major threat to human and animal health (FAO, 2016; WHO, 2017), responsible for thousands of human fatalities annually (WHO, 2014) and economic losses that could reduce global Gross Domestic Product by 1–4% in 2050 (World Bank, 2017). Rising antibiotic resistance is mainly attributed to the overuse of antibiotics in animal production, which continues to rise in low and middle-income countries (Klein et al., 2018; O'Neill, 2016; Van Boeckel et al., 2015). Despite frequent reports of AMR in clinically important bacterial species in domestic animals, there are still considerable gaps in our understanding of how AMR circulates between livestock and the remaining natural environment, including sympatric wildlife species (FAO, 2016; Benavides et al., 2018; Guenther et al., 2011).

Fecal carriage of antimicrobial-resistant Enterobacteriaceae of clinical importance including extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) has been reported in apparently healthy wild birds and mammals worldwide (Benavides et al., 2018; Guenther et al., 2011; Wang et al., 2017). More alarming, *E. coli* isolates found in wild animals can also carry antibiotic-resistant genes to 'last resort' antibiotics such as carbapenems or colistin (Franklin et al., 2020; Köck et al., 2018). Since most wild animals are not treated with third-generation cephalosporins, ESBL-*E. coli* in wildlife is often attributed to contamination from humans or livestock (Arnold et al., 2016; Swift et al., 2019; Guenther et al., 2011). Although the extent that ESBL-*E. coli* circulates in wildlife is uncertain, its mere presence opens the worrying possibility that wildlife could act as a reservoir for future human or domestic animal infections (Dolejska and Literak, 2019; Poeta et al., 2009).

Biological systems characterized by frequent contact between wildlife and livestock provide a powerful opportunity to study the transmission of AMR at the livestock-wildlife interface. Here, we focused on one such interaction – the interaction between common vampire bats (*Desmodus rotundus*) and domestic livestock – which occurs across Latin America and is unusually intense given that these obligately hematophagous bats feed almost nightly on domestic livestock. Both vampire bats and their surrounding livestock prey are known to host ESBL-*E. coli*, but the directionality of transmission between species and the capacity for bats to maintain bacterial circulation is unknown (Benavides et al., 2018). We combined field sampling of vampire bats and sympatric livestock with microbiological and genomic analyses to compare the prevalence, multidrug resistance and pathogenic potential of ESBL-*E. coli* between bats and livestock, and tested whether ESBL-*E. coli* can circulate in the same bat colony over several years.

2. Material and methods

2.1. Sample collection

Vampire bats were captured using mist nets from five colonies located in five districts of the Lima Region in Peru (Barranca, Huacho, Lurin, Mala and Santa Cruz de Las Flores) in 2015 (October), 2017 (March to May) and 2018 (February and March) (Fig. 1). Three colonies were sampled in each of the three years (Barranca, Huacho and Mala) whereas the two other colonies were sampled only in 2017 and 2018. Results from the three bat colonies sampled in 2015 were partially reported by Benavides et al. (2018). Each individual was identified by a unique four digit incoloy wing band using a previously described method (Streicker et al., 2012). Thirty previously tagged bats were recaptured in the same roost across years and resampled. A total of 388 rectal swabs from vampire bats samples were collected (N = 67 in 2015, N = 218 in 2017 and N = 103 in 2018), averaging 30 samples (range: 9–70) per colony per year. To compare the prevalence of antibiotic resistance between vampire bats and livestock, we also collected fresh fecal samples from 134 livestock animals in 2017

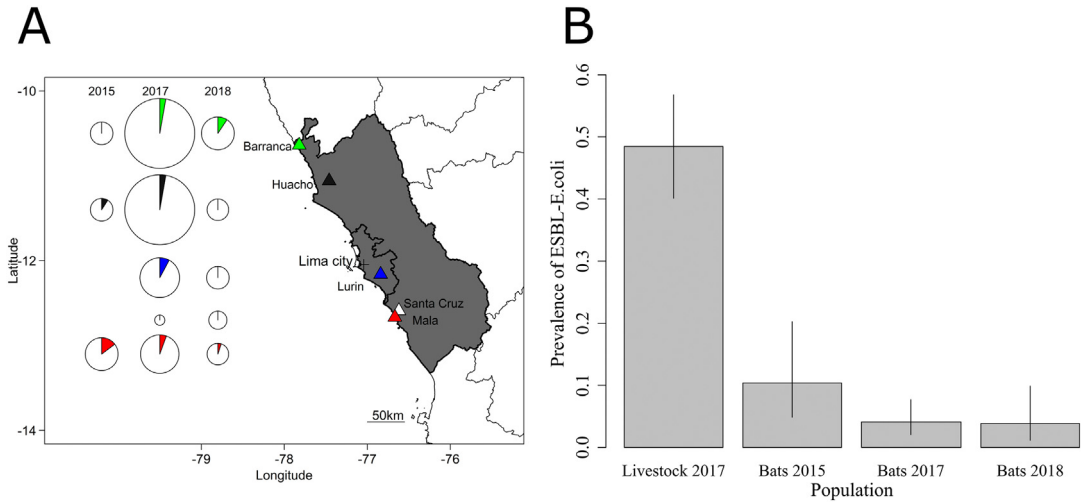

Fig. 1. Prevalence of ESBL-*E. coli* in bats and livestock across colonies and years. A) Location of bat colonies sampled in the Lima region, Peru. Pie-charts illustrate the percentage (colored) of individuals were ESBL-*E. coli* was isolated. The size of the circle is proportional to sample size (range: 9–70 individuals). Colonies located in Lurin and Santa Cruz de las Flores were not sampled in 2015. B) Prevalence ESBL-*E. coli* among livestock and bats (all colonies pooled together) at each sampled year. Bars represent 95% confidence intervals calculated using the binom.confint function (Agresti-Coull method) in the binom package in R 3.6.1.

including 74 cows, 51 pigs, 6 goats, 2 sheep and 1 donkey. Animals belonged to farms located between 0 and 10 km of a sampled vampire bat colony. Previous questionnaires conducted with farmers reported that at least one of their livestock was bitten by vampire bats within the previous 6 months. All fecal/rectal swab samples (one per animal) were collected into Stuart Transport Medium (Deltalab ®) and stored at 4 °C.

2.2. Ethics statement

The Peruvian Government authorized sample collection and use of genetic resources for this study (RD-273-2012-AG-DGFFS-DGEFFS, RD-054-2016-SERFOR-DGGSPFFS). Livestock sampling and bat sampling were approved by the MVLS College Ethics Committee of the University of Glasgow (200140112). Bat sampling was carried out by biologists with over 5 years of experience in capture and handling of bats.

2.3. Microbiology analyses

The same laboratory procedures were conducted for samples obtained from different species. Rectal swabs and fecal samples were cultured within 3 days of sampling. Swabs were screened for ESBL-producing Enterobacteriaceae by direct incubation at 37 °C for 24h in ChromID ESBL agar (bioMérieux, Marcy l'Etoile, France). Samples exhibiting bacterial growth were subcultured on the same medium to confirm growth after 24 h of incubation, and up to 2 colonies with different morphotypes were selected per sample. Isolates were then stored at −80 °C and sent to the Laboratory of Microbiology of the Hospital Arnaud de Villeneuve in Montpellier (France) for bacterial species confirmation by direct sample preparation on a matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry using the MALDI Biotyper database DB 8326 MSP (Bruker Daltonics, Bremen, Germany).

Antimicrobial susceptibility was tested using the disk diffusion method on Müller-Hinton agar according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (Version 7.1, 2017), which were also used to established clinical breakpoints. We tested the susceptibility to 26 antibiotics from 13 categories defined by Magiorakos et al. (2012) including: penicillins (amoxicillin, ticarcillin, piperacillin, and temocillin), penicillins with β-lactamase inhibitors (amoxicillin-clavulanic acid), antipseudomonal penicillins with β-lactamase inhibitors (ticarcillin-clavulanic acid and piperacillin-tazobactam), monobactams (aztreonam), non-extended spectrum cephalosporins (cephalexin), extended-spectrum cephalosporins (cefotaxime, ceftazidime and cefepime), cephamycins (cefoxitin), carbapenems (ertapenem and imipenem), quinolones (nalidixic acid, ofloxacin, ciprofloxacin and levofloxacin), aminoglycosides (gentamicin, tobramycin, netilmicin and amikacin), folate pathway inhibitors (trimethoprim-sulfamethoxazole), phenicol (chloramphenicol), and phosphonic acids (Fosfomycin). Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antibiotic categories (Magiorakos et al., 2012).

2.4. Whole genome sequencing (WGS)

To characterize the genetic origin of the observed resistance profiles and explore genetic similarities between resistant isolates from different bat colonies, species and years, all *E. coli* confirmed as ESBL positive isolates from vampire bats and an equivalent subset of randomly selected ESBL-*E. coli* isolates from livestock were analyzed by shotgun WGS. Briefly, DNA extraction was performed by MicrobesNG (http://www.microbesng.uk, Birmingham, UK) using 2 ml bead tubes containing a mix of pure cultured bacteria (from an entire plate) suspended in cryopreservative. DNA was extracted from five to forty microliters of the suspended mix using a buffer containing lysozyme and RNase A, incubated for 25 min at 37 °C. Proteinase K and RNaseA were added and incubated for 5 min at 65 °C. Preparation of the DNA library was carried out utilizing the Nextera XT library prep kit (Illumina) according to the manufacturer's protocol. The libraries were sequenced using a 2 × 250 bp paired-end reads method with a minimum coverage of 30× on a HiSeq platform (Illumina). Reads were adapter trimmed using Trimmomatic 0.30 (Bolger et al., 2014). Draft genome de novo assemblies were carried out by SPAdesv3.7 (Bankevich et al., 2012) and contigs were annotated using Prokka 1.11 (Seemann, 2014). Genome quality assessment was performed by QUAST (Gurevich et al., 2013). Library preparation, genome sequencing, reads trimming, assembly and quality control were provided by MicrobesNG. The Kraken software was used to confirm bacterial species identity (Wood and Salzberg, 2014). Contigs are available in Genbank (Bioproject ID PRJNA666069).

From assembled contigs, we determined the presence of antibiotic resistance genes, *E. coli* sequence types (STs), plasmid replicon types and virulence genes using the bioinformatic tools of the Center for Genomic Epidemiology (CGE) (http://www.genomicepidemiology.org/) including ResFinder 2.1 (for acquired resistance genes and chromosomal point mutations), MLST 2.0.4 using the PubMLST database (https://pubmlst.org/), PlasmidFinder 2.0, and VirulenceFinder 1.7. A 95% identity threshold and 80% minimum length coverage was used to predict genes and chromosomal point mutations, while 60% coverage was used to predict plasmids. Two isolates with new STs detected by the CGE tool were further submitted and assigned a ST in Enterobase (https://enterobase.warwick.ac.uk/). The MobileElementFinder 1.0.3 tool from CGE was used to assess whether resistance genes were detected in contigs also containing plasmids. Additionally, genetic similarities of *E. coli* isolates were estimated from core genome MLST analysis using hierarchical clustering of cgMLST v1.1.2 in Enterobase, comprising 2513 loci. The resulting phylogenetic tree was edited in FigTree version v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

2.5. Statistical analyses

The prevalence of ESBL-*E. coli*, defined here as the number of individuals harboring at least one ESBL-*E. coli* isolate over the total number of individuals tested, was reported and 95% confidence intervals were calculated using the binom.confint function (Agresti-Coull method) in the binom package in R 3.6.1 (R Development Core Team, 2019). Significant differences in prevalence between two or three populations were tested using the Pearson's chi-squared test or the Fisher's exact test respectively in R.

3. Results

3.1. ESBL-E. coli prevalence in bats and livestock

The prevalence of ESBL-*E. coli* fecal carriage was significantly lower in vampire bats (5% [CI: 3–7%]; 20 out of 388) than livestock (48% [CI: 40–56%]; 65 out of 134; Fig. 1, Pearson's chi-squared test, $p < 0.0001$). ESBL-*E. coli* prevalence remained relatively constant across years among vampire bats (10% in 2015, 4% 2017 and 4% 2018, Fisher's exact test, $p = 0.14$). There were no significant differences in prevalence between the five bat colonies (Fisher's exact test, $p = 0.76$), with ESBL-*E. coli* detected in all but one colony (i.e., Santa Cruz de Las Flores) (Fig. 1). Two bat colonies (Barranca and Huacho) had bats that carried ESBL-*E. coli* over two sampling years, while the bat colony in Mala had at least one bat carrying ESBL-*E. coli* in all three sampling years. Among the thirty recaptured bats, 28 bats did not carry ESBL-*E. coli* in either sampling occasion. Two bats from Barranca harbored ESBL-*E. coli* on the second sampling (2017 and 2018) after no detection in previous years (2015 and 2017, respectively).

3.2. Multidrug resistance of ESBL-E. coli

All ESBL-*E. coli* isolates from bats and livestock were resistant to three or more antibiotic categories and were therefore considered phenotypically MDR (Table S2). Isolates from bats were resistant to an average of 9.0 [range: 5–11] out of 13 categories of antibiotics, while isolates from livestock were resistant to 7.8 [range: 4–11]. More than 60% of isolates were

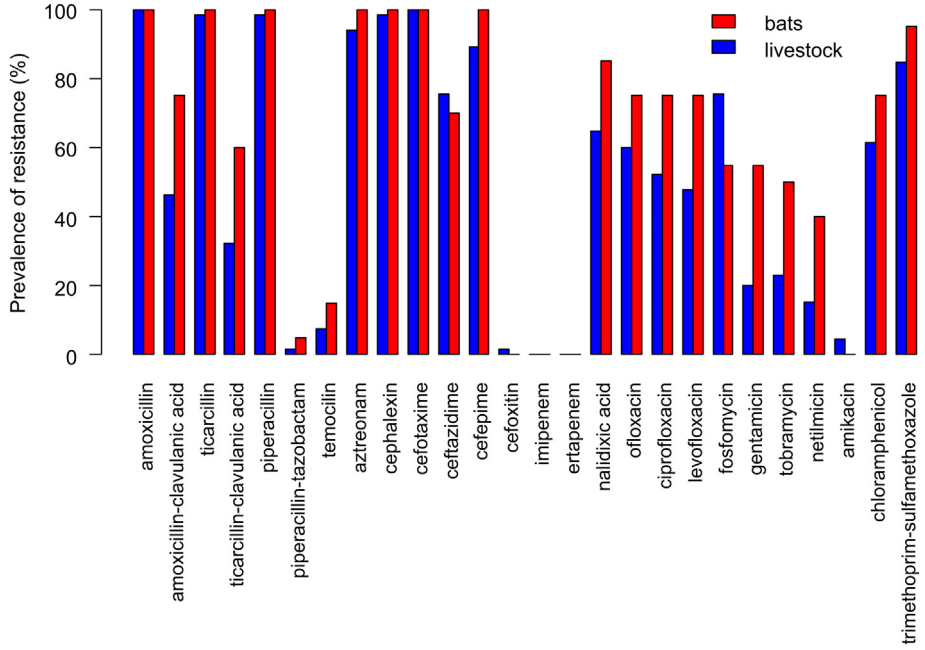

Fig. 2. Antimicrobial resistance phenotypic profiles of ESBL-*E. coli* in bats and livestock. Prevalence of resistance to each of the 26 antibiotics tested.

resistant to aztreonam, nalidixic acid, chloramphenicol, trimethoprim + sulfamethoxazole and several 2nd and 3rd generation cephalosporins (Fig. 2). In contrast, no resistance was observed against carbapenems (Fig. 2). Among ESBL isolates, the prevalence of resistance to each antibiotic was highly correlated between bats and livestock (Spearman's test, Rho = 0.94, $p < 0.0001$), but bats had a slightly higher prevalence of resistance to most antibiotics compared to livestock, particularly for quinolones and aminoglycosides.

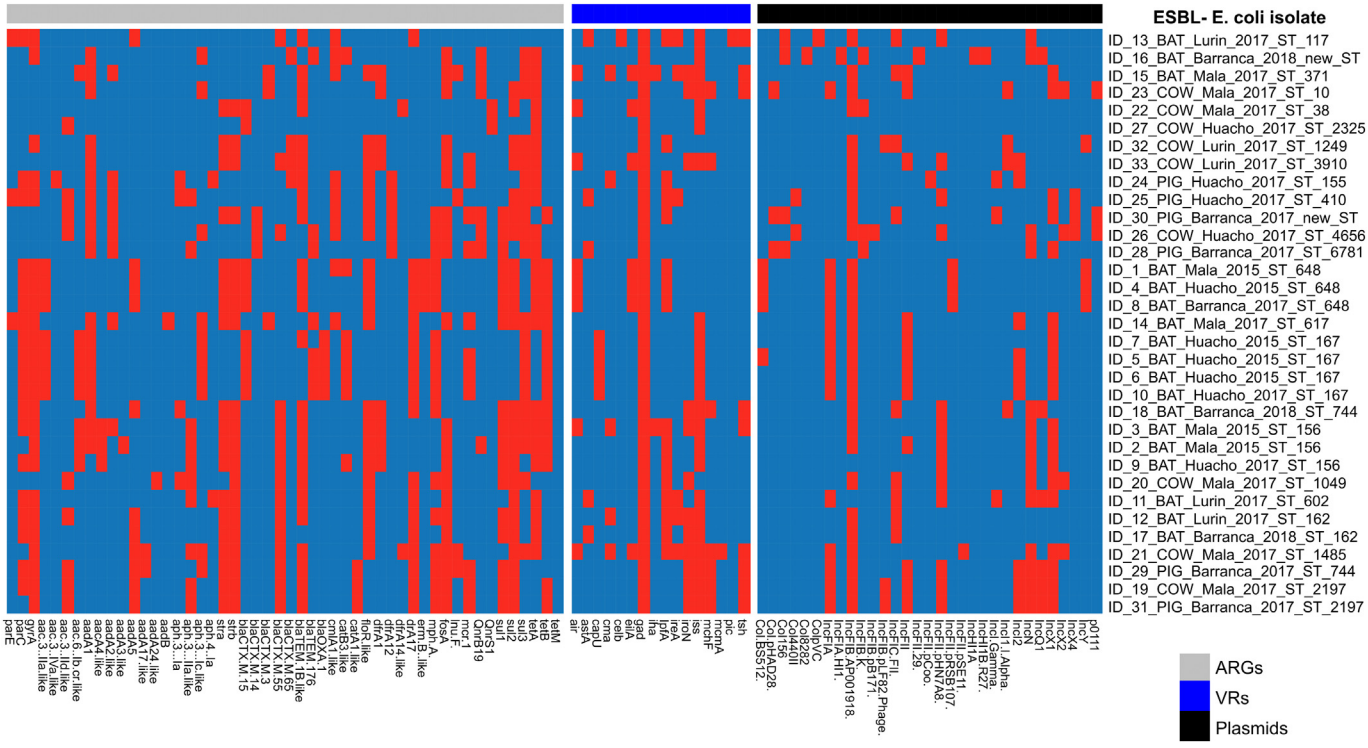

Fig. 3. Antibiotic resistance genes, virulence genes and plasmids estimated in silico by whole genome sequencing. Heat map illustrating the presence/absence of (i) antibiotic resistance genes and chromosomal mutations (in genes *parE*, *parC* and *gyrA*) (labeled ARGs) among 33 isolates from bats and livestock detected using the online tool ResFinder 2.1 from the Center for Genomic Epidemiology, (ii) virulence genes (VRs) using VirulenceFinder 1.7, and (ii) plasmids found using PlasmidFinder 2.0. Red squares correspond to the presence of a given ARGs, VRs or Plasmids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

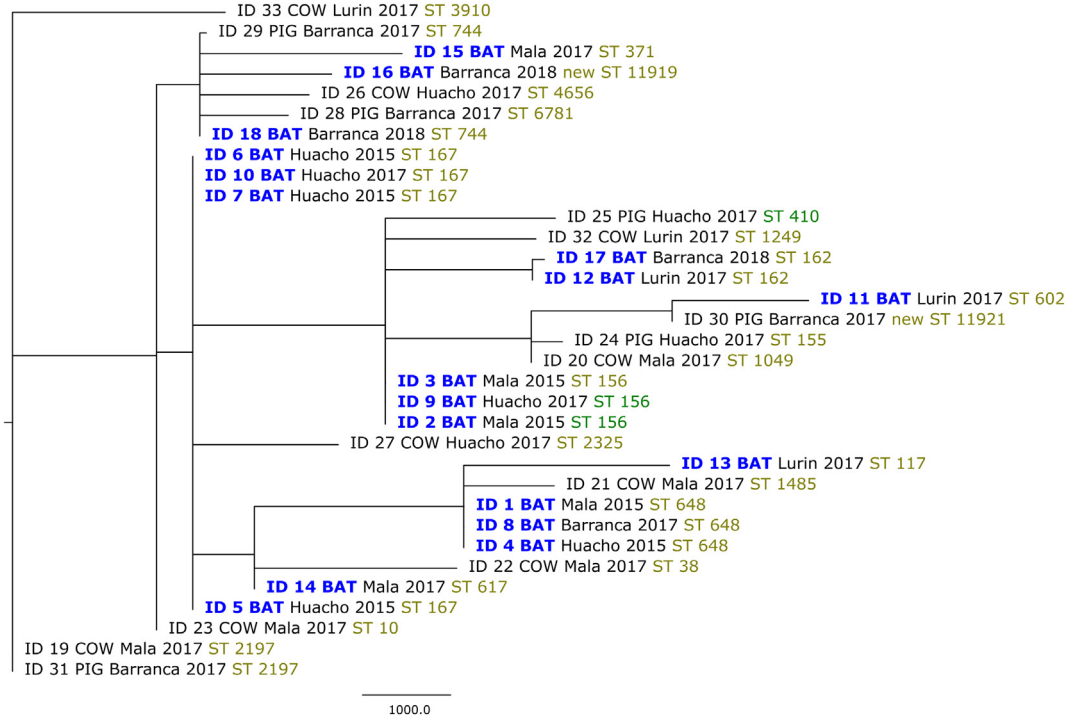

Fig. 4. Core-genome MLST reconstruction of 33 ESBL-*E. coli* isolates from bats and livestock. The phylogeny was reconstructed by the cgMLST hierarchical clustering method v1.1.2 available in Enterobase (https://enterobase.warwick.ac.uk/) using 2513 loci. The resulting phylogenetic tree was edited in FigTree version v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/). Branch length represents differences in the number of MLST loci. Each label shows the isolate's species, locality, year of sampling and *E. coli* Sequence Type (ST).

3.3. Antibiotic resistance genes and plasmids in ESBL-E. coli from bats and livestock

A total of 33 isolates including 18 ESBL-*E. coli* isolates (out of 20 positive isolates) from bats and 15 (out of 20) isolates from livestock were successfully analyzed by WGS. The seven excluded isolates had insufficient sequencing data quality or were suspected to represent mixed cultures following genome quality control (genome length and n50 value). Details of WGS, including reads, coverage and contigs information for each isolate are given in Supplementary Table S1. All isolates from bats belonged to different animals (one isolate/animal) whereas only two isolates (ID19 and ID20) belonged to the same sampled cow in Mala and had different antibiotic resistance patterns and genetic characteristics. *E. coli* species identity was confirmed by Kraken for all isolates. The mean genome size of sequenced isolates was 5.2Mbp [range: 4.6–5.9], the mean GC content was 50.5% [range: 50.1–50.7] and the mean n50 was 170,139 [58,213-699,396] (Table S1).

From the above contigs, 46 acquired resistance genes belonging to 11 antibiotic classes were identified by ResFinder (Fig. 3 and Table S2). ESBL-*E. coli* carried an average of 13 resistance genes [range: 5–18 in bats and 4–18 in livestock], and 30 resistance genes (65%) were detected in isolates from bats and livestock. Eighteen genes conferring resistance to aminoglycosides were detected at prevalence ranging from 3% (*aadB*) to 55% (*aadA1*) (Fig. 3). Eight different β-lactamases genes were detected including $bla_{\text{TEM-B}}$ (72% prevalence), $bla_{\text{TEM-176}}$ (21%), $bla_{\text{OXA-1}}$ (12%), and ESBL genes $bla_{\text{CTX-M-3}}$ (9%), $bla_{\text{CTX-M-14}}$ (9%), $bla_{\text{CTX-M-15}}$ (27%), $bla_{\text{CTX-M-55}}$ (45%) and $bla_{\text{CTX-M-65}}$ (12%) (Fig. 3). Six β-lactamases genes (i.e., $bla_{\text{TEM-B}}$, $bla_{\text{TEM-176}}$, $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-65}}$) were found in isolates from both bats and livestock. The *mcr-1* gene, which confers resistance to colistin, was detected in 11 isolates (33%) including four isolates from bats. Chromosomal point mutations conferring resistance to quinolones ciprofloxacin and nalidixic acid were detected in 24 isolates (73%), including 17 isolates from bats (94%) and 7 isolates from livestock (47%) (Fig. 3 and Table S2). Among 11 categories of antibiotics for which we tested clinical phenotypic resistance and the presence of genes conferring resistance by WGS (i.e., aminoglycosides, carbapenems, cephamycin, extended-spectrum cephalosporins, folate pathway inhibitor, quinolones, monobactam, non-extended spectrum cephalosporin, penicillin, phenicol and phosphonic), antibiotic phenotypic resistance (i.e., resistance to at least one antibiotic of that category) matched genotype resistance (i.e., presence of at least one gene or mutation conferring resistance to that antibiotic category) in 83% of combinations (301 out of 363), while phenotypic resistance was observed without genotypic resistance in 4% of combinations, and phenotypic resistance was absent in 13% of combinations detecting genotype resistance.

A total of 31 plasmids replicon types from were detected among isolates (Fig. 3), with 14 replicon types (45%) found in isolates from bats and livestock. ESBL-*E. coli* isolated from bats carried an average of 5 plasmid replicon types [range: 2–9] and isolates from livestock an average of 6 [0–9] (Table S2). The most prevalent plasmids replicon types included IncFIB. AP001918 (85%), IncX1 (58%) and IncN (42%). IncF-like, IncX1 and IncN plasmids were predominant across years. MobileElementFinder detected that three $bla_{\text{TEM-176}}$ genes were associated with plasmid replicon IncX1, two *sul*2 genes were associated with plasmid replicon IncQ1, two *aph*(3′)-Ia-like genes were associated with plasmid IncX1, and all *mcr-1* genes were associated with plasmid Incl2 except for one *E. coli* where *mcr-1* was associated with plasmid IncHI1B(R27).

3.4. Pathogenic potential of ESBL-E. coli

A total of 16 virulence genes were detected by VirulenceFinder (Fig. 3). Isolates from bats and livestock shared 11 virulence genes (69%) and carried an average of four virulence genes [range: 2–9 in bats and 1–11 in livestock], including glutamate decarboxylase (*gad*) (100% prevalence), iron-regulated adhesin (*iha*), serum survival gene (*iss*), extraintestinal infections toxins (*tsh*, *pic*, *astA*), *Salmonella* HilA homolog (*eilA*), and enteroaggregative immunoglobulin repeat protein (*air*) (Fig. 3 and Table S2).

3.5. ESBL-E. coli sequence types circulating in bats and livestock

MLST and cgMLST analyses showed a high diversity of STs in both bats and livestock with 23 different STs according to the PubMLST database reported among 33 isolates, including two newly assigned STs (Fig. 4). Vampire bat isolates harbored 10 known STs and 1 new ST (ST 11919 in Enterobase), while livestock harbored 14 STs, and 1 new ST (ST 11921 in Enterobase) (Fig. 4). The cgMSLT phylogenomic reconstruction showed no universal isolate clustering by host species, year or sampling area (Fig. 4). However, *E. coli* isolates showing 100% identity on the cgMLST (out of 2513 loci) were collected in the same bat colony at different years (i.e., ST 167 collected in Huacho in 2015 and 2017). Three of the four *E. coli* ST 167 carried the same *bla* genes ($bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, $bla_{TEM\text{-}156}$), *mcr-1* gene, virulence genes and had five plasmid replicon types in common (IncFIA, IncFIB(AP001918), IncFII, IncI2, IncX1) (Table S2). Likewise, bat isolates showing 99% identity on cgMLST (i.e., differ by one or less loci) were isolated from different bat colonies in different years (e.g., ST 156 collected in Huacho in 2015 and Mala in 2017; ST 648 collected in Mala in 2015, Huacho in 2015 and Barranca in 2017). Similarly, two isolates with 100% identity on the cgMLST (ST 2197) were isolated from a pig and a cow located in different localities (Mala and Barranca) (Fig. 4). ESBL-*E. coli* ST 744 with 97% identify on the cgMLST (i.e., differ by 74 loci) was isolated from a bat sampled in 2018 and a pig sampled in 2017 within the same location of Barranca (Fig. 4 and Table S2).

4. Discussion

Fecal carriage of multidrug-resistant (MDR) and clinically relevant Enterobacteriacae in wildlife has been reported worldwide, but the role of wildlife in the circulation of these bacteria at the livestock-wildlife interface remains poorly understood. This study shows that MDR ESBL-*E. coli* circulate in common vampire bat colonies, but occurred at a lower prevalence (5%) than observed in livestock (48%). ESBL-*E. coli* from bats belonged to different *E. coli* STs and harbored multiple antibiotic resistance genes, plasmids and virulence genes, with a majority of these genes also found in ESBL-*E. coli* from livestock. Genomic sequencing of isolates suggests the potential for long-term maintenance of ESBL-*E. coli* in bats as well as common contamination and/or cross-species transmission of strains and/or genetic material between bats and livestock.

Although many studies have reported antibiotic-resistant *E. coli* in wildlife (Wang et al., 2017), few have explored the maintenance of these bacteria over time (Swift et al., 2019; Williams et al., 2011). In this study, ESBL-*E. coli* strains with identical cgMLST (ST 167) were detected in the same vampire bat colonies two years apart (Fig. 4), suggesting the long-term circulation of ESBL-*E. coli* strains within the same bat colony. We also observed that the three bat-derived isolates of ST 648 from 2015 and 2017 had almost identical virulome and plasmids despite being in bat colonies located more than 100 km apart (Fig. 3). This finding suggests either multiple introductions of the same ST in bats across different localities or previous introduction and long-term circulation of strains by dispersal of colonized individuals. Our data from re-captured individuals suggests transmission of ESBL-*E. coli* among bats rather than prolonged colonization in individual bats since bats acquired novel strains through time, though we emphasize that larger sample sizes of recaptured bats are needed to confirm this hypothesis. Overall, the maintenance of ESBL-*E. coli* in bat colonies rises new challenges to reduce the dissemination of these bacteria in both natural and agricultural environments.

Several lines of evidence from our study also suggest cross-species transmission of *E. coli* strains and/or genetic material conferring ESBL resistance between bats and livestock, or contamination from a common unknown source. First, no clear clustering by bats or livestock was detected in the phylogenomic reconstruction, even if several bat isolates clustered together. Furthermore, *E. coli* strain ST 744 detected in a bat was also found in a pig in the same locality one year earlier, with 97% identity in cgMLST between strains. Second, the prevalence of resistance to each antibiotic was highly correlated between bats and livestock. The sharing of similar resistance genes was confirmed by the detection of the same six *bla* genes in both bats and livestock, although these genes are also common among humans, companion animals and other wildlife (Bevan et al., 2017; Salgado-Caxito et al., 2021; Zeballos-Gross et al., 2021). Finally, bats and livestock also shared 11 virulence genes and 14 plasmids replicon types. The high prevalence of ESBL-*E. coli* in livestock (48%) and their nightly contact with vampire bats suggest that livestock could be a reservoir of ESBL-*E. coli* for bats, although future research should also evaluate other potential sources such as humans. The possible exchange of AMR between small-scale livestock and sympatric wild bats confirms the need to develop One Health approaches to tackle AMR circulation in areas of high contact between domestic and wild animals.

The multidrug resistance observed in ESBL-*E. coli* isolated from bats and livestock implies several treatment challenges if these bacteria infect humans or domestic animals. First, ESBL-*E. coli* were resistant to seven antibiotics considered of critical importance for human medicine (WHO, 2019). Second, multidrug resistance was associated to a high diversity of antibiotic resistance genes including ESBL *bla* genes ($bla_{CTX\text{-}M\text{-}3}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}55}$ and $bla_{CTX\text{-}M\text{-}65}$) shared by bats and livestock that have been previously reported in South American humans and animals (Cantón et al., 2012; Cantón and Coque, 2006; Pallecchi et al., 2007; de Carvalho et al., 2020; Fernandes et al., 2018; Sartori et al., 2017; Palma et al., 2017). Third, our study suggests that different plasmids could be involved in disseminating antibiotic resistance genes such as replicon type IncFI and IncFII, previously reported in ESBL-*E. coli* from wild gulls of Argentina and our previous study in this same population of bats (Benavides et al., 2018; Dolejska and Papagiannitsis, 2018; Liakopoulos et al., 2016b). Highly prevalent plasmids replicons IncFIB.AP001918 and IncFII found in our study are also known to carry MDR and virulence genes (Johnson and Nolan, 2009). Finally, chromosomal mutations conferring higher phenotypic resistance to quinolones in bats compared to livestock was also detected, with 94% of isolates from bats carrying mutations on *gyr*A and/or parC/E compared to 47% of isolates in livestock. The overall moderate consistency between the detection of phenotype and genotypic resistance (83% of combinations), compared to other studies finding over 90% consistency (Ellington et al., 2017), was mainly due to resistance genes detected by WGS not conferring phenotypic resistance, suggesting an insufficient expression of resistance genes failing to confer resistance to the clinical antibiotic concentrations tested, as previously shown for aminoglycosides (Ellington et al., 2017; Moran et al., 2017; Tyson et al., 2015).

The public health concern of *ESBL-E. coli* circulating among bats and livestock is also supported by the detection of the *mcr-1* gene conferring resistance to colistin, a last resort antibiotic used to treat human infections by multidrug-resistant Enterobacteriaceae (Lentz et al., 2021). To our knowledge, this is the first study to detect the *mcr-1* gene in *ESBL-E. coli* in a neotropical bat (Lentz et al., 2021), confirming its dissemination among wildlife worldwide (Ahmed et al., 2019; Liakopoulos et al., 2016a). The detection of ESBL-*E. coli* carrying the *mcr-1* gene in Peruvian livestock also suggests its potential dissemination in the Peruvian community, since this gene was previously isolated in *E. coli* from a European tourist returning from Peru (Arcilla et al., 2016) and a few clinical isolates (Deshpande et al., 2019). The origins of *mcr-1* in both bats and livestock are uncertain. In particular, the use of colistin and other antibiotics by small farmers of low-income countries is unknown (Cuong et al., 2018), and the two available studies of cattle in Peru did not report the use of colistin (Benavides et al., 2021; Redding et al., 2014), suggesting selective pressure of antibiotics in animals may be unlikely. Future studies should confirm whether *mcr-1* is carried by the IncI2 plasmid replicon as suggested by our analysis and previous studies (Poirel et al., 2017), as well as the public health implications of its dissemination.

Other than concerns over a reduction in treatment efficiency during infection, specific ESBL-*E. coli* strains can also be associated with pathogenicity. Genomic analyses suggest that different lineages of *E. coli* of high pathogenic potential are circulating in wild mammals of South America (Fuentes-Castillo et al., 2020). Several *E. coli* STs found in this study in

bats or livestock such as ST 167, ST 117 and ST 648 are considered pathogenic, MDR and have been previously isolated from clinically ill humans, domestic and wild animals (Ewers et al., 2014; Fernandes et al., 2018; Guenther et al., 2010; Hasan et al., 2016). For example, *E. coli* ST 167 was associated with clinical infections in China (He et al., 2017; Zong et al., 2018), *E. coli* ST 10 and ST 156 were reported in clinical human samples (Mushtaq et al., 2011; Oteo et al., 2009), and ST 117 can cause extra-intestinal infections in poultry and sepsis in humans (Mora et al., 2012). Very few studies have characterized the *E. coli* STs circulating among patients in Peru (e.g., ST 165 (Tamariz et al., 2018)) and no study has focused on farmers. The presence of several virulence genes among ESBL-*E. coli* strains found in this study also suggests their pathogenic potential. For example, *eilA* found in *E. coli* from bats and cattle is associated with enteroaggregative *E. coli* producing diarrhea in people (Sheikh et al., 2006), whereas both *iroN* and *tsh* genes are associated with avian-pathogenic *E. coli* (APEC) (Caza et al., 2008; Dozois et al., 2000). Thus, future research should quantify whether these bacteria is impacting the health of humans and domestic animals in these agricultural settings.

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CRediT authorship contribution statement

Julio A. Benavides: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Sylvain Godreuil:** Methodology, Writing – review & editing. **Andrés Opazo-Capurro:** Formal analysis, Writing – review & editing. **Oumar O. Mahamat:** Methodology. **Nestor Falcon:** Funding acquisition. **Katarina Oravcova:** Methodology, Writing – review & editing. **Daniel G. Streicker:** Conceptualization, Resources, Funding acquisition, Writing – review & editing. **Carlos Shiva:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Project administration, Funding acquisition.

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.

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