First Report of a Multidrug-Resistant ST58 *Escherichia coli* Harboring Extended-Spectrum Beta-Lactamase of the CTX-M-1 Class in a Fecal Sample of a Captive Baird's Tapir (*Tapirus bairdii*) in Costa Rica, Central America

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Objective: This study's main objective was to analyze the antibiotic susceptibility profile of *Escherichia coli* isolates obtained from a fecal sample of a captive Baird's tapir (*Tapirus bairdii*) in Costa Rica.

Materials and Methods: The fecal sample was collected inside the enclosure on March 3, 2017, right after the animal defecated. Samples were cultured on MacConkey agar plates nonsupplemented and supplemented with $2 \mu g/mL$ of cefotaxime. Bacterial identification and antibiotic susceptibility were performed with the Vitek 2 Compact System and the Kirby Bauer disk diffusion method, respectively. Polymerase chain reaction amplification was performed to detect bla_{CTX-M} beta-lactamase genes. Resistant isolates were subjected to whole-genome sequencing (WGS).

Results: After evaluating several antibiotic classes, a multidrug-resistant *E. coli* strain with extended-spectrum beta-lactamase phenotype was isolated. Resistance to cefotaxime, cefepime, ampicillin, ampicillin/sulbactam, and tetracycline was detected. WGS analysis showed the presence of $bla_{CTX-M-1}$, bla_{TEM-1B}, and $tet(B)$ genes. The presence of IncN plasmids and Col156 was also detected.

Conclusion: Our findings are according with the notion that animals' high density enhances the spread of resistant determinants in a captive environment in a limited space, where the likelihood of direct or indirect contact with other animals and humans is more frequent.

Keywords: *Escherichia coli*, antibiotic resistance, ESBL, captivity, *Tapirus bairdii*

Introduction

TAPIRS (*TAPIRUS* SPP.), AS STRICT herbivores, play an essential ecological role as an ecosystem engineer, mainly through efficient long-distance seed dispersal and selective browsing, the reason why they are called the "gardeners of the forest" contributing to mitigate climate change.[1–3] Currently, Baird's tapir (*Tapirus bairdii*) is classified as an endangered species, according to International Union for Conservation of Nature.[4] With <5,000 living individuals in all its distribution range, due to threats such as habitat fragmentation, hunting, and recently vehicle collisions.[4–6]

It has been calculated that <1,500 free-ranging individuals inhabit Costa Rica.[5] Approximately 15 captive tapirs are estimated in the country (A. Sánchez-Núñez [Wildlife Coordinator, National System of Conservation Areas, SINAC], personal communication). There are several reasons why some tapirs are held in captivity in Costa Rica. However, most of the time, these are animals that have been captured to receive medical attention, and after treatment, they are not good release candidates. Despite the reasons they are held in confinement, little is known about captive tapirs' health status in Costa Rica. Nevertheless, it is known that the systematic collection of data related to their health status

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is essential to develop conservation strategies for this endangered species in the wild.[7]

The characterization of the antibiotic resistome of captive wild animals is not commonly performed as a part of the health status assessment; however, several reports support the notion that animals in captivity are more likely to acquire resistant determinants.[8] This is relevant not only because these individuals may develop infections with antibiotic-resistant bacteria but also because they may act as reservoirs. When these animals are eventually released, there is a potential risk of transmission and dissemination of resistance determinants throughout the environment as well as other animals or humans.[8–10]

Escherichia coli constitutes a high percentage of the large intestine microbiota from mammals. It is a reservoir for antibiotic resistance genes, which may have an essential role in the epidemiology of resistance determinants spill-over.[8,10–12] Given that *E. coli* easily become resistant due to point mutations or horizontal transfer of genetic material, these bacteria have been used to measure antimicrobial resistance prevalence.[11] Among the resistant mechanisms in Gram-negative (GN) bacteria, *E. coli* producing extended-spectrum beta-lactamases (ESBL), mainly from the CTX-M type, are considered an exciting topic in public health since they have caused many outbreaks and infections in humans worldwide.[13]

We previously reported 98% (59/60) of pansusceptible *E. coli* isolates from fecal samples free-ranging Baird's tapirs in Costa Rica.[14] Conversely, multidrug-resistant *E. coli* from lung and fecal isolates were reported in captive South American tapirs (*Tapirus terrestris*) in Colombia and the Czech Republic, respectively.[15,16] Besides multidrug-resistant phenotype, Klimes *et al.* also detected an *E. coli* producing ESBL with the phenotype CTX-M-15 in the captive South American tapir.[16] In addition, phenotype CTX-M-1 was recently isolated in the same species in captivity.[17]

In this study, we report the isolation of a fecal ESBL-producing *E. coli* in a captive Baird's tapir (*T. bairdii*) from Costa Rica and its subsequent genetic characterization using WGS. To our knowledge, this is the first report related to the detection of a multidrug-resistant *E. coli* harboring ESBL of the CTX-M-1 class in a fecal sample of a captive Baird's tapir in Costa Rica, Central America.

Materials and Methods

Sample collection

A female captive tapir (weighing ∼250 kg) had biological, nutritional, and veterinary care. The enclosure where the female was held, consisting of an area of ∼600 m^2, with several trees, a small water tank (8 m^2) and next to it, several enclosures with different animal species such as coyotes (*Canis latrans*), red fox (*Urocyon cinereoargenteus*), collared peccaries (*Tayassu tajacu*), puma (*Puma concolor*), mantled howler monkey (*Alouatta palliata*), and spider monkey (*Ateles geoffroyi*), among others.

During a routine physical examination, the animal presented a good physical condition, no lesions in the eyes, skin, or hooves. However, near a month before examination, the animal had been orally treated with ceftiofur (5 mg/kg per

300 kg) due to a submandibular abscess of unknown etiology. For economic reasons, the oral treatment was suspended and changed to topical. After the physical examination, two fecal samples were collected inside the enclosure on March 3, 2017, right after the animal defecated. After collection, samples were stored at 4°C and transported to the Bacteriology Laboratory of the Escuela de Medicina Veterinaria, Universidad Nacional de Costa Rica, for analysis.

E. coli *isolation*

Samples were directly cultured on standard MacConkey (Oxoid, Hampshire) agar plates nonsupplemented and supplemented with 2 μg/mL of cefotaxime (Sigma-Aldrich, MO) and incubated for 24 hr at 37°C. Typical lactose positive *E. coli* colonies were identified with Vitek Compact 2 automated system (BioMèriux, Marcy-l'Étoile) using the GN card. *E. coli* ATCC 25922 (cefotaxime susceptible) and *Klebsiella pneumoniae* ATCC 700603 (cefotaxime resistant, ESBL positive) strains were used for quality control of the culture media.

Antimicrobial susceptibility testing

The antibiotic susceptibility test was performed with Vitek Compact 2 automated system (BioMèriux) using the AST-N279 card that includes the following antibiotics: amikacin, gentamicin, cefotaxime, ceftazidime, cefepime, ampicillin, piperacillin/tazobactam, ampicillin/sulbactam, imipenem, meropenem, ciprofloxacin, nalidixic acid, sulfonamide/trimethoprim, colistin, and nitrofurantoin. Susceptibility to tetracycline (30 μg) (Oxoid, Hampshire) was tested by Kirby Bauer disk diffusion method. M100 from CLSI (2020) was used for interpretation.[18]

Detection of ESBL genes

ESBL screening test was performed using the combination disk test with cefotaxime (30 μg) and ceftazidime (30 μg) disks with and without clavulanate (10 μg).[14] CTX-M detection was performed using the methodology previously described.[19] In brief, CTX-M polymerase chain reaction (PCR) amplification of bla_{CTX-M} alleles was carried out with primers CTX-MU1 (5′-ATG TGC AGY ACC AGT AAR GT-3′) and CTX-MU2 (5′-TGG GTR AAR TAR GTS ACC AGA-3′). These PCR primers allow the amplification of a 593 bp fragment present in different types of CTX-M, including $bla_{CTX-M-1}$ to $bla_{CTX-M-30}$.

Whole-genome sequencing analysis

An isolate with an ESBL phenotype was subjected to whole-genome sequencing (WGS). WGS was performed using Illumina next-generation sequencing. Reads quality was checked with FastQC and genome assembly with Unicycler,[20] with assembly quality check using Quast[21] and annotation with Prokka.[22] The Serotype was determined using Short Read Sequencing Typing (SRST2) tool[23] against EcOH database from Holt lab. Clermont Phylogroup was determined using the *In Silico* Clermont Phylotyper tool. Antimicrobial Resistance Identification By Assembly (ARIBA) tool[24] was used for multilocus sequence typing (MLST) analysis according to the Achtman scheme and with the ResFinder tool[25] reference database to identify

TABLE 1. FEATURES OF *E. COLI* ISOLATE HARBORING EXTENDED-SPECTRUM
BETA-LACTAMASE AND TETRACYCLINE RESISTANCE

Isolate	Serotype	MLST sequence type	Clermont phylogroup	Resistance profile	Resistance genes	Plasmids
H024-B	0153:H40	58	B1	CTX- FEP-AMP- SAM-TET	$aph(3')$-ia, $aph(6)$-id, $bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1b}$, $mdfA$, $tet(B)$	Col156, IncN

AMP, ampicillin; CTX, cefotaxime; FEP, cefepime; MLST, multilocus sequence typing; SAM, ampicillin/sulbactam; TET, tetracycline.

resistance genes. Point mutation resistance was investigated using AMRFinderPlus[26] and plasmid detection and typing using PlasmidFinder.[27]

Results

The culture of the tapir's fecal sample yielded one multidrug-resistant *E. coli* isolate. This *E. coli* isolate was resistant to cefotaxime, cefepime, ampicillin, ampicillin/sulbactam, and tetracycline (Table 1).

Owing to the resistance against third-generation cephalosporins and a positive ESBL test (using the combination disk test), we performed a PCR toward $bla_{CTX\text{-}M}$ genes. The presence of a $bla_{CTX\text{-}M}$ gene was detected (Fig. 1). This multidrug-resistant isolate, harboring the gene encoding the production of the ESBL, was subjected to WGS to determine the serotype, the MLST sequence type, the Clermont phylogroup and the presence or absence of resistance genes, resistance point mutations, and plasmids (Table 1).

The detection of resistance genes $bla_{CTX\text{-}M\text{-}1}$, $bla_{TEM\text{-}1b}$, $mdfA$, tet(B) (using the ResFinder tool) confirmed the phenotypic resistance to cefotaxime, cefepime, ampicillin, ampicillin/sulbactam, tetracycline, and the ESBL phenotype. We also detected additional resistance genes to the
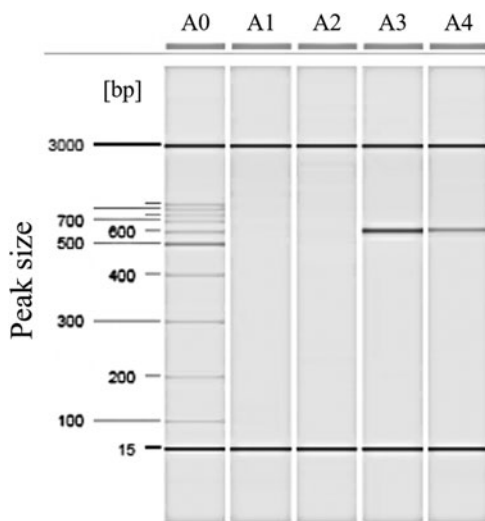

FIG. 1. Detection of ESBL type CTX-M. A0: molecular weight marker, A1: reaction mixture control, A2: negative control (*E. coli* ATCC 25922), A3: positive control (*Shigella flexneri* OPS 187), A4: *E. coli* isolate H024B. ESBL, extended-spectrum beta-lactamase.

aminoglycosides group [$aph(3'')$-Ia, $aph(6)$-id] (Fig. 1). The aminoglycosides gentamicin and amikacin were classified as susceptible by phenotypic methods. This susceptibility is expected since [$aph(3'')$-Ia ot $aph(6)$-id] do not confer resistance to these two antibiotics.[28] No resistance-related point mutations were found for any antibiotic class.

The serotype and MLST sequence type determined by WGS showed that this *E. coli* isolate belonged to the sequence type 58 (ST58) and the serotype O08:H21, respectively (Table 1). The conjugative plasmids Col156 and IncN were also detected (Table 1).

Discussion

In this study, we report a multidrug-resistant ST58 *E. coli* harboring an ESBL of the CTX-M-1 class in a fecal sample of a captive Baird's tapir (*T. bairdii*) in Costa Rica. *E. coli* harboring ESBL has been increasingly reported in several wildlife species in the past two decades in wild and captive conditions, including reports in >30 species shedding ESBL in diverse geographical landscapes.[10,29] Although resistant bacteria seem to correlate with anthropogenic activity, some studies report the prevalence of ESBL in pristine habitats.[30–32] In addition, some of the driving factors for the occurrence of resistant bacteria in wildlife are not fully understood; current evidence suggests that species' biology and ecology, including feeding and nesting near human-dominated landscapes, are among the key factors.[29,33]

In contrast, captive animals are in close contact with humans. Food manipulation, runoff, disinfectant use, and frequent antibiotic therapy are suggested to be the main drivers for resistant bacteria transmission (i.e., mainly through horizontal gene transfer mechanisms) in these facilities.[29,31,34]

Similar to human and veterinarian medicine, the most detected type of ESBL in wild animals corresponds to the $bla_{CTX\text{-}M}$ gene-family. Most of the studies conducted have identified $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$, $bla_{CTX\text{-}M\text{-}13}$, $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}32}$, $bla_{CTX\text{-}M\text{-}55}$, and $bla_{CTX\text{-}M\text{-}65}$.[10,12,16,17] In addition to the $bla_{CTX\text{-}M}$ gene-family also the $bla_{SHV\text{-}12}$ and $bla_{TEM\text{-}52}$ were identified.[10] The most commonly reported non-ESBL phenotypes showed resistance to ampicillin, streptomycin, and tetracycline.[10]

E. coli phylogenetic analysis was formerly based on a triplex PCR determining four main groups (A, B1, B2, and D).[35] The expanding body of multilocus sequence data and genome data for *E. coli* has contributed significantly to the better understanding of phylogroups for this bacteria into eight groups (A, B1, B2, C, D, E, F, belonging to *E. coli* sensu stricto; and *Escherichia* cryptic clade I).[36] Specifically, phylogenetic group B1 is often detected in *E. coli*

isolates from captive animals, including Giant anteater (*Myrmecophaga tridactyla*), Geoffroy's cat (*Leopardus geoffroyi*), ocelot (*Leopardus pardalis*), and South American tapir (*T. terrestris*).[17,37]

ST58 has been reported as one of the main ESBL-producing *E. coli* circulating at the human–animal–environment[38]; however, there are very few reports of ST58 in wildlife.[10] ST58 was previously reported in a fatal case of a captive critically endangered Brazilian merganser (*Mergus octosetaceus*) in several organs[34] and in peri-urban South American coatis (*Nasua nasua*) in Brazil.[38]

IncN has been associated with disseminating several bla_{CTX-M} variants, such as $bla_{CTX-M-1}$, $bla_{CTX-M-3}$, $bla_{CTX-M-15}$, $bla_{CTX-M-32}$, $bla_{CTX-M-40}$, and $bla_{CTX-M-65}$.[39] In several European countries, IncN plasmids largely contributed to the spread of $bla_{CTX-M-1}$ among humans, animals, and the environment.[40] To our knowledge, the only reports related to IncN plasmids in wild animals were performed in European free-ranging waterbirds,[41,42] in which they detected IncN and IncI1 harboring $bla_{CTX-M-1}$ and IncI1 and IncFIA-FIB harboring $bla_{CTX-M-15}$. In both surveys, they also detected the genes bla_{TEM-1B} and *tet*(B). The broad-host-range IncN plasmids might contribute to the spillover of isolates producing CTX-M-1-like enzymes among other members of Enterobacteriaceae.[41]

The detection of an IncN plasmid in the fecal microbiota of a wild animal held in captivity is considered a public health concern.[10] This individual may act as a reservoir, contributing to this resistance determinant's potential dissemination throughout the facilities and the environment.[9,11] The possible source for the acquisition of these resistance determinants in this tapir is unknown. It is relevant to note that, in addition to the frequent contact with the staff that takes care of the animals in the facilities, its enclosure locates near several species' enclosures. This species included coyotes (*C. latrans*), red fox (*U. cinereoargenteus*), collared peccaries (*T. tajacu*), puma (*P. concolor*), mantled howler monkey (*A. palliata*), and spider monkey (*A. geoffroyi*), where water runoff occurs during rain or cleaning of the enclosures. Besides, many wild bird species settled or moved around the facilities that might function as vectors or reservoirs of resistance determinants. Therefore, close contact between humans and animals nearby occurs regularly and can disseminate antibiotic resistance genes. The isolation of a multidrug-resistant *E. coli* harboring ESBL contrasts with our previous results in free-ranging Baird's tapirs, in which 98% of *E. coli* isolates obtained from fecal samples were pansusceptible.[14]

To monitor the animals' health status, we propose monitoring the fecal resistome to prevent resistance determinants spillover in the facilities or in animal candidates to be released. Our findings concern veterinarians to be aware of possible complications in treating captive animals infected with resistant bacteria.[9,10]

Conclusion

Our results provide evidence that hygienic practices, appropriate and responsible use of antibiotics in the rehabilitation of wild animals is essential when releasing them into the wild to prevent the dissemination of resistant determinants into the environment.[9] These findings support the notion that places with a high density of animals in a relatively small area promote high dissemination of resistance determinants, mainly through water runoff or direct or indirect contact among humans and animals.

References

1. O'Farrill, G., M. Galetti, and A. Campos-Arceiz. 2013. Frugivory and seed dispersal by tapirs: an insight on their ecological role. Integr. Zool. 8:4–17.
2. Bello, C., M. Galetti, M.A. Pizo, et al. 2015. Defaunation affects carbon storage in tropical forests. Sci. Adv. 1:1–11.
3. Paolucci, L.N., R.L. Pereira, L. Rattis, et al. 2019. Lowland tapirs facilitate seed dispersal in degraded Amazonian forests. Biotropica 51:245–252.
4. García, M., C. Jordan, G. O'Farril, et al. 2016. *Tapirus bairdii*. The IUCN Red List of Threatened Species 2016: e.T21471A45173340. Available at https://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T21471A45173340.en (accessed June 18, 2021).
5. Schank, C.J., E.Y. Arima, M.V. Cove, et al. 2020. Population status, connectivity, and conservation action for the endangered Baird's tapir. Biol. Conserv. 245:108501.
6. Brenes-Mora, E. 2018. Activity patterns, habitat selection and knockdowns of tapir (*tapirus bairdii*) in a forest complex sectioned by a road in the Talamanca mountain range, Costa Rica [in Portuguese]. Universidad Nacional de Costa Rica, Heredia, Costa Rica. Available at http://hdl.handle.net/11056/14856 (accessed June 18, 2021).

7. Mangini, P.R., E.P. Medici, and R.C. Fernandes-Santos. 2012. Tapir health and conservation medicine. Integr. Zool. 7:331–345.

8. Stoddard, R.A., E.R. Atwill, P.A. Conrad, *et al*. 2009. The effect of rehabilitation of northern elephant seals (*Mirounga angustirostris*) on antimicrobial resistance of commensal *Escherichia coli*. Vet. Microbiol. 133:264–271.

9. Carroll, D., J. Wang, S. Fanning, and B.J. Mcmahon. 2015. Antimicrobial resistance in wildlife: implications for public health. Zoonoses Public Health 62:534–542.

10. Guenther, S., C. Ewers, and L.H. Wieler. 2011. Extended-spectrum beta-lactamases producing *E. coli* in wildlife, yet another form of environmental pollution? Front. Microbiol. 2:1–13.

11. Mosquito, S., J. Ruiz, J.L. Bauer, and T.J. Ochoa. 2011. Molecular mechanisms of antibiotic resistance in *Escherichia coli*—associated diarrhea. Rev. Peru. Med. Exp. Salud Publica 28:648–656.

12. Carvalho, I., M.T. Tejedor-Junco, M. González-Martín, *et al*. 2020. Molecular diversity of extended-spectrum β-lactamase-producing *Escherichia coli* from vultures in Canary Islands. Environ. Microbiol. Rep. 12:540–547.

13. Carattoli, A. 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrob. Agents Chemother. 53:2227–2238.

14. Rojas-Jiménez, J., E. Brenes-Mora, P. Alcázar-García, R. Arguedas-Porras, and E. Barquero-Calvo. 2019. Pansusceptible *Escherichia coli* isolates obtained from faeces of free-ranging Baird's tapirs (*Tapirus bairdii*) suggests a low selective pressure for resistance determinants in the northwestern region of the Talamanca Mountain Range, Costa Rica. J. Glob. Antimicrob. Resist. 16:140–143.

15. Vargas, J., S. Máttar, and S. Monsalve. 2010. Bacterias patógenas con alta resistencia a antibióticos: estudio sobre reservorios bacterianos en animales cautivos en el zoológico de Barranquilla. Infectio 14:6–19.

16. Klimes, J., M. Machalkova, M. Dolejska, *et al*. 2013. *Escherichia coli*-producing extended-spectrum beta-lactamase CTX-M-15 in a captive South American tapir (*Tapirus terrestris*). J. Zoo Wildl. Med. 44:173–175.

17. Witte, C.D., N. Vereecke, S. Theuns, *et al*. 2021. Presence of broad-spectrum beta-lactamase-producing Enterobacteriaceae in Zoo Mammals. Microorganisms 9:1–15.

18. CLSI. 2020. Performance Standards for Antimicrobial Susceptibility Testing; document M100. Clinical and Laboratory Standards Institute, Wayne.

19. Pagani, L., E. Dell'Amico, R. Migliavacca, *et al*. 2003. Multiple CTX-M-type extended-spectrum beta-lactamases in nosocomial isolates of Enterobacteriaceae from a hospital in Northern Italy. J. Clin. Microbiol. 41:4264–4269.

20. Wick, R.R., L.M. Judd, C.L. Gorrie, and K.E. Holt. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13:e1005595.

21. Gurevich, A., V. Saveliev, N. Vyahhi, and G. Tesler. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075.

22. Seemann, T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069.

23. Inouye, M., H. Dashnow, L.A. Raven, *et al*. 2014. SRST2: Rapid genomic surveillance for public health and hospital microbiology labs. Genome Med. 6:90.

24. Hunt, M., A.E. Mather, L. Sánchez-Busó, *et al*. 2017. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. Microb. Genomics 3:e000131.

25. Zankari, E., H. Hasman, S. Cosentino, *et al*. 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67:2640–2644.

26. Feldgarden, M., V. Brover, D.H. Haft, *et al*. 2019. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob Agents Chemother. 63:e00483-19.

27. Carattoli, A., E. Zankari, A. Garciá-Fernández, *et al*. 2014. *In Silico* detection and typing of plasmids using plasmidfinder and plasmid multi-locus sequence typing. Antimicrob. Agents Chemother. 58:3895–3903.

28. Ramirez, M.S., and M.E. Tolmasky. 2010. Aminoglycoside modifying enzymes. Drug Resist. Updat. 13:151–171.

29. Vittecoq, M., S. Godreuil, F. Prugnolle, P. *et al*. 2016. REVIEW: antimicrobial resistance in wildlife. J. Appl. Ecol. 53:519–529.

30. Skurnik, D., R. Ruimy, A. Andremont, *et al*. 2006. Effect of human vicinity on antimicrobial resistance and integrons in animal faecal *Escherichia coli*. J. Antimicrob. Chemother. 57:1215–1219.

31. Allen, H., and J. Donato. 2010. Call of the wild: antibiotic resistance genes in natural environments. Nat. Rev. 8:251–259.

32. Hernandez, J., J. Bonnedahl, I. Eliasson, *et al*. 2010. Globally disseminated human pathogenic *Escherichia coli* of O25b-ST131 clone, harbouring $bla_{\text{CTX-M-15}}$, found in Glaucous-winged gull at remote Commander Islands, Russia. Environ. Microbiol. Rep. 2:329–332.

33. Dolejska, M., and I. Literak. 2019. Wildlife is overlooked in the epidemiology of medically important antibiotic-resistant bacteria. Antimicrob. Agents Chemother. 63:1–5.

34. Fuentes-Castillo, D., P.E. Navas-Suárez, M.F. Gondim, *et al*. 2021. Genomic characterization of multidrug-resistant ESBL-producing *Escherichia coli* ST58 causing fatal colibacillosis in critically endangered Brazilian merganser (*Mergus octosetaceus*). Transbound. Emerg. Dis. 68:258–266.

35. Clermont, O., S. Bonacorsi, and E. Bingen. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl. Environ. Microbiol. 66:4555–4558.

36. Clermont, O., J.K. Christenson, E. Denamur, and D.M. Gordon. 2013. The Clermont *Escherichia coli* phylotyping method revisited: improvement of specificity and detection of new phylo-groups. Environ. Microbiol. Rep. 5:58–65.

37. Furlan, J.P.R., R. Lopes, I.H.L. Gonzalez, P.L. Ramos, and E.G. Stehling. 2020. Comparative analysis of multidrug resistance plasmids and genetic background of CTX-M-producing *Escherichia coli* recovered from captive wild animals. Appl. Microbiol. Biotechnol. 104:6707–6717.

38. de Carvalho, M.P.N., M.R. Fernandes, F.P. Sellera, *et al*. 2020. International clones of extended-spectrum-β-lactamase (CTX-M)-producing *Escherichia coli* in peri-urban wild animals, Brazil. Transbound. Emerg. Dis. 67:1804–1815.

39. Novais, A., R. Cantón, R. Moreira, L. Peixe, F. Barquero, and T.M. Coque. 2007. Emergence and dissemination of Enterobacteriaceae isolates producing CTX-M-1-like enzymes in Spain are associated with IncFII (CTX-M-15) and broad-host-range (CTX-M-1, -3, and -32) plasmids. Antimicrob. Agents Chemother. 51:796–799.

40. García-Fernández, A., L. Villa, A. Moodley, *et al*. 2011. Multi-locus sequence typing of IncN plasmids carrying extended-spectrum β-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. J. Antimicrob. Chemother. 66:1987–1991.

41. Literak, I., M. Dolejska, D. Janoszowska, *et al*. 2010. Antibiotic-resistant *Escherichia coli* bacteria, including strains with genes encoding the extended-spectrum beta-lactamase and QnrS, in waterbirds on the Baltic Sea Coast of Poland. Appl. Environ. Microbiol. 76:8126–8134.

42. Dolejska, M., L. Villa, H. Hasman, L. Hansen, and A. Carattoli. 2013. Characterization of IncN plasmids carrying *bla*$_{\text{CTX-M-1}}$ and qnr genes in *Escherichia coli* and *Salmonella* from animals, the environment and humans. J. Antimicrob. Chemother. 68:333–339.

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