Science in One Health 3 (2024) 100069

Contents lists available at ScienceDirect

Science in One Health

journal homepage: www.journals.elsevier.com/science-in-one-health

Full length article

Emergence of plasmid-mediated tigecycline resistance *tet*(X4) gene in *Enterobacterales* isolated from wild animals in captivity

Lei Lei [a,1], Panfeng Xiong [a,b,1], Zelin Yan [b], Yanyan Zhang [b], Yuchen Wu [b], Gongxiang Chen [b], Houhui Song [a,**], Rong Zhang [b,*]

[a] Key Laboratory of Applied Technology on Green-Eco-Healthy Animal Husbandry of Zhejiang Province, Zhejiang Provincial Engineering Laboratory for Animal Health Inspection & Internet Technology, Zhejiang International Science and Technology Cooperation Base for Veterinary Medicine and Health Management, China-Australia Joint Laboratory for Animal Health Big Data Analytics, College of Animal Science and Technology & College of Veterinary Medicine, Zhejiang Agriculture and Forestry University, Hangzhou, Zhejiang 311300, China

[b] Department of Clinical Laboratory, The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang 310009, China

ARTICLE INFO

Keywords:
Wild animal
tet(X4)
Enterobacterales
Enterotoxin
Tigecycline resistance
Epidemiology

ABSTRACT

Background: Over the past few decades, antimicrobial resistance (AMR) has emerged as a global health challenge in human and veterinary medicine. Research on AMR genes in captive wild animals has increased. However, the presence and molecular characteristics of *tet*(X)-carrying bacteria in these animals remain unknown.

Methods: Eighty-four samples were collected from captive wild animals. *tet*(X) variants were detected using polymerase chain reaction and the isolates were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. All isolated strains were subjected to antimicrobial susceptibility testing and whole-genome sequencing. The virulence of an *Escherichia coli* strain carrying enterotoxin genes was assessed using a *Galleria mellonella* larval model.

Results: We isolated two *tet*(X4)-positive *E. coli* strains and one *tet*(X4)-positive *Raoultella ornithinolytica* strain. Antimicrobial susceptibility tests revealed that all three *tet*(X4)-carrying bacteria were sensitive to the 13 tested antimicrobial agents, but exhibited resistance to tigecycline. Notably, one *tet*(X4)-carrying *E. coli* strain producing an enterotoxin had a toxic effect on *G. mellonella* larvae. Whole-genome sequencing analysis showed that the two *tet*(X4)-carrying *E. coli* strains had more than 95% similarity to *tet*(X4)-containing *E. coli* strains isolated from pigs and humans in China.

Conclusion: The genetic environment of *tet*(X4) closely resembled that of the plasmid described in previous studies. Our study identified *tet*(X4)-positive strains in wildlife and provided valuable epidemiological data for monitoring drug resistance. The identification of enterotoxin-producing *E. coli* strains also highlights the potential risks posed by virulence genes.

1. Introduction

The World Health Organization has declared antimicrobial resistance (AMR) as one of the top ten global public health threats. Over the past few decades, AMR has emerged as an urgent global health challenge for both human and veterinary medicine [1]. The presence of multidrug-resistant (MDR) gram-negative bacteria, such as carbapenem-resistant *Enterobacterales*, extended-spectrum-beta-lactamase-producing *Enterobacterales*, and colistin-resistant gram-negative bacteria mediated by mobile colistin resistance (MCR), has significantly compromised the efficacy of commonly used antibiotics, including cephalosporins, and the last-resort antibiotics, carbapenems and colistin [2–4]. Tigecycline, which is effective against

Abbreviations: AK, amikacin; AMR, antimicrobial resistance; ATM, aztreonam; CAV, ceftazidime/avibactam; CAZ, ceftazidime; CFU, colony-forming units; CIP, ciprofloxacin; CMZ, cefmetazole; CTX, cefotaxime; ETP, ertapenem; FEP, cefepime; IMP, imipenem; MDR, multidrug-resistant; MEM, meropenem; MIC, minimum inhibitory concentration; PB, polymyxin B; SCF, cefoperazone/sulbactam; TGC, tigecycline; TZP, piperacillin/tazobactam; MCR, mobile colistin resistance.

* Corresponding author.

** Corresponding author.

E-mail addresses: leilei910@zafu.edu.cn (L. Lei), xiongpf@stu.zafu.edu.cn (P. Xiong), 22218111@zju.edu.cn (Z. Yan), 12018247@zju.edu.cn (Y. Zhang), 12218313@zju.edu.cn (Y. Wu), chengongxiang@zju.edu.cn (G. Chen), songhh@zafu.edu.cn (H. Song), zhang-rong@zju.edu.cn (R. Zhang).

[1] These authors contributed equally to this article.

https://doi.org/10.1016/j.soh.2024.100069

Received 14 November 2023; Accepted 11 April 2024

serious infections caused by MDR gram-negative bacteria, particularly extensively drug-resistant *Enterobacterales* and *Acinetobacter* species, has become a last-resort antimicrobial agent [5].

Tigecycline, a third-generation tetracycline antibiotic, exhibits an expanded spectrum of activity against both gram-negative and gram-positive bacteria. However, the discovery in 2019 of plasmid-encoded *tet*(X3) and *tet*(X4) genes, which mediate tetracycline-inactivating monooxygenase and confer high levels of tigecycline resistance, has raised public health concerns [6,7]. Subsequently, a range of plasmid-mediated *tet*(X) variants, from *tet*(X5) to *tet*(X22) [8], have been identified worldwide, with *tet*(X4) being the dominant genotype. Epidemiological investigations have revealed the presence of *tet*(X4) in bacteria isolated only from humans, livestock, poultry, wild animals, the food production chain, and the environment [9–11]. Additionally, *tet*(X4) has been detected in *Acinetobacter* spp. and *Enterobacterales*, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella* spp [12]. The widespread presence of *tet*(X4)-positive bacteria in various countries and regions has significantly increased the associated public health burden. Therefore, investigating the prevalence of *tet*(X) variants in different animals and environments is important.

Previous studies have revealed that animal husbandry and the food production chain are key transmission routes and reservoirs of *tet*(X) variants [13]. Food containing AMR bacteria, selective pressure from the use of antimicrobial agents, and contact with animals or environments harboring AMR genes or bacteria are significant pathways for animals and humans to acquire AMR genes or bacteria. While *tet*(X4) has been identified in farmed animals and the environment, both nationally and internationally, its prevalence in wildlife and zoo animals has seldom been reported [14,15]. Our study focused on the presence and molecular characteristics of *tet*(X)-carrying bacteria in captive wild animals with the aim of providing epidemiological data on AMR in these animals.

2. Methods

2.1. Sample collection and isolation

Fecal samples were collected from captive wildlife at a zoo in Hangzhou, Zhejiang Province, China, in November 2022. The samples comprised 84 wild animals, including 15 *Muntiacus crinifron*, 2 *Balaerica regulorum*, 2 *Ciconia boyciana*, 11 *Grus japonensis*, 8 *Anthropoides virgo*, 7 *Phoenicopteridae*, 11 *Rhinopithecus roxellana*, 10 *Pan troglodyte*, 10 *Hylobatidae*, and 8 other primates. Except for the crowned cranes and oriental storks, which shared habitats, the other animals were housed in separate enclosures. A matchhead-sized sample was inoculated in 5 mL of Luria–Bertani broth and incubated at 35 °C for 18 to 20 h. The enrichment culture was then grown on China Blue agar containing 2 mg/L tigecycline at 37 °C for 18 to 20 h. The isolates were purified and species identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *tet*(X1)–*tet*(X10) genes were detected by polymerase chain reaction.

2.2. Antimicrobial susceptibility testing

All *E. coli* isolates were subjected to antimicrobial susceptibility testing. The isolates were screened against the following 15 clinical antimicrobial agents: imipenem, ceftazidime-avibactam, meropenem, ertapenem, cefepime, cefmetazole, polymyxin B, ceftazidime, tigecycline, cefotaxime, amikacin, piperacillin-tazobactam, ciprofloxacin, cefoperazone-sulbactam, and aztreonam. The results were interpreted in accordance with the European Committee on Antimicrobial Susceptibility Testing Breakpoints, version 13.0, and the Clinical and Laboratory Standards Institute's Document M100-ED33.

2.3. Whole-genome sequencing analysis

Genomic DNA was extracted and sequenced on a HiSeq X platform (Illumina, San Diego, CA, USA) using a paired-end strategy. The draft genomes were assembled using SPAdes software (http://bioinf.spbau.ru/spades). Sequence types, AMR genes, and virulence genes were identified using ABRicate software (https://github.com/tseemann/abricate). Genomes collected before April 2023 were downloaded for analysis. A phylogenetic tree, comprising strains from captive animals and those available in the NCBI database (http://www.ncbi.nlm.nih.gov/), was generated using Snippy (https://github.com/tseemann/snippy) and IQ-TREE (https://github.com/iqtree/iqtree2) based on core-genome alignments and was visualized using iTOL (https://itol.embl.de/). Contigs containing *tet*(X4) were examined to determine their Inc type using the PlasmidFinder tool (https://cge.cbs.dtu.dk/services/). Virulence islands were analyzed using the computational tool IslandViewer 4 (http://www.pathogenomics.sfu.ca/islandviewer2/query.php). The genetic environment of *tet*(X4) and associated pathogenicity islands was explored using the web-based GenBank data visualization and comparison tool GBKviz (https://gbkviz.streamlit.app/) and the utility program Chiplot (https://www.chiplot.online/). In addition to whole-genome sequencing analysis, we compared the plasmid sequences and genetic environments with those of other *tet*(X4)-containing plasmids to determine their potential sources and transmission routes.

2.4. Galleria mellonella infection model

G. mellonella (*G. mellonella*) larvae were infected as previously reported to confirm the *in vivo* virulence of *tet*(X)- and *eltA*-containing strains. Bacterial suspensions in the logarithmic growth phase were diluted in sterile physiological saline solution to a concentration of 10^7 colony-forming units (CFU)/mL. A sterile physiological saline solution served as the negative control, whereas CVCC195 was used as the positive control. A 10 μL volume of the diluted bacterial suspension was injected into the last pro-legs of the larvae and incubated at 37 °C for 72 h. The number of dead larvae that were unresponsive to touch and black in color was recorded at 8, 16, 24, 32, 40, 48, 56, 64, and 72 h. Kaplan–Meier survival curves were generated using Prism version 8.0.2 (GraphPad, San Diego, CA, USA).

3. Results

3.1. Sample collection and isolation

A total of 84 fecal samples were collected from captive wildlife at a zoo in Hangzhou, China, in November 2022. Three *tet*(X)-positive strains were isolated: two *E. coli* strains and one *Raoultella ornithinolytica* strain. All strains carried the *tet*(X4) gene. One *E. coli* strain was isolated from a tufted deer and the other from a gibbon, whereas *R. ornithinolytica* was isolated from chimpanzees.

3.2. Antimicrobial susceptibility profiles

The antimicrobial susceptibility testing results showed that the three *tet*(X4)-carrying bacteria were sensitive to the tested antimicrobial agents, but displayed resistance to tigecycline (Table 1). Specifically, the *tet*(X4)-carrying *R. ornithinolytica* strain, designated 33, was resistant to ciprofloxacin. Additionally, one *tet*(X4)-carrying *E. coli* strain, labeled T8, was highly resistant to cefotaxime, whereas the other *E. coli* strain was sensitive to ciprofloxacin.

3.3. Genetic diversity of tet(X4)-positive strains

We analyzed the genetic diversity of the three *tet*(X4)-positive strains and compared them to those from the National Center for Biotechnology Information (NCBI) database. Whole-genome sequencing revealed that the two *E. coli* strains identified in our study were ST195 and ST5420. The *tet*(X4)-positive *R. ornithinolytica* strain carried 11 AMR genes, including the rifampin-resistance gene *arr-3*, the aminoglycoside-resistance genes [*aac(6′)-Ib-cr, aadA16* and *dfrA27*], the beta-lactam-resistance gene

Table 1
Antimicrobial susceptibility profiles of three *tet*(X4)-carrying bacteria.

ID	MIC(μg/mL)														
	IMP	CAV	MEM	ETP	FEP	CMZ	PB	CAZ	TGC	CTX	CIP	TZP	AK	SCF	ATM
33	≤1	≤8/4	≤1	≤2	≤4	≤2	≤0.5	≤2	≥4	≤4	4	≤8/4	≤4	≤8/4	≤4
9	≤1	≤8/4	≤1	≤2	≤4	≤2	≤0.5	≤2	≥4	≤4	≤1	≤8/4	≤4	≤8/4	≤4
T8	≤1	≤8/4	≤1	≤2	≤4	≤2	≤0.5	≤2	≥4	>128	≤1	≤8/4	≤4	≤8/4	≤4

Abbreviations: MIC, minimal inhibitory concentration; IMP, imipenem; MEM, meropenem; ETP, ertapenem; CMZ, cefmetazole; CAZ, ceftazidime; CTX, cefotaxime; TZP, piperacillin/tazobactam; SCF, cefoperazone/sulbactam; CAV, ceftazidime/avibactam; FEP, cefepime; PB, polymyxin B; TGC, tigecycline; CIP, ciprofloxacin; AK, amikacin; ATM, aztreonam.

(bla_{PLA1a}), the florfenicol-resistance gene (*floR*), the fosfomycin-resistance gene (*fos*A), the quinolone-resistance gene (*qnr*B6), the sulfonamide-resistance gene (*sulI*), and the tetracycline-resistance genes [*tet*(A) and *tet*(X4)]. The two *tet*(X4)-positive *E. coli* strains, named 9 and T8, carried 10 and 14 AMR genes, respectively. Both strains harbored the aminoglycoside-resistance genes *aadA2* and *dfrA12*, the beta-lactam-resistance gene bla_{TEM-1B}, the chloramphenicol-resistance gene *cmlA*, the florfenicol-resistance gene *floR*, the quinolone-resistance gene *qnr*S1, the sulfonamide-resistance gene *sulIII*, and the tetracycline resistance genes *tet*(A) and *tet*(X4). However, strain 9 carried *lnu*(G), while T8 carried *aac(3)-IId*, *aph(3')-Ia*, $bla_{CTX-M-14}$, *lnu(F)*, and *mef(B)*.

Two isolates, 9 and T8, were analyzed and compared with other *tet*(X4)-carrying *E. coli* strains obtained from the NCBI database. These strains originated from various sources, including pigs, cows, humans, chickens, birds, and captive animals (Fig. 1).

Isolate T8 exhibited >95% similarity to 13 pig-origin isolates collected from Jiangsu ($n = 9$), Guangxi ($n = 1$), and Sichuan ($n = 3$). Similarly, isolate 9 showed >95% similarity with two pig isolates from Henan and Jiangsu and one isolate from a urine sample from a patient in Zhejiang Province. AMR gene analysis revealed that the strains isolated from captive animals possessed fewer AMR genes than those from other sources.

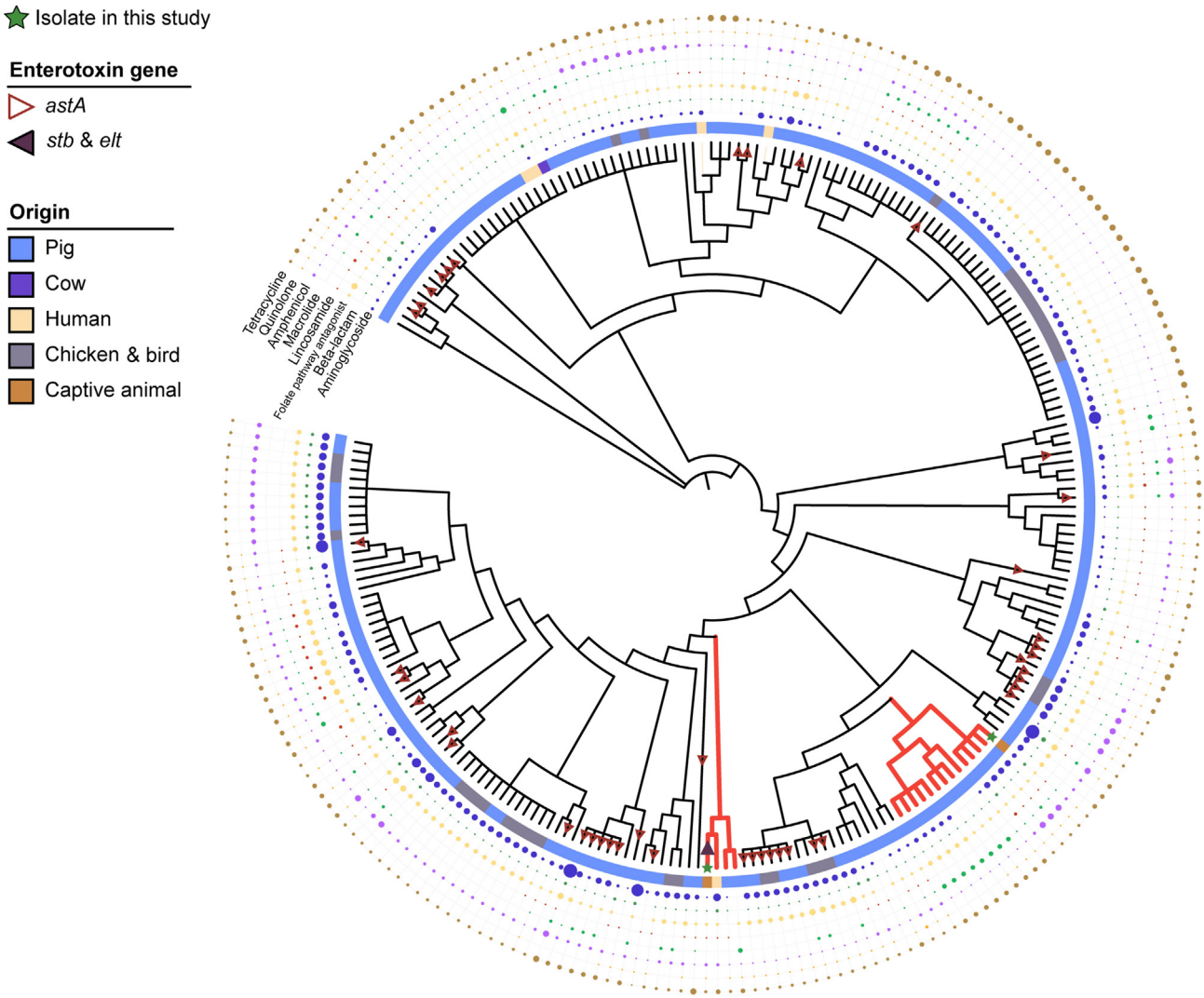

Fig. 1. Phylogenetic tree of *E. coli* strains from captive animals and various origins. In the graphical representation, circles of different colors denote the antimicrobial susceptibility profile of each strain. Furthermore, the size of the circle correlates with the number of resistance genes present in the strain.

3.4. Plasmid sequencing and genetic environment analysis

The *tet*(X4)-containing sequences were consistent with the partial sequences of plasmids from waterfowl manure (*E. coli* strain MY1-8 plasmid pSCMY3, accession No. CP122520.1) and pig slaughterhouses (*E. coli* strain JS1-EC05 plasmid pEC05-X4, accession No. MN436006.1). All genetic contexts of *tet*(X4) shared the conserved structure *estT-tet*(X4)-IS*CR2-floR* (Fig. 2), which is consistent with previous reports. The Inc types identified in the sequences were distinct from those in the *tet*(X4) contigs. Col(pHAD28) and IncX1 were detected in isolates 9 and T8. Isolate 9 also contained IncFIA/FIB/FII, IncHI1A/HI1B, and IncR.

3.5. Utilizing G. mellonella larvae as a model to investigate the virulence of E. coli isolate 9

An analysis of virulence genes revealed that isolate 9 possessed the heat-labile toxin gene *eltAB* and the heat-stable toxin gene *stb*, which are responsible for the production of their respective toxins. *G. mellonella* larvae were used to assess the virulence of this isolate, and the larvae were infected (Fig. 3). These results indicated that isolate 9 caused mortality in *G. mellonella* larvae at a concentration of 10^7 CFU/mL, but exhibited less virulence than the standard strain CVCC195, which produces heat-labile toxins.

IslandViewer 4 (http://www.pathogenomics.sfu.ca/islandviewer2/query.php) was utilized to identify the pathogenicity island containing the virulence genes *eltAB* and *stb* of *E. coli* isolate 9. The analysis revealed that these virulence genes were located within a single pathogenicity island. Additionally, the regions upstream and downstream of *eltAB* and *stb* contained mobile genetic elements, suggesting the potential for the transfer of these virulence genes (Fig. 4). However, the virulence genes *eltAB* and *stb* were interspersed with mobile genetic elements, including IS*1351*, IS*1414*, IS*2*, IS*EC18*, and IS*911*, which can migrate to different genetic structures.

4. Discussion

Tetracyclines are one of the most commonly used classes of antibiotics in veterinary medicine [16]. However, the use of tetracyclines in animals has led to the emergence of tigecycline-resistant bacteria [17]. Consequently, monitoring these resistant strains in animals is crucial for public health [18]. In our study, *tet*(X4) was found in *E. coli* strain ST195 isolated from a migratory bird in Zhejiang, and has previously been found in strains from pork and slaughterhouses in Shanghai, Jiangsu, Shanxi, and Guangdong, and in humans [19,20]. *E. coli* strain ST195 identified in this study is widely distributed in China and warrants significant attention [21]. In addition, a less commonly reported virulent *tet*(X4)-positive *E. coli* strain, ST5420, was identified. The emergence of virulent AMR bacteria poses a threat to the clinical treatment of animals. However, there is no definitive evidence to suggest that this strain initially carried virulence or resistance genes, making the control of these strains a continued challenge.

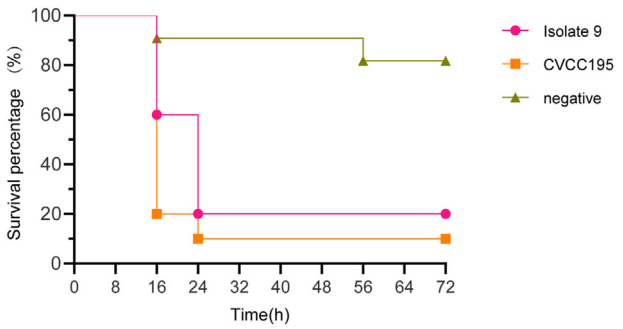

Fig. 3. Virulence potential of *E. coli* isolate 9 using a *G. mellonella* infection model. The horizontal axis of the graph represents the survival time of *G. mellonella* larvae and the vertical axis indicates the survival percentage (%).

The conserved genetic context of the strains identified in our study was consistent with that of previous studies [22,23]. However, some studies have reported that *tet*(X4)-carrying plasmids contain numerous mobile elements, such as IS*26*, IS*3000*, and IS*1*, along with other resistance genes, including *aadA22*, $bla_{\text{TEM-1B}}$, *sulI*, and *dfrA12* [24,25]. To the best of our knowledge, only a limited number of studies have explored structures containing *tet*(A) and *tet*(R) [26] isolated from animal food and environmental samples. Additionally, *tet*(X4) has been found in plasmids with Inc types, such as IncX1, IncFII, IncFIA/FIB, and IncHI1A/HI1B, which are consistent with the Inc types reported in our study [27].

In the *tet*(X4)-containing *E. coli* isolate 9, two enterotoxin genes were identified on the same pathogenicity island positioned adjacent to each other. The insertion sequences flanking the *stb* gene suggested that *stb*

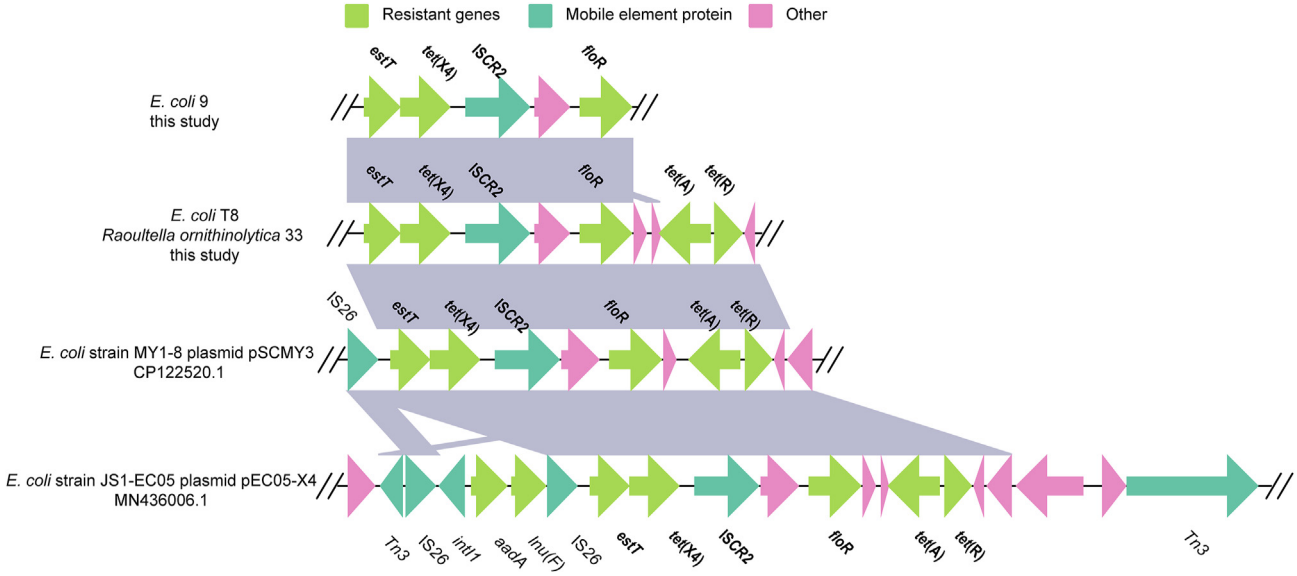

Fig. 2. Genetic contexts of *tet*(X4)-*carried strains* from captive animals. We performed linear comparisons of the genetic architecture surrounding *tet*(X4) from various sources. The *tet*(X4) regions in isolates 9 and T8 were compared with sequences from *E. coli* plasmids pSCMY3 (originating from waterfowl manure) and pEC05-X4 (derived from pigs).

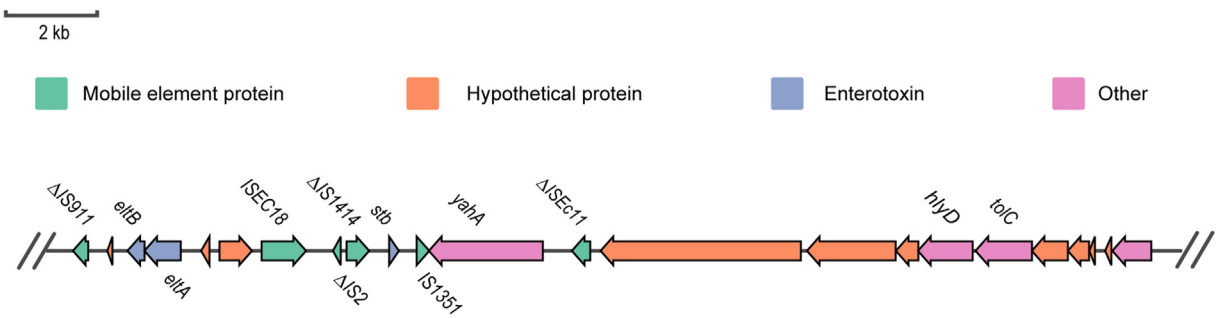

Fig. 4. Genetic environment of the pathogenicity island in *E. coli* isolate 9. The different colors are used to denote the distinct genomic features surrounding the enterotoxin genes.

may have been integrated into this pathogenicity island. *E. coli* isolate 9 exhibited lower virulence than the standard strain CVCC195, which produces only heat-labile toxins. However, the expression levels of these toxins may differ and the factors that influence their expression remain unknown. Translocations between virulence genes can result in the altered expression of virulence genes, but the specific mechanisms involved require detailed investigation [28].

The persistence of AMR in animals poses a challenge to public health and affects clinical therapy [29]. There are few differences in the feeding patterns of farm and zoo animals. In addition to similar activity spaces, captive wild animals in zoos have similar classifications and methods of medication as farm animals. Unlike food-producing animals, zoo animals do not adhere to standard medication practices, and the drug dosage used for captive wild animals is not necessarily lower than that used for farm animals. Research on AMR bacteria isolated from wild animals has increased in recent years [30], and the presence of virulent bacteria carrying AMR genes has been reported [31,32]. Furthermore, AMR bacteria in the environment can transmit plasmids and strains to wild and zoo animals, posing hidden health risks. Therefore, monitoring virulent AMR bacteria is crucial, and the mechanisms of interaction between virulence genes and AMR genes within bacteria warrant further in-depth investigation.

5. Conclusion

The *tet*(X4) gene was identified in *R. ornithinolytica* and *E. coli* strains isolated from captive wild animals. One *E. coli* strain carrying *tet*(X4) possessed virulence genes and exhibited virulence potential. This study reported *tet*(X4)-harboring *E. coli* and *R. ornithinolytica* in captive wildlife. The discovery of enterotoxigenic *E. coli* highlights the potential threat of virulence. Additionally, the genetic structure of *tet*(X4) was similar to that of human and animal isolates. This study broadens the known host range of *tet*(X4) and provides new insights for monitoring drug resistance in wildlife. These findings underscore the need for interdisciplinary approaches to address these issues.

Funding

This work was supported by the National Key Research and Development Program of China [grant number: 2022YFD1800403]; National Natural Science Foundation of China [grant number: 82072341].

Authors' contributions

HS and RZ: Conceptualization. LL and PX: Data curation; and Writing - original draft. LL, ZY, YZ, and CW: Formal analysis. HS and RZ: Writing - review & editing; GC and RZ: Funding acquisition.

Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author upon reasonable request.

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.

Acknowledgments

We would like to express our sincere gratitude the staff at the Hangzhou Safari Park for their invaluable contributions to the successful completion of this study.

References

[1] M. Ferri, E. Ranucci, P. Romagnoli, V. Giaccone, Antimicrobial resistance: a global emerging threat to public health systems, Crit. Rev. Food Sci. Nutr. 57 (13) (2017) 2857–2876, https://doi.org/10.1080/10408398.2015.1077192.
[2] Y. Wang, G.-B. Tian, R. Zhang, Y.-B. Shen, J.M. Tyrrell, X. Huang, et al., Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1* -positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study, Lancet Infect. Dis. 17 (4) (2017) 390–399, https://doi.org/10.1016/S1473-3099(16)30527-8.
[3] R.F. Potter, A.W. D'Souza, G. Dantas, R.L. Guest, E.A. Court, J.L. Waldon, et al., The rapid spread of carbapenem-resistant Enterobacteriaceae, Drug Resist. Updates 29 (2016) 30–46, https://doi.org/10.1016/j.drup.2016.09.002.
[4] J.P. Rueda Furlan, D. Fuentes-Castillo, E. Guedes Stehling, N. Lincopan, F.P. Sellera, The emergence of *tet*(X) variants highlight challenges for the global genomic surveillance of tigecycline resistance, Lancet Microbe (2023), https://doi.org/10.1016/S2666-5247(23)00249-5. S2666-5247(23) (2023) 00249–5.
[5] L.-X. Fang, C. Chen, C.-Y. Cui, X.-P. Li, Y. Zhang, X.-P. Liao, et al., Emerging high-level tigecycline resistance: novel tetracycline destructases spread via the mobile Tet(X), Bioessays 42 (8) (2020) e2000014, https://doi.org/10.1002/bies.202000014.
[6] T. He, R. Wang, D.-J. Liu, T.R. Walsh, R. Zhang, Y. Lv, et al., Emergence of plasmid-mediated high-level tigecycline resistance genes in animals and humans, Nat. Microbiol. 4 (9) (2019) 1450–1456, https://doi.org/10.1038/s41564-019-0445-2.
[7] J. Sun, C. Chen, C.-Y. Cui, Y. Zhang, X. Liu, Z.-H. Cui, et al., Plasmid-encoded *tet*(X) genes that confer high-level tigecycline resistance in *Escherichia coli*, Nat. Microbiol. 4 (9) (2019) 1457–1464, https://doi.org/10.1038/s41564-019-0496-4.
[8] X. Lu, L. Zhang, K. Peng, Q. Wang, R. Liu, Z. Wang, et al., Characterisation of a novel tigecycline resistance gene *tet*(X22) and its coexistence with bla_{NDM-1} in a *Pseudomonas caeni* isolate, Int. J. Antimicrob. Agents 62 (5) (2023) 106961, https://doi.org/10.1016/j.ijantimicag.2023.106961.
[9] M. Mohsin, B. Hassan, W.M.B.S. Martins, R. Li, S. Abdullah, K. Sands, et al., Emergence of plasmid-mediated tigecycline resistance *tet*(X4) gene in *Escherichia coli* isolated from poultry, food and the environment in South Asia, Sci. Total Environ. 787 (2021) 147613, https://doi.org/10.1016/j.scitotenv.2021.147613.
[10] R. Li, K. Peng, Y. Li, Y. Liu, Z. Wang, Exploring *tet*(X)-bearing tigecycline-resistant bacteria of swine farming environments, Sci. Total Environ. 733 (2020) 139306, https://doi.org/10.1016/j.scitotenv.2020.139306.
[11] C. Chen, C.-Y. Cui, Y. Zhang, Q. He, X.-T. Wu, G. Li, et al., Emergence of mobile tigecycline resistance mechanism in *Escherichia coli* strains from migratory birds in China, Emerg. Microb. Infect. 8 (1) (2019) 1219–1222, https://doi.org/10.1080/22221751.2019.1653795.
[12] N.K. Abd El-Aziz, Y.H. Tartor, R.M.A. Gharieb, A.M. Erfan, E. Khalifa, M.A. Said, et al., Extensive drug-resistant *Salmonella enterica* isolated from poultry and humans: prevalence and molecular determinants behind the co-resistance to ciprofloxacin and tigecycline, Front. Microbiol. 12 (2021) 738784, https://doi.org/10.3389/fmicb.2021.738784.
[13] L. Li, Y. Xiao, C. Wang, R.H. Olsen, H. Meng, L. Shi, Exploring the resistome, virulome, mobilome and microbiome along pork production chain using metagenomics, Int. J. Food Microbiol. 371 (2022) 109674, https://doi.org/10.1016/j.ijfoodmicro.2022.109674.

[14] J.P.R. Furlan, E.G. Stehling, Draft genome sequence of a multidrug-resistant *Escherichia coli* ST189 carrying several acquired antimicrobial resistance genes obtained from Brazilian soil, J. Glob. Antimicrob. Resist. 17 (2019) 321–322, https://doi.org/10.1016/j.jgar.2019.05.018.
[15] E. Abdeen, W. Elmonir, I.I.A. Suelam, W.S. Mousa, Antibiogram and genetic diversity of *Salmonella enterica* with zoonotic potential isolated from morbid native chickens and pigeons in Egypt, J. Appl. Microbiol. 124 (2018) 1265–1273, https://doi.org/10.1111/jam.13697.
[16] Y. Xu, C. Guo, J. Lv, S. Hou, Y. Luo, Y. Zhang, et al., Spatiotemporal profile of tetracycline and sulfonamide and their resistance on a catchment scale, Environ. Pollut. 241 (2018) 1098–1105, https://doi.org/10.1016/j.envpol.2018.06.050.
[17] D. Wu, S. Dai, H. Feng, S.H.P.P. Karunaratne, M. Yang, Y. Zhang, Persistence and potential risks of tetracyclines and their transformation products in two typical different animal manure composting treatments, Environ. Poll. 341 (2024) 122904, https://doi.org/10.1016/j.envpol.2023.122904.
[18] L. Ma, H. Zhao, L.B. Wu, Z. Cheng, C. Liu, Impact of the microbiome on human, animal, and environmental health from a One Health perspective, Sci. One Health 2 (2023) 100037, https://doi.org/10.1016/j.soh.2023.100037.
[19] R. Li, X. Lu, A. Munir, S. Abdullah, Y. Liu, X. Xiao, et al., Widespread prevalence and molecular epidemiology of *tet*(X4) and *mcr-1* harboring *Escherichia coli* isolated from chickens in Pakistan, Sci. Total Environ. 806 (2022) 150689, https://doi.org/10.1016/j.scitotenv.2021.150689.
[20] Y. Li, X. Sun, X. Xiao, Z. Wang, R. Li, Global distribution and genomic characteristics of *tet*(X)-positive *Escherichia coli* among humans, animals, and the environment, Sci. Total Environ. 887 (2023) 164148, https://doi.org/10.1016/j.scitotenv.2023.164148.
[21] C. Yue, X. Gao, L. Lu, Y.-Y. Liu, L.-C. Lv, X. He, et al., First detection of *tet*(X4)-positive *Enterobacterales* in retail vegetables and clonal spread of *Escherichia coli* ST195 producing Tet(X4) in animals, foods, and humans across China and Thailand, Int. J. Food Microbiol. 391–393 (2023) 110145, https://doi.org/10.1016/j.ijfoodmicro.2023.110145.
[22] W. Zhang, X. Lu, S. Chen, Y. Liu, D. Peng, Z. Wang, et al., Molecular epidemiology and population genomics of *tet*(X4), bla_{NDM} or *mcr-1* positive *Escherichia coli* from migratory birds in southeast coast of China, Ecotoxicol. Environ. Saf. 244 (2022) 114032, https://doi.org/10.1016/j.ecoenv.2022.114032.
[23] N. Dong, Y. Zeng, C. Cai, C. Sun, J. Lu, C. Liu, et al., Prevalence, transmission, and molecular epidemiology of *tet*(X)-positive bacteria among humans, animals, and environmental niches in China: an epidemiological, and genomic-based study, Sci. Total Environ. 818 (2022) 151767, https://doi.org/10.1016/j.scitotenv.2021.151767.
[24] M. Li, H. Zhang, W. Zhang, Y. Cao, B. Sun, Q. Jiang, et al., One global disseminated 193 kb high-risk hybrid plasmid harboring *tet*(X4), *mcr* or bla_{NDM} threatening public health, Sci. Total Environ. 876 (2023) 162807, https://doi.org/10.1016/j.scitotenv.2023.162807.
[25] Y. Li, P. Wang, X. Xiao, R. Li, Z. Wang, Genomic characterization of tigecycline-resistant *tet*(X4)-positive *E. coli* in slaughterhouses, Vet. Microbiol. 276 (2023) 109606, https://doi.org/10.1016/j.vetmic.2022.109606.
[26] J. Feng, M. Su, K. Li, J. Ma, R. Li, L. Bai, et al., Extensive spread of *tet*(X4) in multidrug-resistant *Escherichia coli* of animal origin in western China, Vet. Microbiol. 269 (2022) 109420, https://doi.org/10.1016/j.vetmic.2022.109420.
[27] C.-Y. Cui, X.-J. Li, C. Chen, X.-T. Wu, Q. He, Q.-L. Jia, et al., Comprehensive analysis of plasmid-mediated *tet*(X4)-positive *Escherichia coli* isolates from clinical settings revealed a high correlation with animals and environments-derived strains, Sci. Total Environ. 806 (2022) 150687, https://doi.org/10.1016/j.scitotenv.2021.150687.
[28] A. Knöppel, M. Knopp, L.M. Albrecht, E. Lundin, U. Lustig, J. Näsvall, et al., Genetic adaptation to growth under laboratory conditions in *Escherichia coli* and *Salmonella enterica*, Front. Microbiol. 9 (2018) 756, https://doi.org/10.3389/fmicb.2018.00756.
[29] S.P. Djordjevic, V.M. Jarocki, T. Seemann, M.L. Cummins, A.E. Watt, B. Drigo, et al., Genomic surveillance for antimicrobial resistance — a One Health perspective, Nat. Rev. Genet. (2023) 1–16, https://doi.org/10.1038/s41576-023-00649-y.
[30] A. Di Francesco, D. Salvatore, F. Bertelloni, V.V. Ebani, Tetracycline resistance genes in wild birds from a wildlife recovery centre in central Italy, Animals 13 (2022) 76, https://doi.org/10.3390/ani13010076.
[31] J. Hu, J. Li, X. Huang, J. Xia, M. Cui, Y. Huang, et al., Genomic traits of multidrug resistant enterotoxigenic *Escherichia coli* isolates from diarrheic pigs, Front. Microbiol. 14 (2023) 1244026, https://doi.org/10.3389/fmicb.2023.1244026.
[32] Y. Xu, X. Bai, Y. Jin, B. Hu, H. Wang, H. Sun, et al., High prevalence of virulence genes in specific genotypes of atypical Enteropathogenic *Escherichia coli*, Front. Cell. Infect. Microbiol. 7 (2017) 109, https://doi.org/10.3389/fcimb.2017.00109.