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Prevalence and antimicrobial susceptibility of CTX-M-type-producing *Escherichia coli* from a wildlife zoo in China

Ziyue Zeng[1,2] | Jie Yang[1,2] | Jinrong Gu[1,2] | Zhihong Liu[1,2] | Jufang Hu[1,2] | Xiangyong Li[3] | Xiaojun Chen[1,2] | Zhiliang Sun[1,2] | Jiyun Li[1,2]

[1] College of Veterinary Medicine, Hunan Agricultural University, Changsha, Hunan, China

[2] Hunan Engineering Technology Research Center of Veterinary Drugs, Hunan Agricultural University, Changsha, Hunan, China

[3] Changsha Ecological Zoo, Changsha, Hunan, China

Correspondence
Zhiliang Sun and Jiyun Li, College of Veterinary Medicine, Hunan Agricultural University, Changsha, Hunan, China.
Email: sunzhiliang1965@aliyun.com; lijiyuny@foxmail.com

Ziyue Zeng and Jie Yang have contributed equally to this work.

Abstract

Background: Wildlife zoos provide the opportunity for children and adults to interact with animals, However, it's unknown that the risk of contact with animals, which carried zoonotic pathogens and antimicrobial resistant bacteria.

Objectives: This study aimed to investigate the prevalence and antimicrobial susceptibility of extended-spectrum β-lactamases *Escherichia coli* (ESBLs-EC) from a wildlife zoo in China.

Methods: A total of 93 wildlife faecal samples were collected from a wildlife zoo. Agar dilution method was used to determine the resistant phenotype. Whole genomes sequencing and bioinformatic analysis were employed to evaluate the molecular typing and genetic relationships of ESBLs-EC.

Results: A total of 23 CTX-M-positive ESBLs-EC were isolated from swan (n = 14), squirrel monkey (n = 5), black hat hanging monkey (n = 2), gibbon monkey (n = 1) and phoenicopteridae (n = 1) respectively. All ESBLs-EC strains were resistant to cefotaxime, tetracycline, ciprofloxacin and trimethoprim-sulfamethoxazole, but susceptible to colistin, tigecycline, meropenem and amikacin. By screening whole genome sequences, ESBLs-EC strains main carried $bla_{\text{CTX-M-55}}$ (34.8%, 8/23) and $bla_{\text{CTX-M-14}}$ (26.0%, 6/23), following by $bla_{\text{CTX-M-27}}$ (21.7%, 5/23), $bla_{\text{CTX-M-15}}$ (13.0%, 3/23) and $bla_{\text{CTX-M-121}}$ (4.3%, 1/23). ESBLs-EC strains mainly belonged to phylogroup A (60.9%, 14/23), and ST48, ST746 and ST616 (3 strains respectively, 13.0%) were major ST types. Core genome-based single nucleotide polymorphism (SNP) analysis suggested that strains from the swan, over the phylogenetic tree, have a closer genetic relationship with strains from other animals (black hat hanging monkey, gibbon monkey, phoenicopteridae and squirrel monkey).

Conclusions: CTX-M type ESBLs-EC can transmit between animals in wildlife zoos, which may be a risk of spread to animal keepers, veterinarians and visitors when

contact with animals. Our study provides that the importance of hygiene measures to minimise the risk of transmission of ESBLs-EC to visitors in wildlife zoos.

KEYWORDS
CTX-M, *E. coli*, ESBLs, wildlife, zoo

1 | INTRODUCTION

Antimicrobial agents are commonly used for the treatment of bacterial infections and prevent animal diseases, which are widely used in healthcare, agricultural farming and zoo animal (Conrad et al., 2018). China is one of the worldwide largest producers and consumers of antimicrobials, with a total consumption of 162,000 tons of antimicrobials in 2013, more than half of which were veterinary antimicrobials (Qian-Qian Zhang et al., 2015). The irrational use of antimicrobials has led to increased antimicrobial resistance and potential threats to the health of humans and animals (Nkansa-Gyamfi et al., 2019).

The β-lactams, including penicillins, carbapenems, cephalosporins and mono-bactams, are essential to the practice of medicine in the 21st centry (Bush & Bradford, 2016). Although different parts of different resistance, the threat remains that any new resistance mechanism may spread rapidly to other areas (Bush & Bradford, 2020). With the clinical use of these β-lactams, the emergence of extended-spectrum β-lactamases (ESBLs) that hydrolyze third generations of cephalosporins, including cefotaxime, ceftriaxone and ceftazidime in the early 1980s (Bauernfeind et al., 1990; Guenther et al., 2010). The emergence of ESBLs-producing bacteria has greatly limited the available drugs for clinical use, which has threatened human health. The number of β-lactamases increased dramatically over time, and this increase was almost entirely attributable to class A and D β-lactamases (Bush, 2010). The main ESBLs are TEM, SHV, CTX-M, VEB and GES. Among them, the largest number of variants described in the past few years is the CTX-M family (Abraham et al., 2018; JH, 2009). In 1989, CTX-M which is different from TEM and SHV, was first appeared (Woerther et al., 2013). Currently, CTX-M type ESBL has become the most common ESBLs worldwide, where the most common host is *Escherichia coli* and *Klebsiella pneumoniae* (Stercz et al., 2021).

In recent years, ESBLs *Escherichia coli* (ESBLs-EC) has been found and reported in a large number of people, animals and the environment around the world, and it suggested that ESBLs-EC can be transmitted among people, animals and environment (Doi et al., 2017). Zoos are reservoirs of drug-resistant bacteria because of their numerous animal species and dense population (Furlan et al., 2020). Humans visiting and contact with animals in wildlife zoos will increase the risk of acquiring resistance bacteria, so the distribution of resistance bacterial in zoos is very important to the local public health security. Herein, our study aims to investigate the prevalence and antimicrobial susceptibility of ESBLs-EC in zoo wildlife and further genomic epidemiology of ESBLs-EC.

2 | MATERIALS AND METHODS

2.1 | Sample collection, isolation and identification

In 2020, we collected 93 animal faecal samples including swan, gibbon monkey, squirrel monkey, phoenicopteridae, black hat hanging monkey, peacock, alpaca and guinea fowl from a wildlife zoo, Hunan, China. Sterile cotton swabs were used to collect all faecal samples and maintained in sterile LB broth (Solarbio, China). All samples were inoculated onto MacConkey agar (Solarbio, China) containing 2 mg/L cefotaxime and 30 mg/L vancomycin. After incubated overnight at 37°C, single cefotaxime-resistant clones were picked into 0.5 mL LB broth (Solarbio, China) at 37°C for 18 h. Bacterial species were confirmed by using 16S rRNA gene sequencing as previously described (Li et al., 2019). The polymerase chain reaction (PCR) products were subsequently confirmed by Sanger sequencing and BLAST analysis. CTX-M genes were detected by universal primer (CTX-all-F: 5′-TTTGCGATGTGCAGTACCAGTAA-3′ and CTX-all-R: 5′-CGATATCGTTGGTGGTGCCATA-3′) as previously described (Zheng et al., 2015).

2.2 | Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of 11 antimicrobial agents (amikacin, cefotaxime, ceftazidime, ciprofloxacin, colistin, florfenicol, gentamicin, meropenem, tetracycline, tigecycline and trimethoprim-sulfamethoxazole) were assessed by agar dilution method according to the American Clinical and Laboratory Standards (CLSI) (CLSI, 2020). *E. coli* ATCC 25922 was used as a quality control strain as recommended.

2.3 | Genomes sequencing and bioinformatic analysis

Whole genomic DNA was extracted from fresh ESBLs-EC strains using TIANamp Bacteria DNA Kit (Tiangen Biotech Company). The DNA libraries were constructed using a KAPA Hyper Prep Kit (Roche, Basel, Switzerland) and 150 bp paired-end reads were sequenced using an Illumina HISEQ XTen platform (Illumina) (Annoroad, Beijing, China). Reads were de novo assembled using Spades 3.14 (Bankevich et al., 2012), and fasta files uploaded to the Centre for Genomic Epidemiology (CGE) for screening for antimicrobial resistance genes, plasmid

TABLE 1 The minimum inhibitory concentration of 23 ESBLs-EC strains

				Antimicrobial agents (mg/L)[a]										
Strains	Origin	CTX-M type	MLST	CS	FFC	GEN	CTX	MEM	STX	AMK	CIP	TET	CAZ	TGC
20p07tr	Gibbon monkey	$bla_{CTX-M-55}$	ST48	1	8	<0.125	**32**	0.03	<0.25	1	**64**	**128**	4	0.06
20p11tr	Phoenicopteridae	$bla_{CTX-M-121}$	ST12219	0.5	8	<0.125	**8**	0.015	**32**	1	**32**	**64**	0.5	0.06
20p21tr	Squirrel monkey	$bla_{CTX-M-55}$	ST4162	0.125	<0.25	**64**	**32**	0.015	2	2	0.06	**128**	4	0.5
20p22tr	Squirrel monkey	$bla_{CTX-M-27}$	ST10	0.125	4	0.25	**8**	0.03	<0.25	2	0.06	**128**	1	0.5
20p28tr	Squirrel monkey	$bla_{CTX-M-121}$	ST12218	0.5	2	0.25	**32**	0.015	**32**	1	**2**	**32**	4	0.25
20p34tr	Squirrel monkey	$bla_{CTX-M-55}$	ST746	<0.062	<0.25	0.5	**16**	<0.004	**32**	1	1	**32**	2	0.25
20p35tr	Squirrel monkey	$bla_{CTX-M-27}$	ST6775	0.125	4	<0.125	**32**	0.03	0.25	1	0.015	0.5	4	0.25
20p56tr	Black hat hanging monkey	$bla_{CTX-M-121}$	ST744	0.5	4	0.5	**16**	0.03	**32**	1	**128**	**64**	2	0.06
20p62tw	Black hat hanging monkey	$bla_{CTX-M-27}$	ST2169	0.5	8	0.25	**4**	0.03	<0.25	2	**2**	**64**	0.5	0.03
20p69tr	Swan	$bla_{CTX-M-55}$	ST6018	1	**>128**	<0.125	**4**	0.03	**32**	1	**2**	**64**	1	0.25
20p72tr	Swan	$bla_{CTX-M-55}$	ST6050	0.125	**32**	**16**	**32**	0.015	**64**	1	**32**	**128**	**8**	2
20p74tr	Swan	$bla_{CTX-M-14}$	ST10	0.5	8	0.25	**8**	0.015	**32**	4	**32**	**64**	0.5	0.5
20P78tr	Swan	$bla_{CTX-M-14}$	ST746	<0.062	8	<0.125	**16**	0.015	**32**	1	**4**	**128**	0.5	0.5
20p79tr	Swan	$bla_{CTX-M-121}$	ST162	1	**>128**	4	**16**	0.03	**32**	2	**128**	**64**	0.5	1
20p80tr	Swan	$bla_{CTX-M-27}$	ST165	0.5	4	0.25	**8**	0.03	<0.25	1	**64**	**64**	2	0.25
20p81tr	Swan	$bla_{CTX-M-15}$	ST616	0.5	<0.25	<0.125	**4**	0.015	**32**	1	**2**	**32**	0.5	0.25
20p83tr	Swan	$bla_{CTX-M-55}$	ST6050	0.5	4	0.25	**8**	0.03	**32**	1	**2**	**64**	**8**	2
20p84tr	Swan	$bla_{CTX-M-55}$	ST48	0.125	**>128**	<0.125	**32**	0.03	**32**	2	**128**	**128**	4	0.25
20p85tr	Swan	$bla_{CTX-M-55}$	ST48	0.5	**>128**	<0.125	**32**	0.03	**32**	1	**128**	**64**	4	0.25
20p86tr	Swan	$bla_{CTX-M-15}$	ST616	0.125	4	0.25	**16**	0.015	**32**	4	**2**	**32**	0.5	0.5
20p87tr	Swan	$bla_{CTX-M-15}$	ST616	0.5	4	<0.125	**4**	0.015	**32**	1	**2**	**128**	0.5	0.125
20p88tr	Swan	$bla_{CTX-M-14}$	ST746	0.125	4	0.25	**8**	0.03	0.5	2	**4**	**64**	1	0.25
20p89tr	Swan	$bla_{CTX-M-27}$	ST1202	0.5	**>128**	**32**	**64**	0.03	**32**	2	**64**	**128**	**8**	0.25

[a]Abbreviations and resistance breakpoints: CS: colistin (R > 2 mg/L), FFC: florfenicol (R > 16 mg/L), GEN: gentamicin (R > 4 mg/L), CTX: cefotaxime (R > 2 mg/L), MEM: meropenem (R > 8 mg/L), SXT: trimethoprim-sulfamethoxazole (R > 4 mg/L), AMK: amikacin (R > 16 mg/L), CIP: ciprofloxacin (R > 1 mg/L), TET: tetracycline (R ≥ 16mg/L), CAZ: ceftazidime (R > 4 mg/L), TGC: tigecycline (R > 4 mg/L). Bold formatting indicates resistance to the respective antimicrobial agents.

replicon and multilocus sequence typing (MLST) (http://www.genomicepidemiology.org/services/). *E. coli* phylogenetic groups were determined by ClermonTyping scheme (Beghain et al., 2018). All draft genomes were used for constructing a phylogenetic tree using Parsnp (Todd J Treangen et al., 2014), and the result was finally visualised using the online tool iTOL (Letunic & Bork, 2019).

3 | RESULTS

3.1 | Isolation of ESBLs-EC

A total of 46 cefotaxime-resistant strains were isolated from 93 wildlife animal faecal samples, of 23 strains, from swan ($n = 14$), squirrel monkey ($n = 5$), black hat hanging monkey ($n = 2$), gibbon monkey ($n = 1$) and phoenicopteridae ($n = 1$), were identified as *E. coli* by 16S rRNA gene sequencing. All 23 ESBLs-EC strains carried bla_{CTX-M} gene.

3.2 | Antimicrobial susceptibility profiles

All ESBLs-EC have resistance to cefotaxime (100.0%), tetracycline (95.7%), ciprofloxacin (82.6%) and trimethoprim-sulfamethoxazole (69.6%) (Tables 1 and 2). Mild resistance was observed against florfenicol (26.0%), gentamicin (13.1%) and ceftazidime (13.0%). No strains showed resistance to colistin, tigecycline, meropenem and amikacin.

3.3 | Molecular typing and antimicrobial resistance genes

Phylogenetic analysis showed that these ESBLs-EC strains from zoo animals samples have belonged to phylogroup A (60.9%, 14/23), phylogroup B1 (34.8%, 8/23) and phylogroup F (4.3%, 1/23). The first two phylogroups (A/B1) mostly belonged to commensal *E. coli*, which mainly harboured enterobactin (*fes*, *entB*, *entC*, *entE*, *fepABCD*) responsible for

TABLE 2 Antimicrobial susceptibility profiles of bla_{CTX-M}-positive *E. coli* strains ($n = 23$)

Antimicrobial	MIC_{50} (mg/L)	MIC90 (mg/L)	Range (mg/L)	R%	I%	S%
CS	0.5	1	<0.062–1	0.0%	0.0%	100.0%
FFC	4	>128	<0.25-> 128	26.0%	0.0%	74.0%
GEN	0.25	32	<0.125–64	13.1%	4.3%	82.6%
CTX	16	32	4–64	100.0%	0.0%	0.0%
MEM	0.015	0.03	<0.004–0.08	0.0%	0.0%	100.0%
SXT	32	32	<0.25–64	69.6%	0.0%	30.4%
AMK	1	2	1–4	0.0%	0.0%	100.0%
CIP	2	128	0.015–128	82.6%	4.3%	13.1%
TET	64	128	0.5–128	95.7%	0.0%	4.3%
CAZ	2	8	0.5–8	13.0%	39.1%	47.9%
TGC	0.25	1	0.06–2	0.0%	8.7%	91.3%

CS: colistin, FFC: florfenicol, GEN: gentamicin, CTX: cefotaxime, MEM: meropenem, SXT: trimethoprim-sulfamethoxazole, AMK: amikacin, CIP: ciprofloxacin, TET: tetracycline, CAZ: ceftazidime, TGC: tigecycline; R: resistance, I: intermediary, S: susceptible.

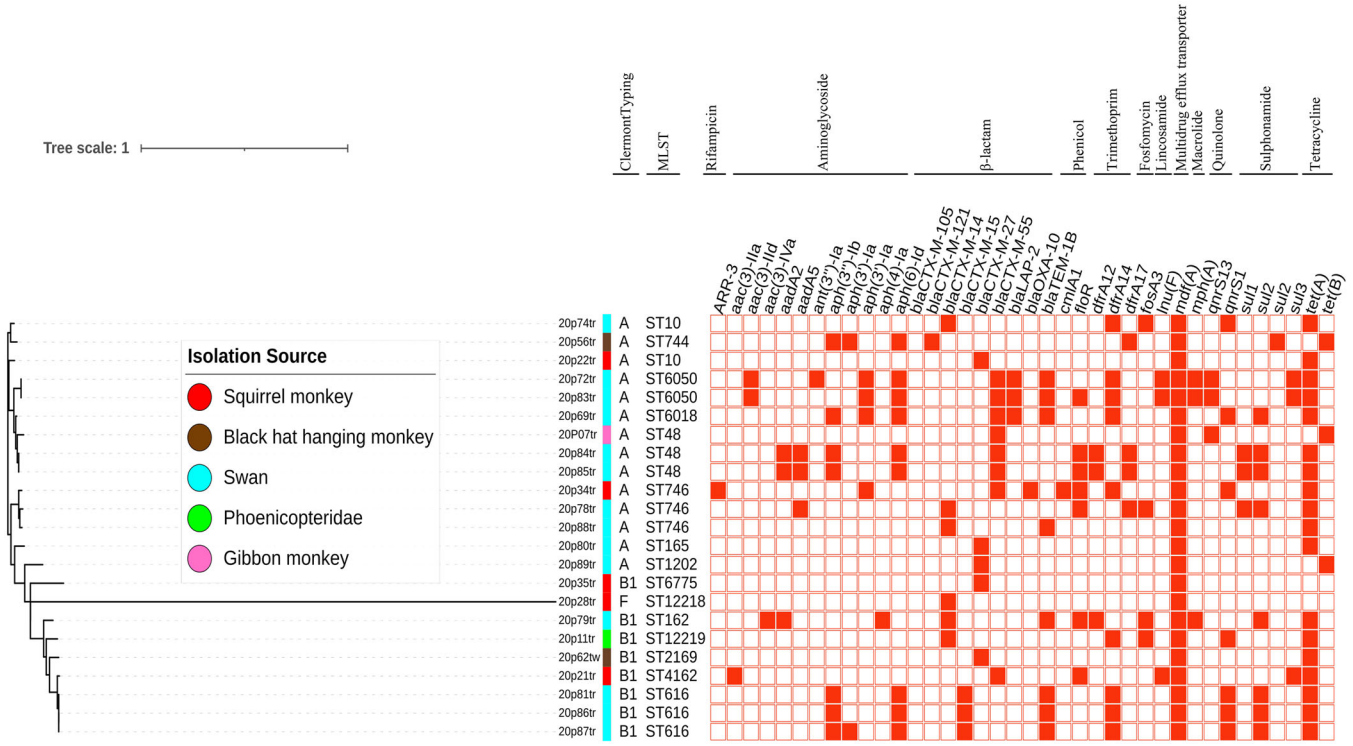

FIGURE 1 Phylogenetic tree and distribution of ESBLs-EC multilocus sequencing types, Clermont typing and antimicrobial resistance genes from zoo wildlife. The sources of the strains are differentiated by colour. Antimicrobial resistance genes are denoted by filled squares for presence and empty squares for absence

iron uptake (Figure S1), and few strains relating to causing human infections (Sauget et al., 2014). However, hypervirulent phylogroup F *E. coli*, which was more frequently associated with human extraintestinal pathogenic *E. coli* pathotypes, exhibiting zoonotic potential which could cause multiple diseases in animal models of avian colibacillosis and human infections (Zhuge et al., 2021), was isolated from a squirrel monkey, carrying Type III secretion system (*escD*, *escF* etc.) contributing to infect the human gut (Slater et al., 2018). Fifteen different STs (Figure 1) were detected among the 23 ESBLs-EC strains. ST48, ST746 and ST616 (3 strains respectively, 13.0%) were major ST types, followed by ST10 (2 strains; 8.7%) and ST6050 (2 strains; 8.7%). By submitting to EnteroBase (https://enterobase.warwick.ac.uk/species/index/ecoli), two novel STs (ST12218 and ST12219) were determined in squirrel monkey and phoenicopteridae. All strains carried one of β-lactam resistance genes including $bla_{CTX-M-55}$ (34.8%, 8/23), $bla_{CTX-M-14}$ (26.0%, 6/23), $bla_{CTX-M-15}$ (13.0%, 3/23) and $bla_{CTX-M-27}$

(21.7%, 5/23) $bla_{CTX\text{-}M\text{-}121}$ (4.3%, 1/23) (Figure 1). The most abundant drug resistance gene in all strains was multidrug efflux transporter gene *mdf*(A) (100.0%, 23/23) and tetracycline resistance gene *tet*(A) (78.3%, 18/23), following by the trimethoprim resistance gene *drfA14* (39.1%, 9/23), the aminoglycoside resistance gene *ant*(3'')-*like* (43.5%, 10/23), *aph*(6)-*Id* (39.1%, 9/23) and *aph*(4)-*Ia* (4.3%, 1/23). Notably, rifampicin resistance gene *arr-3* (4.3%, 1/23), *β*-lactam resistance gene $bla_{OXA\text{-}10}$ (4.3%, 1/23), phenicol resistance gene *cmlA1* (4.3%, 1/23) and sulphonamide resistance gene *sul2* (4.3%, 1/23) were also detected in ESBLs-EC strains. By plasmid replicon analysis, Incp0111 (7/23, 30.4%) and ColRNAI (6/23, 26.1%) were the most common plasmid replicon types, followed by IncFIB (5/23, 21.8%), IncHI1B (4/23, 17.4%), Col440I (3/23, 13.0%) and IncFIB(K) (3/23,13.0%).

3.4 | Phylogenetic tree of ESBLs-EC

The core genome-based single nucleotide polymorphism (SNP) analysis showed that all ESBLs-EC strains share a total of 173,734 SNPs site and the SNPs between the two strains were from 0 to 122,296 bp. Furthermore, the core-genome SNP phylogenetic tree suggested that the ESBLs-EC strains from swan have three clone group (sample number: 20p72tr & 20p83tr, 20p84tr & 20p85tr, 20p81tr & 20p86tr & 20p87tr) (Figure 1). In addition, strains from swan, distributed all over the phylogenetic tree (Figure 1), have a closer genetic relationship with strains from the rest four kinds of animals (black hat hanging monkey, gibbon monkey, phoenicopteridae and squirrel monkey) (Figure 1).

4 | DISCUSSION

In our study, all ESBLs-EC strains presented resistance to a third-generation cephalosporin (cefotaxime), also exhibited resistance to other important antimicrobials including ciprofloxacin, trimethoprim-sulfamethoxazole and tetracycline. We found that oxytetracycline was used in the treatment of swans' diarrhoea 6 months ago, which shows that antimicrobial treatment may be a risk factor for faeces shedding of ESBL producers in zoos (Shnaiderman-Torban et al., 2019). When antimicrobial drugs are used in large quantities, most of these will enter the ecosystem as metabolites, affecting the normal activities of plants, animals and microorganisms, and ultimately posing a threat to public health and human health, so it should reduce the use of antimicrobials when treating animals (Kluytmans et al., 2013).

All 23 ESBLs-EC strains contained multiple antimicrobial resistance genes in this study. The $bla_{CTX\text{-}M\text{-}55}$ and $bla_{CTX\text{-}M\text{-}15}$ genes were found among the 11 strains. Interestingly, $bla_{CTX\text{-}M\text{-}55}$ is a variant of $bla_{CTX\text{-}M\text{-}15}$ by differing in one nucleotide at position 239 resulting in A77V being identified (Hayashi et al., 2018). The $bla_{CTX\text{-}M\text{-}55}$ was the majority of CTX-M gene in ESBLs-EC, followed by $bla_{CTX\text{-}M\text{-}14}$. However, $bla_{CTX\text{-}M\text{-}14}$ was among animal ESBL producers in China and other Asian countries (Zhang et al., 2014), which suggested that $bla_{CTX\text{-}M\text{-}55}$ may turn into the main CTX-M gene in Chinese zoo wildlife.

ESBLs-EC, from swans and squirrel monkey, gibbon monkey, have the same ST (ST746, ST10 and ST48). Further, ESBLs-EC from swans showed a closer genetic relationship with strains from other animals (black hat hanging monkey, gibbon monkey, phoenicopteridae and squirrel monkey). These suggested that the transmission of CTX-M type ESBLs-EC have occurred between animals in wildlife zoo. Besides, eight CTX-M-55-producing *E.coli* were detected from three types of wildlife (swans, gibbon monkey and squirrel monkey) located in conjugative IncX1 (2/8, 25.0%) and IncX2 (1/8, 12.5%) (Guo et al., 2019), which suggested antimicrobial resistance genes could migrate into other pathogenic bacteria via various mobile genetic elements such as plasmids, transposons and integrons, posing a potential threat to public health when human contact with wildlife in the zoo (Furlan et al., 2020). Besides, CTX-M-55-producing *E. coli* was detected from three types of wildlife (swans, gibbon monkey and squirrel monkey), which suggested antimicrobial resistance genes could migrate into other pathogenic bacteria via various mobile genetic elements such as plasmids, transposons and integrons, posing a potential threat to public health when human contact with wildlife in zoo (Furlan et al., 2020).

5 | CONCLUSION

Our study provided further evidence of the prevalence of ESBLs-EC in wildlife zoo and expanded our knowledge of ESBLs-EC from wildlife animals from the zoo. The present study reveals a high prevalence of CTX-M type ESBLs from a variety of animals in a wildlife zoo. It will be a potentially serious threat to public health when humans were exposed to zoo wildlife with pathogenic potential multidrug-resistant *E. coli*, such as CTX-M. In summary, more measures must be implemented in the zoo to minimise the risk to public health, such as reducing the dissemination/contamination of antimicrobial resistant bacteria in the zoo's veterinary clinics and more disinfection for visitors.

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CONFLICT OF INTEREST

The authors report no declarations of interest.

DATA AVAILABILITY STATEMENT

All genome assemblies of the 23 ESBLs-EC strains were deposited in GenBank under the BioProject accession number PRJNA742108.

AUTHOR CONTRIBUTIONS

J.L. and Z.S. designed the research. Z.Z., J.G., Z.L., J.H. and X.L. collected the data. J.Y. and Z.Z. analyzed and interpreted the data. Z.Z. drafted the manuscript, J.L., Z.S. and X.C. revised the article. All authors read and approved the manuscript.

ORCID

Jiyun Li https://orcid.org/0000-0003-4877-8199

REFERENCES

Abraham, S., Kirkwood, R. N., Laird, T., Saputra, S., Mitchell, T., Singh, M., Linn, B., Abraham, R. J., Pang, S., Gordon, D. M., Trott, D. J., & O'Dea, M. (2018). Dissemination and persistence of extended-spectrum cephalosporin-resistance encoding IncI1-$bla_{CTX\text{-}M\text{-}1}$ plasmid among *Escherichia coli* in pigs. *The ISME Journal*, *12*(10), 2352–2362.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, *19*(5), 455–477.<./bib>

Bauernfeind, A., Grimm, H., & Schweighart, S. (1990). A new plasmidic cefotaximase in a clinical isolate of *Escherichia coli*. *Infection 18*(5), 294–298.

Beghain, J., Bridier-Nahmias, A., Le Nagard, H., Denamur, E., & Clermont, O. (2018). ClermonTyping: An easy-to-use and accurate in silico method for *Escherichia* genus strain phylotyping. *Microbial Genomics*, *4*(7), e000192.

Bush, K. (2010). Alarming β-lactamase-mediated resistance in multidrug-resistant *Enterobacteriaceae*. *Current Opinion in Microbiology*, *13*(5), 558–564.

Bush K, & Bradford, P. (2016). *β-Lactams and β-lactamase inhibitors: An overview*. Cold Spring Harbor Laboratory Press.

Bush, K., & Bradford, P. A. (2020). Epidemiology of β-lactamase-producing pathogens. *Clinical Microbiology Reviews*, *33*(2), e00047.

CLSI. (2020). Performance standards for antimicrobial susceptibility testing (30th ed.). *CLSI supplement M100*. Wayne, PA: CLSI.

Conrad, C. C., Stanford, K., Narvaez-Bravo, C., Neumann, N. F., Munns, K., Tymensen, L., Jokinen, C., & McAllister, T. A. (2018). Zoonotic fecal pathogens and antimicrobial resistance in canadian petting zoos. *Microorganisms*, *6*(3), 70.

Doi, Y., Iovleva, A., & Bonomo, R. A. (2017). The ecology of extended-spectrum beta-lactamases (ESBLs) in the developed world. *Journal of Travel Medicine*, *24*(suppl_1), S44–S51.

Furlan, J. P. R., Lopes, R., Gonzalez, I. H. L., Ramos, P. L., & Stehling, E. G. (2020). Comparative analysis of multidrug resistance plasmids and genetic background of CTX-M-producing *Escherichia coli* recovered from captive wild animals. *Applied Microbiology and Biotechnology*, *104*(15), 6707–6717.

Guenther, S., Grobbel, M., Beutlich, J., Guerra, B., Ulrich, R. G., Wieler, L. H., & Ewers, C. (2010). Detection of pandemic B2-O25-ST131 *Escherichia coli* harbouring the CTX-M-9 extended-spectrum beta-lactamase type in a feral urban brown rat (*Rattus norvegicus*). *Journal of Antimicrobial Chemotherapy*, *65*(3), 582–584.

Guo, S., Tay, M. Y. F., Thu, A. K., Seow, K. L. G., Zhong, Y., Ng, L. C., & Schlundt, J. (2019). Conjugative IncX1 plasmid harboring colistin resistance gene *mcr-5.1* in *Escherichia coli* isolated from chicken rice retailed in Singapore. *Antimicrobial Agents and Chemotherapy*, *63*(11), e01043.

Hayashi, W., Ohsaki, Y., Taniguchi, Y., Koide, S., Kawamura, K., Suzuki, M., Kimura, K., Wachino, J. I., Nagano, Y., Arakawa, Y., & Nagano, N. (2018). High prevalence of $bla_{CTX\text{-}M\text{-}14}$ among genetically diverse *Escherichia coli* recovered from retail raw chicken meat portions in Japan. *International Journal of Food Microbiology*, *284*, 98–104.

JH, L. (2009). Antimicrobial resistance of *Escherichia colic* O26 and O111 isolates from cattle and their characteristics. *Veterinary Microbiology*, *135*(3-4), 401–405.

Kluytmans, J. A., Overdevest, I. T., Willemsen, I., Kluytmans-van den Bergh, M. F., van der Zwaluw, K., Heck, M., Rijnsburger, M., Vandenbroucke-Grauls, C. M., Savelkoul, P. H., Johnston, B. D., Gordon, D., & Johnson, J. R. (2013). Extended-spectrum beta-lactamase-producing *Escherichia coli* from retail chicken meat and humans: Comparison of strains, plasmids, resistance genes, and virulence factors. *Clinical Infectious Diseases*, *56*(4), 478–487.

Letunic, I., & Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: Recent updates and new developments. *Nucleic Acids Research*, *47*(W1), W256–W259.

Li, J., Bi, Z., Ma, S., Chen, B., Cai, C., He, J., Schwarz, S., Sun, C., Zhou, Y., Yin, J., Hulth, A., Wang, Y., Shen, Z., Wang, S., Wu, C., Nilsson, L. E., Walsh, T. R., Borjesson, S., Shen, J., Sun, Q., & Wang, Y. (2019). Inter-host transmission of carbapenemase-producing *Escherichia coli* among humans and backyard animals. *Environmental Health Perspectives*, *127*(10), 107009.

Nkansa-Gyamfi, N. A., Kazibwe, J., Traore, D. A. K., & Nji, E. (2019). Prevalence of multidrug-, extensive drug-, and pandrug-resistant commensal *Escherichia coli* isolated from healthy humans in community settings in low- and middle-income countries: A systematic review and meta-analysis. *Global Health Action*, *12*(suppl 1), 1815272.

Zhang, Q. Q., Ying, G. G., Pan, C. G., Liu, Y. S., & Zhao, J.-L. (2015). A comprehensive evaluation of antibiotics emission and fate in the river basins of China: Source analysis, multimedia modelling, and linkage to bacterial resistance. *Environmental Science & Technology*, *49*(11), 6772–6782.

Sauget, M., Nicolas-Chanoine, M. H., Cabrolier, N., Bertrand, X., & Hocquet, D. (2014). Matrix-assisted laser desorption ionization-time of flight mass spectrometry assigns *Escherichia coli* to the phylogroups A, B1, B2 and D. *International Journal of Medical Microbiology*, *304*(8), 977–983.

Shnaiderman-Torban, A., Steinman, A., Meidan, G., Paitan, Y., Abu Ahmad, W., & Navon-Venezia, S. (2019). Petting zoo animals as an emerging reservoir of extended-spectrum beta-lactamase and AmpC-producing *Enterobacteriaceae*. *Frontiers in Microbiology*, *10*, 2488.

Slater, S. L., Sagfors, A. M., Pollard, D. J., Ruano-Gallego, D., & Frankel, G. (2018). The type III secretion system of pathogenic *Escherichia coli*. *Current Topics in Microbiology and Immunology*, *416*, 51–72.

Stercz, B., Farkas, F. B., Toth, A., Gajdacs, M., Domokos, J., Horvath, V., Ostorhazi, E., Makra, N., Kocsis, B., Juhasz, J., Ligeti, B., Pongor, S., & Szabo, D. (2021). The influence of antibiotics on transitory resistome during gut colonization with CTX-M-15 and OXA-162 producing *Klebsiella pneumoniae* ST15. *Science Reports*, *11*(1), 6335.

Treangen, T. J., Ondov, B. D., Koren, S., & Phillippy, A. M. (2014). The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biology*, *15*(11), 524.

Woerther, P. L., Burdet, C., Chachaty, E., & Andremont, A. (2013). Trends in human fecal carriage of extended-spectrum beta-lactamases in the community: Toward the globalization of CTX-M. *Clinical Microbiology Reviews*, *26*(4), 744–758.

Zhang, J., Zheng, B., Zhao, L., Wei, Z., Ji, J., Li, L., & Xiao, Y. (2014). Nationwide high prevalence of CTX-M and an increase of CTX-M-55 in *Escherichia coli* isolated from patients with community-onset infections in Chinese county hospitals. *Bmc Infectious Diseases [Electronic Resource]*, *14*, 659.

Zheng, B., Zhang, J., Ji, J., Fang, Y., Shen, P., Ying, C., Lv, J., Xiao, Y., & Li, L. (2015). Emergence of *Raoultella ornithinolytica* coproducing IMP-4 and KPC-2 carbapenemases in China. *Antimicrobial Agents and Chemotherapy*, *59*(11), 7086–7089.

Zhuge, X., Zhou, Z., Jiang, M., Wang, Z., Sun, Y., Tang, F., Xue, F., Ren, J., & Dai, J. (2021). Chicken-source *Escherichia coli* within phylogroup F shares virulence genotypes and is closely related to extraintestinal pathogenic *E. coli* causing human infections. *Transboundary and Emerging Diseases*, *68*(2), 880–895.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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