A Novel Structure Harboring \(\text{bla}_{\text{CTX-M-27}}\) on IncF Plasmids in \textit{Escherichia coli} Isolated from Swine in China

Yan Zhang \(^1,2\), Yin-Huan Sun \(^2\), Jiang-Yang Wang \(^2\), Man-Xia Chang \(^2\), Qiu-Yun Zhao \(^2\) and Hong-Xia Jiang \(^1,2,*\)

\(^1\) Guangdong Laboratory for Lingnan Modern Agriculture, Guangzhou 510642, China; yz_scau@163.com
\(^2\) Guangdong Key Laboratory for Veterinary Drug Development and Safety Evaluation, College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, China; sunyh0210@163.com (Y.-H.S.); wjy19927533599@163.com (J.-Y.W.); 13424457247@163.com (M.-X.C.);
qiuyunzhao123@163.com (Q.-Y.Z.)

* Correspondence: hxjiang@scau.edu.cn; Tel.: +86-20-8528-3934

Abstract: The aim of this study was to elucidate the prevalence of \(\text{bla}_{\text{CTX-M-27}}\)-producing \textit{Escherichia coli} and transmission mechanisms of \(\text{bla}_{\text{CTX-M-27}}\) from swine farms in China. A total of 333 \textit{E. coli} isolates were collected from two farms from 2013 to 2016. Thirty-two CTX-M-27-positive \textit{E. coli} were obtained, and all were multidrug-resistant. Pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) profiles indicated a wide range of strain types that carried \(\text{bla}_{\text{CTX-M-27}}\), and the sequence type ST10 predominated. Conjugation, replicon typing, S1-PFGE and hybridization experiments confirmed that 28 out of 32 CTX-M-27 positive isolates carried \(\text{bla}_{\text{CTX-M-27}}\) genes on plasmids F18:A-B10 (16) and F24:A-B1 (12). The \(\text{bla}_{\text{CTX-M-27}}\) genes for 24 isolates were transmitted by plasmids with sizes ranging from 40 to 155 kb. A comparative analysis with \(\text{bla}_{\text{CTX-M-27}}\)-plasmids indicated that the \textit{tra-trb} region of F24:A-B1 plasmids was destroyed by insertion of a complex region (eight isolates) and a novel structure containing \(\text{bla}_{\text{CTX-M-27}}\) in the F18:A-B10 plasmids (12 isolates). The novel structure increased the stability of the \(\text{bla}_{\text{CTX-M-27}}\) gene in \textit{E. coli}. This study indicated that the predominant vehicle for \(\text{bla}_{\text{CTX-M-27}}\) transmission has diversified over time and that control strategies to limit \(\text{bla}_{\text{CTX-M-27}}\) transmission in farm animals are necessary.

Keywords: \(\text{bla}_{\text{CTX-M-27}}\); Tn2; plasmid; \textit{Escherichia coli}; ST10

1. Introduction

Production of extended-spectrum \(\beta\)-lactamases (ESBLs) is the principal mechanism of resistance to cephalosporins. CTX-Ms was the largest group of ESBLs, and they have become globally disseminated [1]. A recent study indicated that food-producing animals represent an important source of \(\text{bla}_{\text{CTX-M}}\)-producing \textit{E. coli} isolates [2]. CTX-M-15 and CTX-M-14 are the most prevalent. Additionally, \(\text{bla}_{\text{CTX-M-27}}\) has been detected as increasing rapidly in prevalence [1]. The detection of CTX-M-27 in \textit{Escherichia coli} patient isolates has been increasing, and is especially alarming because of its presence in clonal groups such as ST10, ST69 and ST131 [3–6]. In particular, \textit{E. coli} ST131 isolates are the primary hosts responsible for CTX-M-27 transmission in \textit{E. coli} from companion animals, the environment, farm animals and animal products [3,7–13]. IncF plasmids and transposons are frequently associated with transfer of \(\text{bla}_{\text{CTX-M-27}}\) in \textit{E. coli} and are frequently associated with IncF plasmids and transposons [14,15]. The pressure of antibiotics could facilitate the evolution of the transmission mechanism of \(\text{bla}_{\text{CTX-M}}\). The prevalence of \(\text{bla}_{\text{CTX-M-27}}\) in \textit{Salmonella} isolated from food animals is also increasing, and transduction of \(\text{bla}_{\text{CTX-M-27}}\) in \textit{Salmonella} isolated from pork has been recently demonstrated to be mediated by a P1-like bacteriophage that had integrated a Tn1721-like structure [16]. A prospective study utilizing salmonella and \textit{E. coli} isolates from 2003 to 2014 indicated that \(\text{bla}_{\text{CTX-M-27}}\) could be mobilized via a Tn1721-like structure between \textit{E. coli} plasmids and P1-like bacteriophage in salmonella [17].
Intestinal carriage of CTX-M-producing bacteria (especially pathogenic bacteria) in food-producing animals and retail meat or dairy products contaminated by CTX-M β-lactamases might contribute to increased occurrence of infections with ESBL-producing bacteria in humans [18]. The prevalent vehicle for $\text{bla}_{\text{CTX-M}}$ transmission has diversified over time. This would lead to the further spread of the $\text{bla}_{\text{CTX-M-27}}$ gene and further highlights the importance of controlling the spread of $\text{bla}_{\text{CTX-M-27}}$. In the current work we investigated the prevalence and transmission mechanisms of CTX-M-27 $E.\ coli$ from swine in Guangdong province, China from 2013 to 2016. We identified a novel structure containing $\text{bla}_{\text{CTX-M-27}}$ that contributed to the stability of $\text{bla}_{\text{CTX-M-27}}$ in $E.\ coli$.

2. Results and Discussion

2.1. Phenotypes of $\text{bla}_{\text{CTX-M-27}}$-Carrying Isolates

We surveyed two swine farms in Guangdong province and confirmed the presence of $\text{bla}_{\text{CTX-M-27}}$ in 32 $E.\ coli$ isolates including 62.5% (20) from farm 1 and 37.5% (12) from farm 2 (Figure 1). CTX-M-27 has been only sporadically detected in $E.\ coli$ isolates from food-producing animals, and its prevalence has remained constant at <8% over the past decade [17,19]. The prevalence of $\text{bla}_{\text{CTX-M-27}}$ in our study (9.61%) was higher than in previous studies. Importantly, all 32 CTX-M-27 isolates were multidrug-resistant (MDR) strains (Table S1). The prevalence of ciprofloxacin-resistance in these isolates was 93.75% compared with 77.78% from isolates in 2003 to 2009 in China on swine farms [17], and the prevalence of ciprofloxacin resistance in $\text{bla}_{\text{CTX-M-14}}$ or $\text{bla}_{\text{CTX-M-15}}$-producing $E.\ coli$ almost above 80% [20,21]. Additionally, we detected QRDR (Quinolone Resistance Determining Regions) mutations in $\text{gyrA}$ (S83L and D87N) and $\text{parC}$ (S80I) in high-level quinolone resistant isolates (28), and 14 of these coharbored $\text{qxAB}$ (Figure 1). This was consistent with a previous study where blood isolates of $\text{bla}_{\text{CTX-M-27}}$-producing ST131 $E.\ coli$ possessed QRDR mutations that enabled high level resistance to quinolones [22]. Similarly, $\text{bla}_{\text{CTX-M-14}}$, $\text{bla}_{\text{CTX-M-55}}$, $\text{bla}_{\text{CTX-M-15}}$ and $\text{bla}_{\text{CTX-M-9}}$-producing $E.\ coli$ possessed QRDR mutations and carried PMQR genes that enabled high level resistance to quinolones [23]. In contrast, our isolates possessed a lower MIC$_{\text{CIP}}$ range of 0.25 to 1 µg/mL but did not contain QRDR mutations although either $\text{qnrS}$ or $\text{qxAB}$ were present. This was consistent with a previous study reporting that $\text{bla}_{\text{CTX-M-55}}$-producing salmonella with an MIC$_{\text{CIP}}$ in the 0.5 to 4 µg/mL range did not contain QRDR mutations but all contained $\text{qnrS}$ [24]. In addition, 9/32 (28.1%) of our isolates coharbored $\text{mcr-1}$, and these exhibited resistance to colistin at MIC = 8 µg/mL (Figure 1).

The coexistence or cotransfer of other resistance genes in CTX-M-producing $E.\ coli$ strains increases their probability of survival in the presence of other antibiotics, including the cephalosporins [24–26]. Infections caused by MDR bacteria are becoming common and represents a serious public health concern [27].

2.2. Molecular Typing Analysis

In our group of 32 CTX-M-27-producing $E.\ coli$ isolates, we identified seven sequence types (ST). The ST131 was previously found to be associated with $\text{bla}_{\text{CTX-M-27}}$ in Japan, North America and Europe [28–30]. However, ST131 was not present in any of our isolates. We found that ST10 was most prevalent (50%, 16/32) followed by ST224 (28.1%, 9/32), ST101 (9.4%, 3/32). ST10 and ST224 were detected on both farms. The isolates containing ST46, ST162, ST93 and ST58 were represented by one isolate each (Figure 1). Recent reports documented that ST10 and ST224 $E.\ coli$ are primarily carriers of CTX-M-15, CTX-M-14, CTX-M-1 and CTX-M-8 [31–37]. Our recent study identified ST8900 as the most prevalent ST (Sequence Typing) in CTX-M-27-positive $E.\ coli$ isolates obtained during 2003 to 2009 [17]. These results indicate that the dominant ST of $\text{bla}_{\text{CTX-M-27}}$-producing $E.\ coli$ has changed. ST10 $E.\ coli$ contributes to the distribution of the $\text{bla}_{\text{CTX-M-27}}$ gene on swine farms in China. Previous studies showed that although the epidemiology and population dynamics of Global Extraintestinal Pathogenic $E.\ coli$ (ExPEC) are complex, ST10 was the major ST the ExPEC (Extraintestinal Pathogenic $Escherichia\ coli$) group, and
has remained at a constant level. ST10 has now been linked to multidrug resistance gene carriage or ESBL production [38]. Increased numbers of antibiotic resistance genes have been found and are carried by pathogenic bacteria and this is a human health concern. The 32 CTX-M-27 isolates we found could also be divided into 14 different PFGE clusters. These data indicated that the transmission of \( \text{bla}_{\text{CTX-M-27}} \) among \( E. \ coli \) isolates occurred via clonal expansion and horizontal transmission. The results of both clonal spread of resistant strains and horizontal transmission of the resistance plasmids contributed to the dissemination of \( \text{bla}_{\text{CTX-M-27}} \)-positive salmonella or \( E. \ coli \) isolates were also obtained in previous studies [16,17,39].

**Figure 1.** Characteristics of 32 CTX-M-27-carrying \( E. \ coli \) isolated from 2013 to 2016 in Guangdong, China. The \( \text{bla}_{\text{CTX-M-27}} \) genes were all located on plasmids. Isolate names that are underlined indicate that \( \text{bla}_{\text{CTX-M-27}} \) plasmids could be transferred to strain C600 by conjugation. *: Only the \( \Delta \text{IS}_{\text{ECP1}}-\text{bla}_{\text{CTX-M-27}}-\text{IS}_{\text{903B}} \) region was sequenced. Pulsed field gel electrophoresis (PFGE) patterns with a cutoff at 85% similarity (the dashed line) are considered to be the same PFGE cluster and are indicated as groups I–XIV respectively.

### 2.2. Molecular Typing Analysis

In our group of 32 CTX-M-27-producing \( E. \ coli \) isolates, we identified seven sequence types (ST). The ST131 was previously found to be associated with \( \text{bla}_{\text{CTX-M-27}} \) in Japan, North America and Europe [28–30]. However, ST131 was not present in any of our isolates. We found that ST10 was most prevalent (50%, 16/32) followed by ST224 (28.1%, 9/32), ST101 (9.4%, 3/32). ST10 and ST224 were detected on both farms. The isolates containing ST46, ST162, ST93 and ST58 were represented by one isolate each (Figure 1). Recent reports documented that ST10 and ST224 \( E. \ coli \) are primarily carriers of CTX-M-15, CTX-M-14, CTX-M-1 and CTX-M-8 [31–37]. Our recent study identified ST8900 as the most prevalent ST (Sequence Typing) in CTX-M-27-positive \( E. \ coli \) isolates obtained during 2003 to 2009 [17]. These results indicate that the dominant ST of \( \text{bla}_{\text{CTX-M-27}} \)-producing \( E. \ coli \) has changed. ST10 \( E. \ coli \) contributes to the distribution of the \( \text{bla}_{\text{CTX-M-27}} \) gene on swine farms in China.

Previous studies showed that although the epidemiology and population dynamics of Global Extraintestinal Pathogenic \( E. \ coli \) (ExPEC) are complex, ST10 was the major ST the ExPEC (Extraintestinal Pathogenic \( E. \ coli \)) group, and has remained at a constant level. ST10 has now been linked to multidrug resistance gene carriage or ESBL production [38]. Increased numbers of antibiotic resistance genes have been found and are carried by pathogenic bacteria and this is a human health concern.
the F18:A-:B10 backbone (Figure 2B). These transfer regions were highly similar to other F plasmids and differed by the number of CAACAGCCG tandem repeats in the trnD gene. Our isolates all possessed only one of these repeats and were highly similar to that of a bla<sub>CTX-M-55</sub>-harboring F18:A-:B1 plasmid pCREC-544_1 (NZ_CP024827.1) from a human clinical isolate from Korea, and a bla<sub>NDM-5</sub>-harboring F18:A-:B58 plasmid pMTY18780-2 (NZ_AP023199.1) human isolate from Japan. IncF plasmids have been implicated as main players for transmission of bla<sub>CTX-M-27</sub> [40]. So, we estimate that F18:A-:Bx plasmids are the primary vectors for animal to human transmission of most antibiotic resistance genes, and this has significant health implications for both humans and animals.

The second plasmid pM8-1 (F24:A-:B1) we identified possessed DNA transfer genes that had been altered by insertion of a 17,034 bp region that included TnAs3, IS5075, IS26, intII, mph and IS6100, as well as two IS26 copies in the same orientation. This insertion destroyed tral and tran, and the entire trbH/F/G/B/A/E, traD/T/S/G/H/Q/F region was lost (Figure 2). A similar disruption was previously documented for pHNAH9 that lacked most parts of the tra-trb region due to multiple recombination events, and this most likely accounted for its conjugation failure. Our F24:A-:B1 plasmids were highly similar to pHNAH9 [41]. These results indicate that E. coli ST224 possessing F24:A-:B1 was disseminated prior to its acquisition of bla<sub>CTX-M-27</sub>, similar to the bla<sub>CTX-M</sub>-carrying F1:A2:B20 plasmid in E. coli ST131-H30R1 [42].

The region (Figure 3a) containing bla<sub>CTX-M-27</sub> (17354 bp) was present in both our plasmid types, F18:A-:B10 and F24:A-:B1. This region was identical to the variable region from pMRV15_117 (accession no: NZ_AP017618.1, Figure 3a). The insertion of IS<sub>Ecp1</sub>-bla<sub>CTX-M-27</sub>-IS903B in the tnpA transposition gene of Tn2 might be expected to prevent transposition. However, IR<sub>TEM</sub> had been lost, although the IR<sub>np</sub> and the resolvase (res) sites were intact. Therefore, it is possible that the TnpA function was provided by another Tn<sub>intI1</sub>p1-8, while Tn<sub>intI1</sub> was incomplete and incapable of transfer of bla<sub>CTX-M-27</sub> from In<sub>903B</sub> [43]. These results indicate that E. coli ST224 possessing F24:A-:B1 was disseminated prior to its acquisition of bla<sub>CTX-M-27</sub>, similar to the bla<sub>CTX-M</sub>-carrying F1:A2:B20 plasmid in E. coli ST131-H30R1 [42].

The region (Figure 3a) containing bla<sub>CTX-M-27</sub> (17354 bp) was present in both our plasmid types, F18:A-:B10 and F24:A-:B1. This region was identical to the variable region from pMRV15_117 (accession no: NZ_AP017618.1, Figure 3a). The insertion of IS<sub>Ecp1</sub>-bla<sub>CTX-M-27</sub>-IS903B in the tnpA transposition gene of Tn2 might be expected to prevent transposition. However, IR<sub>TEM</sub> had been lost, although the IR<sub>np</sub> and the resolvase (res) sites were intact. Therefore, it is possible that the TnpA function was provided by another Tn<sub>intI1</sub>p1-8, while Tn<sub>intI1</sub> was incomplete and incapable of transfer of bla<sub>CTX-M-27</sub> from In<sub>903B</sub> [43]. These results indicate that E. coli ST224 possessing F24:A-:B1 was disseminated prior to its acquisition of bla<sub>CTX-M-27</sub>, similar to the bla<sub>CTX-M</sub>-carrying F1:A2:B20 plasmid in E. coli ST131-H30R1 [42].

We also identified another genetic context contained within a 22,178 bp insertion (Figure 3b). This was a novel structure associated with bla<sub>CTX-M-27</sub>. The region responsible for bla<sub>CTX-M-27</sub> transfer possessed a common background region ΔISEcp1-bla<sub>CTX-M-27</sub>-IS903B within the variable region. The region downstream of IS903B was the 312 bp ΔtnpA of Tn2 produced by the insertion of IS15D1 and TnAs3 in pSCU-103 (CP054458.1) (Figure 3d) and differed from p53_A (Figure 3e) (684 bp ΔtnpA/res/tnpR of Tn2). In p4_4.1 (CP023827.1) (Figure 3c), the insertion of IS15 resulted in ΔtnpR/res/tnpAΔ-iroNΔ inversion. The iroN and Tn2 sequences were lost, and R1, R2, R3 and adaA5-qaEΔ1-sul1 were all inverted in p1-8, while intII and dfrA17 from In54 were lost (Figure 3b). Taken together, these results indicate that Tn2 was incomplete and incapable of transfer of bla<sub>CTX-M-27</sub> in these plasmids. Therefore, F18:A-:B10 was one of the primary contributors to the spread of bla<sub>CTX-M-27</sub> in E. coli for our swine isolates and resulted in greater stability of bla<sub>CTX-M-27</sub> in clinical isolates. This indicates that the bla<sub>CTX-M-27</sub> gene was stable in vehicles and its natural host. The plasmid can mediate its transmission in a wider range hosts, which poses a serious threat to public health. The ISEcp1-like family has been shown to act as a promoter region for high-level expression of different CTX-M enzymes in a previous study [44].

The conserved region responsible for transmission of bla<sub>CTX-M-27</sub> was ISEcp1-bla<sub>CTX-M-27</sub>-IS903B. Since more complex structures that mediate transmission of bla<sub>CTX-M-27</sub> have been recently described, and a diversity of media promotes the transmission of bla<sub>CTX-M-27</sub>, it may cause a serious threat to public health.

The F18:A-:B10 plasmids may make it easier for E. coli ST10 to acquire bla<sub>CTX-M-27</sub>, and similar results have been reported for the MDR IncF group F1:A2:B20 plasmid in E. coli ST131-H30R1 [42]. Our recent work revealed that bla<sub>CTX-M-27</sub>-plasmids isolated from 2003 to 2009 contained a genetic context with the Tn1721-like structure ΔISEcp1B-bla<sub>CTX-M-27</sub>-IS903D-iroN-Δmap-Tn1721 [17]. In contrast, the present study indicated that the horizontal transmission of bla<sub>CTX-M-27</sub> was related to Tn2, so that the prevalent vehicle for bla<sub>CTX-M-27</sub> transmission has diversified over time.
Figure 2. Linear comparisons of backbones for the two plasmids identified in this study. Regions of >99% identity are shaded in blue. The fluorescent pink arrows indicate the tra area and the purple arrow indicates the trb area.
Figure 3. Genomic context of \textit{bla}_{CTX-M-27} in pM8-1 and p1-8 and gene structure comparisons of with other plasmids as indicated. Regions of >99% identity are shaded in blue. R1, \textit{orf1}-\textit{sul2}-\textit{aph(3')-Ib}-\textit{aph(6)-Ib}-\textit{orf2}-\textit{tet(A)}-\textit{orf3}; R2, \textit{mphR(A)}-\textit{mrx}-\textit{mph(A)}; R3, \textit{chrA} region; R4, \textit{aadA5}-\textit{qacE}\Delta1-\textit{sul1}. The five-pointed star indicates the insertion element. 

\begin{itemize}
  \item[a] Genomic context of \textit{bla}_{CTX-M-27} in pM8-1 (F24:A-:B1, this study).
  \item[b] Genomic context of \textit{bla}_{CTX-M-27} in p1-8 (F18:A-:B10, this study).
  \item[c] Genomic context of \textit{bla}_{CTX-M-27} in p4_4.1 (F1:A2:B20, accession no: CP023827.1).
  \item[d] Genomic context of \textit{bla}_{CTX-M-27} in pSCU-103 (F36:A4:B58, accession no: CP054458.1).
  \item[e] Genomic context of \textit{bla}_{CTX-M-27} in p53_A (F31:A4:B37, accession no: CP048360.1).
\end{itemize}
3. Materials and Methods

A total of 333 *E. coli* isolates were isolated from swine swabs from Guangdong province, China and were maintained in our laboratory. These were obtained between June 2013 and September 2016 from farm 1 (95/236, 40.25%) and farm 2 (141/236, 59.75%). The presence of the *blaCTX-M-27* gene was confirmed by PCR (Polymerase Chain Reaction) and DNA sequencing [45]. The minimum inhibitory concentration (MIC) of *blaCTX-M-27*-positive isolates was determined for the following: ampicillin (AMP), ceftiofur (CTF), cefotaxime (CTX), ceftazidime (CTZ), meropenem (MRO), florfenicol (FLF), chloramphenicol (CHL), gentamycin (GEN), streptomycin (STR), amikacin (AMI), apramycin (APR), ciprofloxacin (CIP), nalidixic acid (NAL), daloxacin (DF), olaquindox (OQX), tetracycline (TET), fosfomycin (FOS), enrofloxacin (ENR) and kanamycin (KAN), determined by the agar dilution method, and colistin (CL) was determined by the microdilution broth method. *Escherichia coli* ATCC 25,922 was used as a quality control strain. The results were interpreted following Clinical and Laboratory Standards Institution (CLSI) guidelines (2015, M100-S25) and veterinary CLSI (VET01-A4/VET01-S2).

All *blaCTX-M-27* positive isolates were screened for the presence of the β-lactamase genes (*blaTEM*, *blaSHV*, *blaCTX-M-1G*, *blaCTX-M-9C*), carbapenem resistance genes (bla*IMP*, bla*VIM*, bla*NDM*, bla*OXA*) and PMQR genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *aqxAB*, *aac(6′)-Ib-cr*, *qepA*) using previously described primers and protocols [18]. The DNA sequences and deduced amino acid sequences were compared with sequences available at GenBank using BLAST (Basic Local Alignment Search Tool) [https://blast.ncbi.nlm.nih.gov/Blast.cgi] (TET), fosfomycin (FOS), enrofloxacin (ENR) and kanamycin (KAN), determined by the microdilution broth method. *Escherichia coli* ATCC 25,922 was used as a quality control strain. The results were interpreted following Clinical and Laboratory Standards Institution (CLSI) guidelines (2015, M100-S25) and veterinary CLSI (VET01-A4/VET01-S2).

MLST was performed for *blaCTX-M-27*-producing isolates as previously reported [46]. The PCR and DNA sequence analysis of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) was performed, the allelic profiles were determined, and the sequence type (ST) was assigned in accordance with [http://mlst.warwick.ac.uk/mlst/dbs/] (accessed on 15 February 2021). Pulsed field gel electrophoresis (PFGE) patterns with the *XbaI* enzyme were analyzed in experiments following the method of Jiang et al. for genetic relatedness of all *blaCTX-M-27*-harboring isolates [18].

*E. coli* C600 strain (streptomycin-resistant) was used as donor strain and clinical *blaCTX-M-27*-producing strains were used as recipient strains to test the transferability of the *blaCTX-M-27* gene. Transconjugants were selected on MacConkey agar supplemented with 1 mg/L cefotaxime and 2000 mg/L streptomycin [18]. PCR-based replicon typing (PBRT) was performed on all transconjugants with *blaCTX-M-27*-carrying plasmids using primers as previously described [47]. To determine the location of *blaCTX-M-27*, all the transconjugants were subjected to pulsed-field gel electrophoresis (PFGE) with S1 nuclease (Takara) and Southern transfer and probing with a digoxigenin-labelled probes specific for the *blaCTX-M-9C* gene as described previously [16].

Genomic DNA samples from M8-1 and transconjugant C-1-8 were subjected to 250-bp paired-end whole-genome sequencing using the Illumina HiSeq system (Illumina, San Diego, CA, USA), and the paired-end Illumina reads were assembled by SOAPdenovo version 2.04 [48]. To obtain the complete sequence of p1-8, PCR and Sanger sequencing was applied to close all the suspected gaps. Sequence comparisons of M8-1 and pMRY15_117 (NZ_AP017618.1) were performed using BLAST [http://blast.ncbi.nlm.nih.gov (accessed on 15 February 2021)] and Mauve [49], and similar contigs were extracted from the assemblies.

Specific primers used for further analysis of genomic context sequence on the other plasmids carrying *blaCTX-M-27* gene were designed using Primer Premier v5.0 (IS1-8-fw: TCCGACACGATAAGGAAT/IS1-8-rev: TTGCCATCACGACTGTGTC; ISM1-1-fw: CCGTCAGAACTAGTGGTGTC/ISM1-1-rev: GTGGACTGTGGTGATAAGA). Target sequences were identified using PCR (30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C (p1-8)/52.9 °C (pM8-1) for 30 s and extension at 72 °C for 4 min
30 s. This was followed by an additional 10 min extension at 72 °C) and amplicon identities were confirmed by Sanger sequencing [50]. Gene prediction and annotation of contigs were performed using the RAST (Rapid Annotation using Subsystem Technology) server (http://rast.nmpdr.org/ (accessed on 15 February 2021)) [51] and BLAST was used for a manual review of results. The ISfinder program (https://www-is.biotoul.fr/ (accessed on 15 February 2021)) [52] and ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/ (accessed on 15 February 2021)) were used to identify mobile elements and resistance genes. The replicon types of plasmids were analyzed using the plasmid MLST database (http://pubmlst.org/plasmid/ (accessed on 15 February 2021)).

4. Conclusions

In this study we identified ST10 E. coli as one of the dominant vehicles for \(\text{bla}_{\text{CTX-M-27}}\) transmission in China. We report a novel structure containing \(\text{bla}_{\text{CTX-M-27}}\) that contributed to the persistence of this gene in \(E.\) coli. This study identified multidrug-resistant IncF group F18:A-B10 plasmids that contributed to the spread of \(\text{bla}_{\text{CTX-M-27}}\) in \(E.\) coli. \(E.\) coli ST224 possessing F24:A-B1 were likely transmitted before acquiring \(\text{bla}_{\text{CTX-M-27}}\), and Tn2-related events may have contributed to the spread of \(\text{bla}_{\text{CTX-M-27}}\) in F24:A-B1 plasmids. Additionally, the prevalent vehicle for \(\text{bla}_{\text{CTX-M-27}}\) transmission has diversified over time so that that transmission of the \(\text{bla}_{\text{CTX-M-27}}\) gene in farm animals should be closely monitored.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/antibiotics10040387/s1, Table S1: Antimicrobial resistance of 32 CTX-M-27 producing \(E.\) coli from swine in china, Table S2: Primers used in this study.

Author Contributions: Y.-H.S., J.-Y.W. and Y.Z.; the antimicrobial susceptibility tests and PCR amplification; Y.Z. and Y.-H.S.; conjugation experiments, S1-PFGE and southern blotting; Y.Z., Q.-Y.Z. and M.-X.C.; bioinformatics analyses, visualization and wrote the paper; H.-X.J.; acquired funding and edited the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (Grant number 31972734) and the Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (Grant number 2019BT02N054).

Informed Consent Statement: Not applicable.

Data Availability Statement: The complete nucleotide sequence of plasmids p1-8 have been deposited to the GenBank database and assigned accession number MW633522.

Acknowledgments: The authors would like to thank all the laboratory researchers that collected the \(E.\) coli strains over several years.

Conflicts of Interest: The other authors have no conflict of interest to disclose.

References

1. Bevan, E.R.; Jones, A.M.; Hawkey, P.M. Global epidemiology of CTX-M \(\beta\)-lactamases: Temporal and geographical shifts in genotype. \(J.\) Antimicrob. Chemother. \textbf{2017}, \textit{72}, 2145–2155. [CrossRef] [PubMed]
2. Huang, Y.; Zeng, L.; Doi, Y.; Lv, L.; Liu, J.H. Extended-spectrum \(\beta\)-lactamase-producing \(E.\) coli. \textit{Lancet Infect. Dis.} \textbf{2020}, \textit{20}, 404–405. [CrossRef]
3. Matsumura, Y.; Johnson, J.R.; Yamamoto, M.; Nagao, M.; Tanaka, M.; Takakura, S.; Ichiyama, S.; Kyoto, S.C.M.S.; Kyoto-Shiga, C.M.S.G. \(\text{CTX-M-27}\)- and \(\text{CTX-M-14}\)-producing, ciprofloxacin-resistant \(E.\) coli of the H30 subclonal group within ST131 drive a Japanese regional ESBL epidemic. \textit{J. Antimicrob. Chemother.} \textbf{2015}, \textit{70}, 1639. [CrossRef] [PubMed]
4. Rohde, A.M.; Zweigner, J.; Wiese-Posselt, M.; Schwab, F.; Behnke, M.; Kola, A.; Schröder, W.; Peter, S.; Tacconelli, E.; Wille, T.; et al. Prevalence of third-generation cephalosporin-resistant Enterobacteriales colonization on hospital admission and ESBL-genotype-specific risk factors: A cross-sectional study in six German university hospitals. \textit{J. Antimicrob. Chemother.} \textbf{2020}, \textit{75}, 1631–1638. [CrossRef] [PubMed]
5. Flament-Simon, S.; Garcia, V.; Duprilot, M.; Mayer, N.; Alonso, M.P.; García-Meníno, I.; Blanco, J.E.; Blanco, M.; Nicolas-Chanoine, M.; Blanco, J. High Prevalence of ST131 Subclades C2-H30Rx and C1-M27 Among Extended-Spectrum \(\beta\)-Lactamase-Producing \(E.\) coli Causing Human Extraintestinal Infections in Patients from Two Hospitals of Spain and France During 2015. \textit{Front. Cell Infect. Microbiol.} \textbf{2020}, \textit{10}. [CrossRef]
6. García-Meniño, I.; García, V.; Mora, A.; Díaz-Jiménez, D.; Flament-Simon, S.C.; Alonso, M.P.; Blanco, J.E.; Blanco, M.; Blanco, J. Swine Enteric Colibacillosis in Spain: Pathogenic Potential of mcr-1 ST10 and ST131 Escherichia coli Isolates. *Front. Microbiol.* **2018**, *9*. [CrossRef]

7. Kawamura, K.; Sugawara, T.; Matsu, N.; Hayashi, K.; Norizuki, C.; Tamai, K.; Kondo, T.; Arakawa, Y. Spread of CTX-Type Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Isolates of Epidemic Clone B2-O25-ST131 Among Dogs and Cats in Japan. *Microb. Drug Resist.* **2017**, *23*, 1059–1066. [CrossRef] [PubMed]

8. Piccirilli, A.; Pompilio, A.; Rossi, L.; Segatore, B.; Amicosante, G.; Rosatelli, G.; Perilli, M.; Di Bonaventura, G. Identification of CTX-M-15 and CTX-M-27 in Antibiotic-Resistant Gram-Negative Bacteria Isolated from Three Rivers Running in Central Italy. *Microb. Drug Resist.* **2019**, *25*, 1041–1049. [CrossRef]

9. Pepin-Puget, L.; El Garch, F.; Bertrand, X.; Valot, B.; Hoquet, D. Genome analysis of enterobacteriaceae with non-wild type susceptibility to third-generation cephalosporins recovered from diseased dogs and cats in Europe. *Vet. Microbiol.* **2020**, *242*, 108601. [CrossRef]

10. Aguire, L.; Vidal, A.; Seminati, C.; Tello, M.; Redondo, N.; Darwich, L.; Martin, M. Antimicrobial resistance profile and prevalence of extended-spectrum beta-lactamases (ESBL), AmpC beta-lactamases and colistin resistance (*mcr*) genes in *Escherichia coli* from swine between 1999 and 2018. *Porc. Health Manag.* **2020**, *6*. [CrossRef]

11. Tamang, M.D.; Nam, H.; Kim, S.; Chae, M.H.; Jang, G.; Jung, S.; Lim, S. Prevalence and Molecular Characterization of CTX-M β-Lactamase–Producing *Escherichia coli* Isolated from Healthy Swine and Cattle. *Foodborne Pathog. Dis.* **2013**, *10*, 13–20. [CrossRef]

12. Ball, T.A.; Monte, D.F.; Aidara-Kane, A.; Matheu-Alvarez, J.; Ru, H.; Thakur, S.; Horovitz, J.; Ejobi, F.; Lacher, D.W.; Fedorka-Cray, P.J. Phenotypic and Genotypic Characterization of *Escherichia coli* and *Salmonella enterica* from Dairy Cattle Farms in the Wakiso District, Uganda. *Foodborne Pathog. Dis.* **2019**, *16*, 54–59. [CrossRef]

13. Afema, J.A.; Ahmed, S.; Besser, T.E.; Jones, L.P.; Sischo, W.M.; Davis, M.A. Molecular Epidemiology of Dairy Cattle-Associated *Escherichia coli* Carrying *blaCTX-M* Genes in Washington State. *Appl. Environ. Microbiol.* **2018**, *84*. [CrossRef]

14. Matsunura, Y.; Vitout, J.D.D.; Gomi, R.; Matsuda, T.; Noguchi, T.; Yamamoto, M.; Peirano, G.; DeVinney, R.; Bradford, P.A.; Motyl, M.R.; et al. Global *Escherichia coli* Sequence Type 131 Clade with *blaCTX-M-27* Gene. *Emerg. Infect. Dis.* **2016**, *22*, 1900–1907. [CrossRef]

15. Tadesse, D.A.; Li, C.; Mukherjee, S.; Hsu, C.; Bodeis Jones, S.; Gainer, S.A.; Kabera, C.; Loneragan, G.H.; Torrence, M.; Harhay, D.M.; et al. Whole-Genome Sequence Analysis of CTX-M-Producing *Escherichia coli* Isolated from Retail Meats and Cattle in the United States. *Microb. Drug Resist.* **2018**, *24*, 939–948. [CrossRef] [PubMed]

16. Yang, L.; Li, W.; Jiang, G.; Zhang, W.; Ding, H.; Liu, Y.; Zeng, Z.; Jiang, H. Characterization of a P1-like bacteriophage carrying CTX-M-27 in *Salmonella* spp. resistant to third generation cephalosporins isolated from pork in China. *Sci. Rep.* **2017**, *7*, 40710. [CrossRef]

17. Zhao, Q.; Li, W.; Cai, R.; Lu, Y.; Zhang, Y.; Cai, P.; Webber, M.A.; et al. Global *Escherichia coli* Sequence Type 131 Clade with *blaCTX-M-27* between P1-like bacteriophage in *Salmonella* and plasmids in *Escherichia coli* in China. *Vet. Microbiol.* **2021**, *253*, 108944. [CrossRef]

18. Jiang, H.X.; Song, L.; Liu, J.; Zhang, X.H.; Ren, Y.N.; Zhang, W.H.; Zhang, J.Y.; Liu, Y.H.; Webber, M.A.; Ogbolu, D.O.; et al. Multiple transmissible genes encoding fluoroquinolone and third-generation cephalosporin resistance co-located in non-typhoidal *Salmonella* isolated from food-producing animals in China. *Int. J. Antimicrob. Agents* **2014**, *43*, 242–247. [CrossRef]

19. Cormier, A.; Zhang, P.L.C.; Chalmers, G.; Weese, J.S.; Deckert, A.; Mulvey, M.; Allister, T.; Boerlin, P.; Motyl, M.R.; et al. Global *Escherichia coli* Sequence Type 131 Clade with *blaCTX-M* Genes in Washington State. *Appl. Environ. Microbiol.* **2018**, *84*. [CrossRef]

20. Dhanji, H.; Murphy, N.M.; Akhigbe, C.; Dounith, M.; Hope, R.; Livermore, D.M.; Woodford, N. Isolation of fluoroquinolone-resistant O25b:H4-ST131 *Escherichia coli* with CTX-M-14 extended-spectrum beta-lactamase from UK river water. *J. Antimicrob. Chemother.* **2011**, *66*, 512–516. [CrossRef]

21. Peirano, G.; Lynch, T.; Matsumara, Y.; Nobrega, D.; Finn, T.J.; DeVinney, R.; Pitout, J.D.D. Trends in Population Dynamics of *Escherichia coli* Sequence Type 131, Calgary, Alberta, Canada, 2006–2016. *Emerg. Infect. Dis.* **2020**, *26*, 2907–2915. [CrossRef] [PubMed]

22. Zogg, A.L.; Simmen, S.; Zurfluh, K.; Stephan, R.; Schmitt, S.N.; Nuesch-Inderbinen, M. High Prevalence of Extended-Spectrum beta-Lactamase Producing *Enterobacteriaceae* Among Clinical Isolates From Cats and Dogs Admitted to a Veterinary Hospital in Switzerland. *Front. Vet. Sci.* **2018**, *5*, 62. [CrossRef] [PubMed]

23. Zhang, C.; Ding, X.; Lin, X.; Sun, R.; Lu, Y.; Cai, R.; Webber, M.A.; Ding, H.; Jiang, H. The Emergence of Chromosomally Located *blaCTX-M* in *Salmonella* from Foodborne Animals in China. *Front. Microbiol.* **2019**, *10*. [CrossRef] [PubMed]

24. Faccone, D.; Moreo, F.A.; Giacoboni, G.I.; Albornoz, E.; Alarcon, L.; Nievas, V.F.; Corso, A. Multidrug-resistant *Escherichia coli* harbouring mcr-1 and *blaCTX-M* genes isolated from swine in Argentina. *J. Glob. Antimicrob. Resist.* **2019**, *18*, 160–162. [CrossRef]

25. Komatsu, Y.; Kasahara, K.; Inoue, T.; Lee, S.; Muratani, T.; Yano, H.; Kirita, T.; Mikasa, K. Molecular epidemiology and clinical features of extended-spectrum beta-lactamase-or carbanenemase-producing *Escherichia coli* bacteremia in Japan. *PLoS ONE* **2018**, *13*, e022276. [CrossRef]

26. Vivas, R.; Barbosa, A.A.T.; Dolabela, S.S.; Jain, S. Multidrug-Resistant Bacteria and Alternative Methods to Control Them: An Overview. *Microb. Drug Resist.* **2019**, *25*, 890–908. [CrossRef] [PubMed]
28. Matsuo, N.; Nonogaki, R.; Hayashi, M.; Wachino, J.I.; Suzuki, M.; Arakawa, Y.; Kawamura, K. Characterization of blaCTX-M-27/F1:A2:B20 Plasmids Harbored by Escherichia coli Sequence Type 131 Sublineage C1/H30R Isolates Spreading among Elderly Japanese in Nonacute-Care Settings. Antimicrob. Agents Chemother. 2020, 64. [CrossRef]

29. Ghosh, H.; Bunk, B.; Doijad, S.; Schmiedel, J.; Fašinger, L.; Spröer, C.; Imirzalioglu, C.; Overmann, J.; Chakraborty, T. Complete Genome Sequence of blaCTX-M-27-Encoding Escherichia coli Strain H105 of Sequence Type 131 Lineage C1/H30R. Genome Announc. 2017, 5. [CrossRef]

30. Birgy, A.; Levy, C.; Nicolas-Chanoine, M.; Cointe, A.; Hobson, C.A.; Magnan, M.; Bechet, S.; Bidet, P.; Cohen, R.; Bonacorsi, S. Independent Host Factors and Bacterial Genetic Determinants of the Emergence and Dominance of Escherichia coli Sequence Type 131 CTX-M-27 in a Community Pediatric Cohort Study. Antimicrob. Agents Chemother. 2019, 63. [CrossRef]

31. Founou, L.L.; Founou, R.C.; Allam, M.; Ismail, A.; Essack, S.Y. Draft genome sequence of an extended-spectrum beta-lactamase (CTX-M-15)-producing Escherichia coli ST10 isolated from a nasal sample of an abattoir worker in Cameroon. J. Glob. Antimicrob. Resist. 2018, 14, 68–69. [CrossRef]

32. Zahra, R.; Javeed, S.; Malala, B.; Babenko, D.; Toleman, M.A. Analysis of Escherichia coli STs and resistance mechanisms in sewage from Islamabad, Pakistan indicates a difference in E. coli carriage types between South Asia and Europe. J. Antimicrob. Chemother. 2018, 73, 1781–1785. [CrossRef]

33. Day, M.J.; Hopkins, K.L.; Wareham, D.W.; Toleman, M.A.; Elviss, N.; Randall, L.; Teale, C.; Cleary, P.; Wubb, C.; Doumith, M.; et al. Extended-spectrum beta-lactamase-producing Escherichia coli in human-derived and foodchain-derived samples from England, Wales, and Scotland: An epidemiological surveillance and typing study. Lancet Infect. Dis. 2019, 19, 1325–1335. [CrossRef]

34. Song, J.; Oh, S.; Kim, J.; Shin. Extended-spectrum β-lactamase-producing Escherichia coli isolated from raw vegetables in South Korea. Sci. Rep. 2020, 10. [CrossRef]

35. Sghaier, S.; Abbassi, M.S.; Pascual, A.; Serrano, L.; Díaz-Díez-García, J.R.; Huertas, N.; Navarro, F.J.; Mateo, A.B.; Pellejero, E.M.; Illescas, S.; Vidal, M.D.; Del Campo, R. Prevalence and risks factors associated with ESBL-producing faecal carriage in a single long-term-care facility in Spain: Emergence of CTX-M-24- and CTX-M-27-producing Escherichia coli ST131-H30R. J. Antimicrob. Chemother. 2020, 75, 2484–2484. [CrossRef] [PubMed]

36. Silva, M.M.; Seller, F.P.; Fernandes, M.R.; Moura, Q.; Garino, F.; Azevedo, S.S.; Lincopan, N. Genomic features of a highly virulent, cefotiofur-resistant, CTX-M-8-producing Escherichia coli ST224 causing fatal infection in a domestic cat. J. Glob. Antimicrob. Resist. 2018, 15, 252–253. [CrossRef] [PubMed]

37. Silva, K.C.; Moreno, M.; Cabrera, C.; Spira, B.; Cerdeira, L.; Lincopan, N.; Moreno, A.M. First Characterization of CTX-M-15-Producing Escherichia coli Strains Belonging to Sequence Type (ST) 410, ST224, and ST284 from Commercial Swine in South America. Antimicrob. Agents Chemother. 2016, 60, 2505–2508. [CrossRef] [PubMed]

38. Manges, A.R.; Geum, H.M.; Guo, A.; Edens, T.J.; Fibke, C.D.; Pitout, J.D.D. Global Extraintestinal Pathogenic Escherichia coli (ExPEC) Lineages. Clin. Microbiol. Rev. 2019, 32. [CrossRef]

39. Colmenarejo, C.; Hernández-García, M.; Muñoz-Rodríguez, J.R.; Huertas, N.; Navarro, F.J.; Mateo, A.B.; Pellejero, E.M.; Illescas, S.; Vidal, M.D.; Del Campo, R. Prevalence and risks factors associated with ESBL-producing faecal carriage in a single long-term-care facility in Spain: Emergence of CTX-M-24- and CTX-M-27-producing Escherichia coli ST131-H30R. J. Antimicrob. Chemother. 2020, 75, 2484–2484. [CrossRef] [PubMed]

40. Lucas, P.; Jouy, E.; Le Devendec, L.; de Boisseson, C.; Perrin-Guyomard, A.; Jove, T.; Blanchard, Y.; Touzain, F.; Kempf, I. Characterization of plasmids harboring blaCTX-M genes in Escherichia coli from French pigs. Vet. Microbiol. 2018, 224, 100–106. [CrossRef]

41. Wang, J.; Zeng, Z.L.; Huang, X.Y.; Ma, Z.B.; Guo, Z.W.; Lv, L.C.; Xia, Y.B.; Zeng, L.; Song, Q.H.; Liu, J.H. Evolution and Comparative Genomics of F33:A-:B- Plasmids Carrying blactx-M-55 or blactx-M-65 in Escherichia coli and Klebsiella pneumoniae Isolated from Animals, Food Products, and Humans in China. MSphere 2018, 3. [CrossRef]

42. Hayashi, M.; Matsu, M.; Sekizuka, T.; Shima, A.; Segawa, T.; Kuroda, M.; Kawamura, K.; Suzuki, S. Dissemination of IncF group FI:A2:B20 plasmid-harbouring multidrug-resistant Escherichia coli ST131 before the acquisition of blactx-M-15 in Japan. J. Glob. Antimicrob. Resist. 2020, 20, 456–465. [CrossRef]

43. Zong, Z.; Ginn, A.N.; Dobiosova, H.; Iredell, J.R.; Partridge, S.R. Different IncI1 plasmids from Escherichia coli carry ISEcp1-blaCTX-M-15 associated with different Tn2-derived elements. Plasmid 2015, 80, 118–126. [CrossRef]

44. Cantón, R.; Coque, T.M. The CTX-M beta-lactamase pandemic. Curr. Opin. Microbiol. 2006, 9, 466–475. [CrossRef]

45. Jiang, H.X.; Tang, D.; Liu, Y.H.; Zhang, X.H.; Zeng, Z.L.; Xu, L.; Hawkey, P.M. Prevalence and characteristics of beta-lactamase and plasmid-mediated quinolone resistance genes in Escherichia coli isolated from farmed fish in China. J. Antimicrob. Chemother. 2012, 67, 2350–2353. [CrossRef]

46. Hibbert-Rogers, L.C.; Heritage, J.; Gascoyne-Binzi, D.M.; Hawkey, P.M.; Todd, N.; Lewis, I.J.; Bailey, C. Molecular epidemiology of ceftazidime resistant Enterobacteriaceae from patients on a paediatric oncology ward. J. Antimicrob. Chemother. 1995, 36, 65–82. [CrossRef] [PubMed]

47. Carattoli, A.; Bertini, A.; Villa, L.; Falbo, V.; Hopkins, K.L.; Threlfall, E.J. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Meth. 2005, 63, 219–228. [CrossRef]

48. Luo, R.; Liu, B.; Xie, Y.; Li, Z.; Huang, W.; Yuan, J.; He, G.; Chen, Y.; Pan, Q.; Liu, Y.; et al. SOAPdenovo2: An empirically improved memory-efficient short-read de novo assembler. Gigasience 2012, 1, 18. [CrossRef] [PubMed]
49. Darling, A.C.; Mau, B.; Blattner, F.R.; Perna, N.T. Mauve: Multiple alignment of conserved genomic sequence with rearrangements. *Genome Res.* **2004**, *14*, 1394–1403. [CrossRef] [PubMed]

50. Beck, T.F.; Mullikin, J.C.; Biesecker, L.G. Systematic Evaluation of Sanger Validation of Next-Generation Sequencing Variants. *Clin. Chem.* **2016**, *62*, 647–654. [CrossRef] [PubMed]

51. Aziz, R.K.; Bartels, D.; Best, A.A.; DeJongh, M.; Disz, T.; Edwards, R.A.; Formsma, K.; Gerdes, S.; Glass, E.M.; Kubal, M.; et al. The RAST Server: Rapid annotations using subsystems technology. *BMC Genom.* **2008**, *9*, 75. [CrossRef] [PubMed]

52. Siguier, P. ISfinder: The reference centre for bacterial insertion sequences. *Nucleic Acids Res.* **2006**, *34*, D32–D36. [CrossRef] [PubMed]