Characterization of a genomic Island carrying the tet(X4) gene in porcine Acinetobacter towneri co-harboring plasmid-borne blaNDM–1 and blaOXA–58 genes

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Tigecycline and carbapenems are last-resort antimicrobial agents to treat serious infections caused by multi-drug resistant bacterial pathogens. However, the co-occurrence of tigecycline and carbapenem resistance determinants challenges the clinical efficacy of these antimicrobial agents. In this study, we report the co-existence of tet(X4), blaNDM–1 and blaOXA–58 genes in the porcine Acinetobacter towneri isolate 19110F47. Sequence analysis revealed that tet(X4) gene, along with the florfenicol resistance gene floR, was flanked by three copies of IS91–like elements, which can form three different translocatable units (TUs), and were located in a 41,098-bp multidrug resistance region (MDRR) within a novel 100,354-bp genomic island (GI) region. TUs comprising floR-virD2-IVSva3, hp-abh-tet(X4)-IVSva3 and virD2-floR-IVSva3-hp-abh-tet(X4)-IVSva3 can be looped out from the chromosomal DNA and facilitate the transfer of the TU-based resistance genes into other plasmidic or chromosomal sites. In addition, the carbapenemase genes blaNDM–1 and blaOXA–58 were found on different non-conjugative multiresistance plasmids in this isolate, with the genetic contexts ISAbα125-blaNDM–1-bleMBL-tnpR and ΔISAbα3-blaOXA–58-ISAbα3, respectively. The simultaneous occurrence of tet(X4), blaNDM–1 and blaOXA–58 in the same porcine A. towneri isolate emphasizes the importance of antimicrobial resistance surveillance in food-producing animals.

KEYWORDS

tigecycline, carbapenem, resistance, tet(X4), blaNDM–1, blaOXA–58, Acinetobacter towneri
Introduction

Antimicrobial resistance poses a significant threat to public health globally. The presence of extensively drug-resistant (XDR) Gram-negative bacteria, in particular carbapenem-resistant Enterobacteriaceae and Acinetobacter spp., compromises the efficacy of carbapenems. Moreover, the choices of effective antimicrobial agents against carbapenem-resistant bacteria are very limited (1). Tigecycline has been recognized as a last-resort antimicrobial agent to treat infections caused by XDR Gram-negative bacteria (2). However, a variety of plasmid-borne tet(X) variants are found in Acinetobacter spp., Enterobacteriaceae from China (3, 4). The gene tet(X4) was detected in bacteria from food-producing animals, meat for human consumption, migratory birds, humans and environmental samples (5–7).

Acinetobacter spp. are considered as ubiquitous in the nature and have emerged as a major cause of nosocomial infections globally in recent decades (8). In addition, Acinetobacter spp. are not only associated with hospital-acquired infections, but also responsible for community-acquired infections (9). XDR Acinetobacter isolates, especially when they are carbapenem-resistant, are recognized as one of the most troublesome pathogens worldwide (10). Food-producing animals have been regarded as a potential reservoir for Acinetobacter spp. in many countries (11). To date, the tet(X4) gene was mostly reported in E. coli, and sometimes described in other bacterial species, such as Acinetobacter spp. (4, 12, 13). Currently, the genetic basis for co-resistance against tigecycline and carbapenems has been investigated in Acinetobacter spp. The tigecycline resistance genes tet(X) have been reported with carbapenem resistance gene blaTEM in A. baumannii, A. indicus, A. schindleri, A. lwofii, and other Acinetobacter (14–16).

In this study, we investigated a tigecycline- and carbapenem-resistant A. towneri isolate collected from a pig in China for the tigecycline- and carbapenem resistance genes present, their association with mobile genetic elements and their transfer potential.

Materials and methods

Sample collection and bacterial isolation

A total of 1,146 non-duplicate anal swab samples were collected from three unrelated and geographically distant pig farms and one pig slaughterhouse located in the Henan Province of China in 2019. Brain heart infusion (BHI) broth was used as transport medium for the anal swabs. The swabs were streaked on BHI agar plates supplemented with tigecycline (2 mg/L) and meropenem (2 mg/L) and incubated at 37°C for 24 h. Bacteria growing on these double-selective plates were identified to the species level by 16S rRNA amplification and sequencing of the amplicons (17).

Antimicrobial susceptibility testing

The minimal inhibitory concentrations (MICs) of the A. towneri isolate to meropenem, tigecycline, florfenicol, tetracycline, colistin and gentamicin were determined and evaluated using broth microdilution according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (18). E. coli ATCC25922 was used as quality control strain.

Whole-genome sequencing analysis

The A. towneri isolate 19110F47, was sequenced by using the PacBio RS and Illumina MiSeq platforms (Shanghai Personal Biotechnology Co., Ltd, China). The PacBio long reads were assembled with HAP4 and CANU (Version 1.6) and corrected by the Illumina MiSeq short reads with pilon (Version 1.22). The prediction of ORFs and their annotations were performed using Glimmer 3.0. The blast software is used following the procedures at https://blast.ncbi.nlm.nih.gov.

PCR analysis

The presence of translocatable units (TU) was detected by PCR using the primers shown in Table 1. All the PCR products were subjected to Sanger sequencing. The obtained sequences were analyzed by BLAST comparison with the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Transfer experiments

Conjugation assays were performed to assess the transferability of carbapenem and tigecycline resistance genes from A. towneri isolate to the azide-resistant recipient.
E. coli J53 and the rifampicin-resistant recipient E. coli EC600 according to a previously described method with a minor modification (1). Briefly, the donor and recipient strains were mixed at a ratio of 1:4 and incubated on LB agar for 5 h. The mixtures were collected and then plated on LB agar containing two selective markers, including azide (128 µg/mL) and meropenem (2 µg/mL), rifampicin (64 µg/mL) and meropenem (2 µg/mL), azide (128 µg/mL) and tigecycline (2 µg/mL), rifampicin (64 µg/mL) and tigecycline (2 µg/mL), respectively. The transconjugants were confirmed by PCR analysis.

Results
Identification of a carbapenem- and tigecycline-resistant A. towneri isolate

An isolate conferring resistance to both meropenem and tigecycline, designated 19110F47, was identified from swine in nosocomial infections as described in a previous study (16). Antimicrobial susceptibility testing results revealed that it had an expanded resistance profile, including resistances to meropenem (MIC, 16 µg/mL), tigecycline (8 µg/mL), ceftazidime (256 µg/mL), tetracycline (32 µg/mL), gentamicin (64 µg/mL) and florfenicol (128 µg/mL). However, it was classified by the CLSI breakpoints as colistin-intermediate (≤0.5 µg/mL). It is noteworthy that CLSI classifies all isolates without an MDR region being present at the same location in A. towneri, is part of TUs

 tet(X4) was located in the chromosomal DNA in A. towneri and is part of TUs

The tet(X4) gene was found to be a part of a multidrug resistance region (MDRR) located on a 100,354-bp genomic island (GI) that was present in the chromosomal DNA of A. towneri 19110F47. The complete GI shared only a low query coverage (16–37%) with the sequences deposited in GenBank. It was inserted between the DUF3375 and AAA family ATPase encoding genes in the chromosomal DNA of A. towneri 19110F47. Database searches identified a distinctly smaller GI without an MDR region being present at the same location in A. towneri CIP_107472_V1 (accession number GCA_000368785.1) from an activated sludge plant in Australia. A total of 94 ORFs were identified in the GI of the present study. These ORFs encoded proteins responsible for several functions, such as gene regulation, transfer, efflux and antimicrobial resistance (Figure 1). The 41,098-bp MDRR consists of 33 ORFs [from IS26 to aph(6)-lb] (Figure 1). Among them, a total of 12 resistance genes were identified, including tet(X4), the aminoglycoside resistance genes adaA1, adaA2b, aph(3’)-lb, aph(3’)-la and aph(6)-ld, the sulfonamide resistance genes sul1 and sul2, the phenicol resistance genes cmiA1 and floR, the β-lactam resistance gene blactC2 and the trimethoprim resistance gene dfrA16 (Figure 1). In the close vicinity of floR and tet(X4), one truncated and two complete copies of ISVsa3, all in the same orientation, were found (Figure 1), which was similar to the corresponding region on p47EC from a porcine E. coli (MK134376).

Three PCR assays were developed to detect ISVsa3-mediated rolling-circle transposition of tet(X4) and/or floR. The results revealed that these TUs, including TU1 (virD2-floR- ISVsa3, 4,274 bp), TU2 [hp-abh-tet(X4)-ISVsa3, 4,608 bp] and TU3 [virD2-floR-ISCR2-hp-abh-tet(X4)-ISVsa3, 8,882 bp] were formed in A. towneri 19110F47 (Figure 1).

blaNDM-1 and blaOXA-58 were located on novel non-conjugative plasmids

The blaNDM-1 gene was located on a plasmid with a size of 47,094 bp, designated p19110F47-1 (Figure 2A). BLAST analysis revealed that p19110F47-1 showed similarities with two other plasmids, pAT232 (GN014838) and pGX7 (CP071772), present in the GenBank database. However, these similarities did not include the regions covering the antimicrobial resistance genes, nor those with the plasmid replication gene. Overall, a low query coverage (with the highest of 48%) (Supplementary Figure 1) was detected, suggesting that p19110F47-1 is a novel carbapenem resistance plasmid. Further analysis of the flanking regions of blaNDM-1 revealed that the insertion sequence ISAba125 was located upstream of blaNDM-1. Moreover, the blaNDM-1 gene was part of a region that contained also the resistance genes aac(3)-Ia, aphA6 and blcMBL (Figure 2A).

The blaOXA-58-carrying plasmid p19110F47-2 was 143,035 bp in size, also carried the aminoglycoside resistance genes aac(3)-IV, aph(3’)-la and aph(4’)-la, floR, sul2, the tetracycline resistance gene tet(M), the macrolide-lincosamide-streptogramin B resistance gene ermA(B), and the macrolide resistance genes mph(E) and msr(E) (Figure 2B). GenBank database searches identified the complete sequences of seven plasmids from Acinetobacter spp. with p19110F47-2 query coverage ranges from 40 to 87% (Supplementary Figure 2), and most of these plasmids harbored both blaOXA-58 and tet(X) orthologs (19, 20).

Conjugation experiments were performed using A. towneri 19110F47 as donor and two different E. coli strains as recipients.
No transconjugants were obtained, which might be explained by the fact that the blaNDM−1, but also the blaOXA−58-carrying plasmids lack a conjugal transfer region (Figure 2).

Discussion

Acinetobacter spp. are ubiquitous in the natural environment, and have become important opportunistic pathogens, e.g., A. towneri, A. baumannii, A. indicus, and A. Iwoffii. Acinetobacter spp. strains are widely distributed in a variety of environmental sources, including water, soil, foods, and livestock (15, 16, 21). In this study, we report the identification of an Acinetobacter spp. strain co-harboring blaNDM−1, blaOXA−58 and tet(X4) collected from food-producing animals in China. It belonged to Acinetobacter towneri, which is involved in nosocomial infections (16). Carbapenem-resistant Acinetobacter spp. is one of the most dangerous pathogens in the world (21). And Acinetobacter spp. was the major reservoir of tigecycline-resistant tet(X) genes (12). The co-location of tet(X) and carbapenem resistance gene blanDM−1 was previously described in Acinetobacter isolates from animals and the environment (14, 22). The strain 19110F47 had an expanded resistance profile. More attention should be paid to multidrug resistant (MDR) A. towneri isolates, because they have been reported increasingly in recent years, especially in hospital sewage and from livestock (23–26).

The tet(X4) gene conferring resistance to tigecycline was found to be on the chromosome of 19110F47. The floR and tet(X4) were found to be flanked by one truncated and two complete copies of ISVsa3. ISVsa3 is 977 bp in size and represents a member of the IS91 family. This family of insertion sequences differs from others in that they transpose by rolling circle transposition (27). Members of the IS91 family can mobilize genes, including resistance genes that are located in their close vicinity. For this, only a single copy of the IS91 element—not two copies as with most other insertion sequences—is necessary (27). The tet(X4) is the part of active TUs in the chromosomal DNA, may excise from the chromosomal DNA and transfer to other plasmidic or chromosomal sites. Of three TUs identified in A. towneri 19110F47, a sequence indistinguishable from that of TU2 was also found in p47EC (MK134376) from E. coli in a previous study (3), suggesting its mobility across genus boundaries. Of note, TU3 contained both tet(X4) and floR, implying that tigecycline resistance can be co-selected by florfenicol, a veterinary antimicrobial agent commonly used in cattle, pigs, poultry and fish (28).

The blanDM−1 located on the plasmid p19110F47-1. Insertion of blanDM−1 together with ISAba125 have been found
FIGURE 2
Structure of plasmids p19110F47-1 (A) and p19110F47-2 (B) from A. towneri 19110F47. The size scales are given in bp; genes are color-coded, depending on functional annotations: red, antimicrobial resistance; blue, plasmid stability; green, mobile elements; purple, plasmid replication; and black, other coding sequences (CDS).
within the chromosomal DNA and plasmids of *Acinetobacter* isolates (29, 30), suggesting that ISAb125 might be involved in the dissemination of *blaNDM−1* among *Acinetobacter* spp. Numerous studies have documented that the *blaNDM−1* was frequently present adjacent to truncated or intact ISAb125 as well as in other *Enterobacteriaceae*, including *E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Salmonella enterica* (31–33), indicating that ISAb125 plays a vital role in the spread of *blaNDM−1* among different species of bacteria.

The *blaOXA−58* was located on another plasmid p19110F47-2. An analysis for the genetic environments of *blaOXA−58* showed that a truncated ISAb3 element was located upstream of *blaOXA−58* and a complete ISAb3 element downstream of it. The genetic context found in the present study was consistent with that in plasmid pLHC22-2-tetX-162k (CP084298) in a previous study (19). In addition, the presence of two intact ISAb3 copies in opposite orientation up-stream and down-stream of *blaOXA−58* was described in a series of studies and led to the assumption that ISAb3 might play a role in the transfer of *blaOXA−58* (22, 34). It has been documented that transformation of a cloned *blaOXA−58* gene on a low-copy-number vector into a susceptible *A. baumannii* strain increased the MICs of carbapenems, but not to levels considered as resistant (35, 36). For clinical carbapenem resistance in *Acinetobacter* spp., other carbapenem resistance mechanisms in addition to OXA-58-like β-lactamases are required (35). In the present study, the *A. towneri* isolate 19110F47 showed resistance to meropenem with a MIC of 16 µg/mL. It was speculated that *blaNDM−1* played a dominant role in conferring the meropenem resistance phenotype.

The tet(X4), *blaNDM−1* and *blaOXA−58* genes have been documented in multiple studies solely or any two of them (14, 16, 20, 22, 37). Only one study by Zheng et al. described that a tet(X6) variant and the two carbapenemase genes *blaNDM−1* and *blaOXA−58* were located on the same plasmid in an *A. baumannii* isolate of chicken origin (15). In the current study, we identified the occurrence of tet(X4), *blaNDM−1* and *blaOXA−58* in the same *A. baumannii* isolate of swine origin, with tet(X4) being located on a novel chromosomal GI and *blaNDM−1* and *blaOXA−58* carried by two novel plasmids.

Conclusions

In conclusion, we described the co-existence of tet(X4), *blaNDM−1* and *blaOXA58* in a porcine *A. towneri* isolate that displays resistance to carbapenems and tigecycline among numerous other antimicrobial agents. The tet(X4) is part of active translocatable units in the chromosomal DNA, facilitating its transfer into other plasmidic or chromosomal sites. The emergence of chromosomal tet(X) genes combined with plasmid-mediated carbapenem resistance genes in the same isolate, as shown in this study, will further compromise the treatment options of severe infections caused by *Acinetobacter* spp.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Author contributions

X-DD and HY designed the research and supervised the study. AL, WZ, RY, and CL performed the experiments and analyzed the data. AL and HY wrote the manuscript. SS and X-DD reviewed and edited the manuscript. All authors revised the manuscript and approved the final version for submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2022.1002149/full#supplementary-material
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