



# Whole-genome sequencing of *Enterococcus hirae* CQP3-9, a strain carrying the phenicol–oxazolidinone–tetracycline resistance gene *poxtA* of swine origin in China

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## ARTICLE INFO

### Article history:

Received 2 June 2019

Accepted 9 June 2019

Available online 14 June 2019

### Keywords:

*Enterococcus*

*poxtA*

Linezolid

Multiresistance

Whole-genome sequencing

## ABSTRACT

**Objectives:** The aim of this study was to characterise the whole genome sequence of linezolid-intermediate *Enterococcus hirae* strain CQP3-9 isolated from a large-scale swine farm in Sichuan Province, China, in August 2018.

**Methods:** An Illumina MiSeq platform (400-bp paired-end reads with 230-fold average coverage) and PacBio RS II sequencing instrument (100-fold average read depth) were used for genome sequencing. The chromosome and two plasmids were assembled using the software SMRT portal v.3.2.0. Acquired antimicrobial resistance genes were identified using ResFinder 3.1.

**Results:** The genome of *E. hirae* strain CQP3-9 consists of one 2 695 881-bp chromosome, one 125 915-bp plasmid (pCQP3-9\_1) and one 33 132-bp plasmid (pCQP3-9\_2). The genome of CQP3-9 contains 2458 coding sequences and 89 RNA genes. The *poxtA* gene is the only linezolid resistance gene in CQP3-9, located on plasmid pCQP3-9\_2 that co-harbours *erm(B)* (macrolide resistance), *fexB* (chloramphenicol and florfenicol resistance), and *tet(M)* and *tet(L)* (tetracycline resistance).

**Conclusion:** Here we report for the first time the phenicol–oxazolidinone–tetracycline resistance gene *poxtA* in *E. hirae*, located on a plasmid that co-harbours *erm(B)*, *fexB*, *tet(L)* and *tet(M)*. The genome sequence of *E. hirae* CQP3-9 provides valuable information for the dissemination of *poxtA* among enterococci.

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## 1. Introduction

The emergence of linezolid-resistant enterococci has gained extensive attention as linezolid is considered as a last-resort antimicrobial for treating severe infections caused by Gram-positive bacteria, including vancomycin-resistant enterococci [1]. Resistance to linezolid in enterococci is mainly caused by the G2576T mutation (according to *Escherichia coli* numbering) in the 23S rRNA gene. The phenicol–oxazolidinone–tetracycline resistance gene *poxtA* was first reported in a clinical methicillin-resistant *Staphylococcus aureus* isolate in 2018 [2] and has also been

found in *Enterococcus faecium* and *Enterococcus faecalis* [3–5]. Here we report for the first time the *poxtA* gene in *Enterococcus hirae* and characterise the genome of *E. hirae* strain CQP3-9.

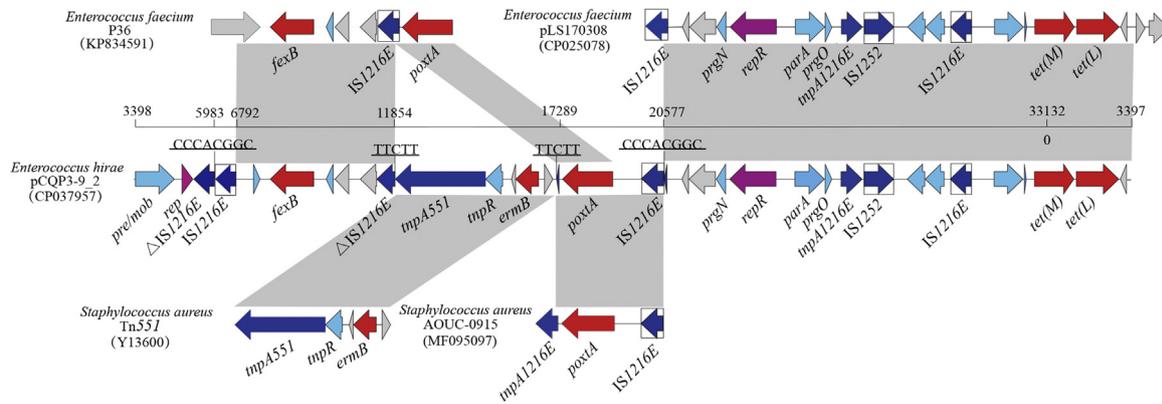
## 2. Methods

*E. hirae* strain CQP3-9 was isolated from a faecal swab from a large-scale swine farm in Sichuan Province, China, and was identified using an automated system (BD Diagnostic Systems, Sparks, MD). Antimicrobial susceptibility testing was performed by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) recommendations. The genome of *E. hirae* CQP3-9 was sequenced using an Illumina MiSeq platform (400-bp paired-end reads with 230-fold average coverage) and PacBio RS II sequencing instrument (100-fold average read depth). The chromosome and plasmids were assembled using the software SMRT portal v.3.2.0. Acquired antimicrobial resistance genes were

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**Fig. 1.** Schematic representation of *poxtA*-harbouring plasmid pCQP3-9\_2 from *Enterococcus hirae* CQP3-9. Structures are drawn to scale from GenBank accession nos. CP037957 (pCQP3-9\_2), KP834591 (*Enterococcus faecium* P36), CP025078 (pLS170308), Y13600 (Tn551) and MF095097 (*Staphylococcus aureus* AUC-0915). Genes and open reading frames (ORFs) are shown as arrows, and their orientations of transcription are indicated by arrowheads. Shared regions of >99% nucleotide sequence identity are indicated in light grey. Antimicrobial resistance genes are in red, transposase or integrase genes are in navy blue, hypothetical protein genes are in grey, replicon genes are in purple, and other genes are in light blue.

identified using ResFinder 3.1 (<https://cge.cbs.dtu.dk/services/ResFinder/>). A conjugation experiment was performed by filter mating using *E. hirae* CQP3-9 as the donor strain and rifampicin-resistant *E. faecalis* JH2-2 as the recipient strain with selection of transconjugants on brain–heart infusion agar plates containing 25 mg/L rifampicin and 8 mg/L florfenicol. Positive transconjugants were further determined by detecting the antimicrobial resistance profile and the presence of the *poxtA* gene.

### 3. Results and discussion

*E. hirae* strain CQP3-9 was intermediate to linezolid (4 mg/L) and tedizolid (1 mg/L) and was resistant to chloramphenicol (16 mg/L), florfenicol (32 mg/L), erythromycin (>128 mg/L), tetracycline (>128 mg/L) and doxycycline (>128 mg/L). The genome of *E. hirae* CQP3-9 contains 2458 coding sequences and 89 RNA genes predicted by the Prokaryotic Genome Annotation Pipeline (PGAP). Genome analyses showed that CQP3-9 consists of one chromosome and two plasmids. The chromosome is 2 695 881 bp in size with a GC content of 37.05%. Plasmid pCQP3-9\_1 has a total length of 125 915 bp with a GC content of 36.37%, and pCQP3-9\_2 is 33 132 bp in size with a GC content of 35.23%. Strain CQP3-9 carries the following antimicrobial resistance genes: *erm*(B) (macrolide resistance); *fexB* (chloramphenicol and florfenicol resistance); *tet*(M) and *tet*(L) (tetracycline resistance); *poxtA* (chloramphenicol, florfenicol, linezolid and tetracycline resistance); and *ant*(6)-*la* and *aac*(6)-*lid* (aminoglycoside resistance). To the best of our knowledge, this is the first report of *poxtA* in *E. hirae*.

The *poxtA* gene is the only linezolid resistance gene in CQP3-9, located on plasmid pCQP3-9\_2 that co-harbours *erm*(B), *fexB*, *tet*(M) and *tet*(L). pCQP3-9\_2 carries two replication-initiator genes (*repR* and *rep*), which belong to the *rep*<sub>2</sub> family and rolling circle replication initiator, respectively. BLAST analysis showed that the 15.95-kb region carrying *repR*, *tet*(M) and *tet*(L) (corresponding to bases 20 578–33 132 joint with 1–3197 in GenBank accession no. **CP037957**) was identical to the corresponding region of plasmid pLS170308 (accession no. **CP025078**) found in *E. faecium* of *Moschus berezovskii* (Forest muck deer) origin in Sichuan Province, indicating that these two plasmids may have the same origin.

The *poxtA* gene in pCQP3-9\_2 is flanked by insertion sequence IS1216E, which is similar to that in *S. aureus* strain AUC-0915 (Fig. 1). However, the IS1216E next to the 3'-end of *poxtA* is disrupted by transposon Tn551 (corresponding to bases 11 855–17 116 in GenBank accession no. **CP037957**), resulting in a 5-bp target site duplication (TTCTT). It is noteworthy that a 14.60-kb

IS1216E-mediated composite transposon (corresponding to bases 5983–20 577 in GenBank accession no. **CP037957**) that is flanked by an 8-bp target site duplication (CCCACGGC) and carries *poxtA*, *erm*(B) and *fexB*, is inserted into IS1216E (Fig. 1). Plasmid pCQP3-9\_2 could be successfully transferred into *E. faecalis* JH2-2, reducing susceptibility to linezolid (2 mg/L), tedizolid (0.5 mg/L), erythromycin (>128 mg/L), chloramphenicol (16 mg/L), florfenicol (16 mg/L) and doxycycline (>128 mg/L).

In conclusion, here we report the whole genome sequence of *poxtA*-carrying *E. hirae* strain CQP3-9. The *poxtA* gene is located on a 33.13-kb plasmid that co-harbours *erm*(B), *fexB*, *tet*(L) and *tet*(M). Co-existence of *poxtA* with other antimicrobial resistance genes on a conjugative plasmid in a Gram-positive bacterium is alarming given that it may lead to co-selection of these genes. The genome sequence of *E. hirae* CQP3-9 provides valuable information for the dissemination of *poxtA* among enterococci.

### 4. GenBank accession nos.

The complete genome of *E. hirae* strain CQP3-9 characterised in this study was submitted to GenBank and assigned accession nos. **CP037955** (CQP3-9 chromosome), **CP037956** (pCQP3-9\_1) and **CP037957** (pCQP3-9\_2).

### Funding

This work was supported by the National Key R&D Program of China [grant no. 2018YFD0500305], the Scientific Research Foundation of Sichuan University [grant no. 2017SCU12006] and the International Collaborative Program of Sichuan Province [grant no. 2018HH0027].

### Competing interests

None declared.

### Ethical approval

Not required.

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