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Genome Note

Draft genome sequence of a multidrug-resistant caprine isolate of *Staphylococcus cohnii* subsp. *urealyticus* from Tanzania encoding *ermB*, *tet*(K), *dfrG*, *fusF* and *fosD*

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ABSTRACT

Objectives: Coagulase-negative staphylococci such as *Staphylococcus cohnii* are opportunistic pathogens of humans and animals. A multidrug-resistant isolate of *S. cohnii* subsp. *urealyticus* (073AN) was isolated from the nasal cavity of a healthy goat in Tanzania. This study produced and analysed a draft genome sequence of strain 073AN to investigate the genetic basis for antimicrobial resistance in this isolate.
Methods: Strain 073AN was sequenced using HiSeq 2000 technology, sequencing reads were assembled using Velvet, and the genome was annotated using Prokka.
Results: The draft genome of strain 073AN is 2 677 652 bp in size with a GC content of 32.5%. The isolate was resistant to several classes of antimicrobials, which correlated with the presence of known antimicrobial resistance genes. Of particular note, the draft genome sequence of strain 073AN represents the first report of *fosD* in *S. cohnii* and the first descriptions of *fosD* and *fusF* in Africa.
Conclusion: The draft genome sequence of *S. cohnii* subsp. *urealyticus* 073AN released here provides an insight into the antimicrobial resistance determinants found in this species and in Tanzania and offers a valuable resource for further studies on staphylococcal genomics and antimicrobial resistance.

1. Introduction

Like many species of coagulase-negative staphylococci, *Staphylococcus cohnii*, comprised of two subspecies, namely *S. cohnii* subsp. *cohnii* and *S. cohnii* subsp. *urealyticus*, is a commensal organism and an opportunistic pathogen of humans and a range of animal hosts [1–4]. Antimicrobial resistance is widely reported among coagulase-negative staphylococci. In this study, the draft genome sequence of a multidrug-resistant caprine isolate of *S. cohnii* subsp. *urealyticus* from Tanzania was analysed to provide an insight into the genetic basis for its antimicrobial resistance.

2. Methods

Staphylococcus cohnii subsp. *urealyticus* strain 073AN was isolated on sheep blood agar from a nasal swab of a healthy goat in Nyakato, Tanzania, in March 2014. Its genome was sequenced using an Illumina HiSeq 2000 sequencing system as described previously [5]. The isolate was incidentally cultured during sampling of healthy animals for carriage of *Staphylococcus aureus*. Genome assembly was performed using Velvet [6] and the genome was annotated using Prokka [7]. Phenotypic profiling and antimicrobial susceptibility testing were performed with VITEK®2 using cards GP and AST-P620, respectively (bioMérieux, Basingstoke, UK) according to the manufacturer's instructions. Antimicrobial susceptibility testing was interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST) v.7.1 guidelines. Antimicrobial resistance genes were identified using

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ResFinder v.3.1 [8] with identity and length thresholds of 60% or by BLAST. Average nucleotide identity (ANI) (http://enve-omics.ce.gatech.edu/ANI/) [9] and genome-to-genome distance (GGD) (https://ggdc.dsmz.de/ggdc.php#) [10] were calculated using online tools with default settings.

3. Results and discussion

The draft genome of strain 073AN comprises 28 scaffolds and a total sequence length of 2 677 652 bp with a GC content of 32.5%. Coverage was 170-fold and the scaffold N_{50} is 471 861 bp. Strain 073AN was confirmed to belong to *S. cohnii* subsp. *urealyticus* on the basis of biochemical profile using VITEK®2 (93% probability). Regarding differentiation of the two *S. cohnii* subspecies, strain 073AN was positive for β-galactosidase and β-glucuronidase activities as well as acid production from lactose, all of which are indicative of *S. cohnii* spp. *urealyticus*. However, atypically for *S. cohnii* subsp. *urealyticus*, strain 073AN was negative for urease activity using both VITEK®2 and urease agar (Oxoid Ltd., Basingstoke, UK). Genes encoding the urease enzyme (*ureABC*), accessory proteins (*ureDEFG*) and urea transporter (*yut*) were present and intact in strain 073AN. Furthermore, their predicted proteins are identical or highly conserved (99% identity) with those of the *S. cohnii* subsp. *urealyticus* type strain DSM6718^T. Although present, these genes may not be expressed and the genetic basis for the apparent lack of phenotypic urease activity in strain 073AN is not yet clear. At the genetic level, comparison of partial 16S rRNA, *rpoB*, *dnaJ* and *tuf* gene sequences [11] were all consistent with the designation of strain 073AN as *S. cohnii* subsp. *urealyticus*. Finally, at the whole genome level, both ANI and GGD were also consistent with the designation of strain 073AN as *S. cohnii* subsp. *urealyticus*. Strain 073AN displays an ANI of 98.87% and a GGD of 90.00% compared with *S. cohnii* subsp. *urealyticus* DSM6718^T. The corresponding figures for comparison of strain 073AN with *S. cohnii* subsp. *cohnii* ATCC 49330^T are 90.00% and 46.30%.

ResFinder identified the following acquired antimicrobial resistance genes: *ermB* (100% match to **U18931**); *tet*(K) (98.55% match to **U38428**); *dfrG* (100% match to **AB205645**); and *fosD* (100% match to **KC989517**). Using VITEK®2, strain 073AN was susceptible to ciprofloxacin, daptomycin, gentamicin, linezolid, mupirocin, oxacillin, benzylpenicillin, rifampicin, teicoplANIn, tigecycline and vancomycin and was negative in the cefoxitin resistance screen. Corresponding with this phenotypic susceptibility profile, ResFinder did not identify any resistance determinants for these antimicrobial agents. Strain 073AN was resistant to clindamycin, erythromycin, fusidic acid, tetracycline and trimethoprim. Fosfomycin susceptibility was not included in the VITEK®2 panel and was therefore tested by Etest (bioMérieux), with strain 073AN being resistant showing a minimum inhibitory concentration (MIC) of 192 mg/L. Phenotypic resistance to clindamycin and erythromycin correlated with the presence of the *ermB* gene, as did resistance to tetracycline and trimethoprim in the case of *tet*(K) and *dfrG*, respectively. Likewise, fosfomycin resistance corresponded with the presence of *fosD*. To the best of our knowledge, this represents the first description of *fosD* in *S. cohnii* and the first report of this antimicrobial resistance determinant in Africa. No acquired resistance genes conferring resistance to fusidic acid were identified using ResFinder. However, the ResFinder 3.1 database at the time of use (March 2019) contained the resistance determinants *fusB* and *fusC* but not *fusD* or *fusF*. A BLAST search of the strain 073AN genome with *fusD* and *fusF* was therefore performed. Strain 073AN lacked *fusD*, which appears unique and intrinsic to *Staphylococcus saprophyticus* [12,13], but was positive for *fusF* with a 99% (641/645) match to the corresponding gene from the *S. cohnii* subsp. *urealyticus* ATCC 49330^T (**AB934903.1** position 2020–2664). The *fusF* gene has only been reported in *S. cohnii* with reports limited to Taiwan to date [13,14], thus this report of the draft genome of strain 073AN represents the first description of *fusF* in Africa.

Here we report the draft genome sequence of a multidrug-resistant veterinary isolate of *S. cohnii* subsp. *urealyticus* from Tanzania. The draft genome sequence provides a valuable resource for further studies on staphylococci and represents the first report of *fosD* in *S. cohnii* and the first reports of *fosD* and *fusF* in Africa.

Nucleotide sequence accession no

The draft genome sequence of *S. cohnii* subsp. *urealyticus* 073AN had been deposited at DDJB/ENA/GenBank under accession no. **FMPF01000000**. The version described in this paper is **FMPF00000000.1**. The HiSeq sequencing reads from strain 073AN are available under accession no. **ERR829963**.

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Competing interests

None declared.

Ethical approval

Not required.

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