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Complete genome sequence and antimicrobial resistance analysis of ESBL-producing *Shigella sonnei* carrying small cryptic plasmids isolated in northern Italy

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ABSTRACT

Objectives: Herein, we sequenced and assembled the genome of a *Shigella sonnei* isolate carrying several small plasmids using a hybrid approach that combined Oxford Nanopore Technologies and Illumina platforms.

Methods: Whole-genome sequencing was conducted using the Illumina iSeq 100 and Oxford Nanopore MinION systems, and the resulting reads were used for hybrid genome assembly via Unicycler. Coding sequences were annotated using RASTtk, while genes involved in antimicrobial resistance and virulence were identified using AMRFinderPlus. Plasmid nucleotide sequences were aligned to the NCBI non-redundant database using BLAST, and replicons were identified using PlasmidFinder.

Results: The genome consisted of 1 chromosome (4 801 657 bp), 3 major plasmids (212 849 bp, 86 884 bp, and 83 425 bp, respectively) and 12 small cryptic plasmids (ranging from 8390 bp to 1822 bp). BLAST analysis revealed that all plasmids were highly similar to previously deposited sequences. Genome annotation predicted 5522 coding regions, including 19 antimicrobial resistance genes and 17 virulence genes. Four of the antimicrobial resistance genes were located in small plasmids, and four of the virulence genes were located in a large virulence plasmid.

Conclusion: The presence of antimicrobial resistance genes in small cryptic plasmids may represent an overlooked mechanism for the propagation of these genes among bacterial populations. Our work provides new data on these elements that may inform the development of new strategies to control the spread of extended spectrum β-lactamase–producing bacterial strains.

1. Introduction

Shigella Spp. are a major cause of bloody mucoid diarrhoea, or shigellosis. While generally self-limiting, *Shigella* Spp. infections can lead to severe complications such as encephalopathy. *Shigella* Spp. strains producing extended spectrum β-lactamases (ESBLs) have been reported worldwide; to optimize clinical outcomes for individuals affected by shigellosis, new antimicrobial compounds are needed [1]. *Shigella* Spp. are known to harbour small cryptic plasmids (SCPs), which can be maintained in high copy numbers despite lacking a clear function [2]. These plasmids can integrate antimicrobial resistance genes (ARGs) in their backbone, facilitating their dissemination [3]. Herein, we describe the genome of an ESBL-producing *Shigella sonnei* clinical strain carrying an unusually high number of SCPs. We used a hybrid assembly approach to accurately characterise these plasmids.

2. Materials and methods

The *S. sonnei* strain, named SO87, was isolated in November 2021 from a stool sample collected from a patient hospitalised at Policlinico Sant'Orsola-Malpighi in Bologna, Italy. Identification was performed using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS; Bruker Daltonik, Germany). Antimicrobial susceptibility testing was performed using an automated MicroScan Walkaway 96 platform (Beckman Coulter, California). Minimum inhibitory concen-

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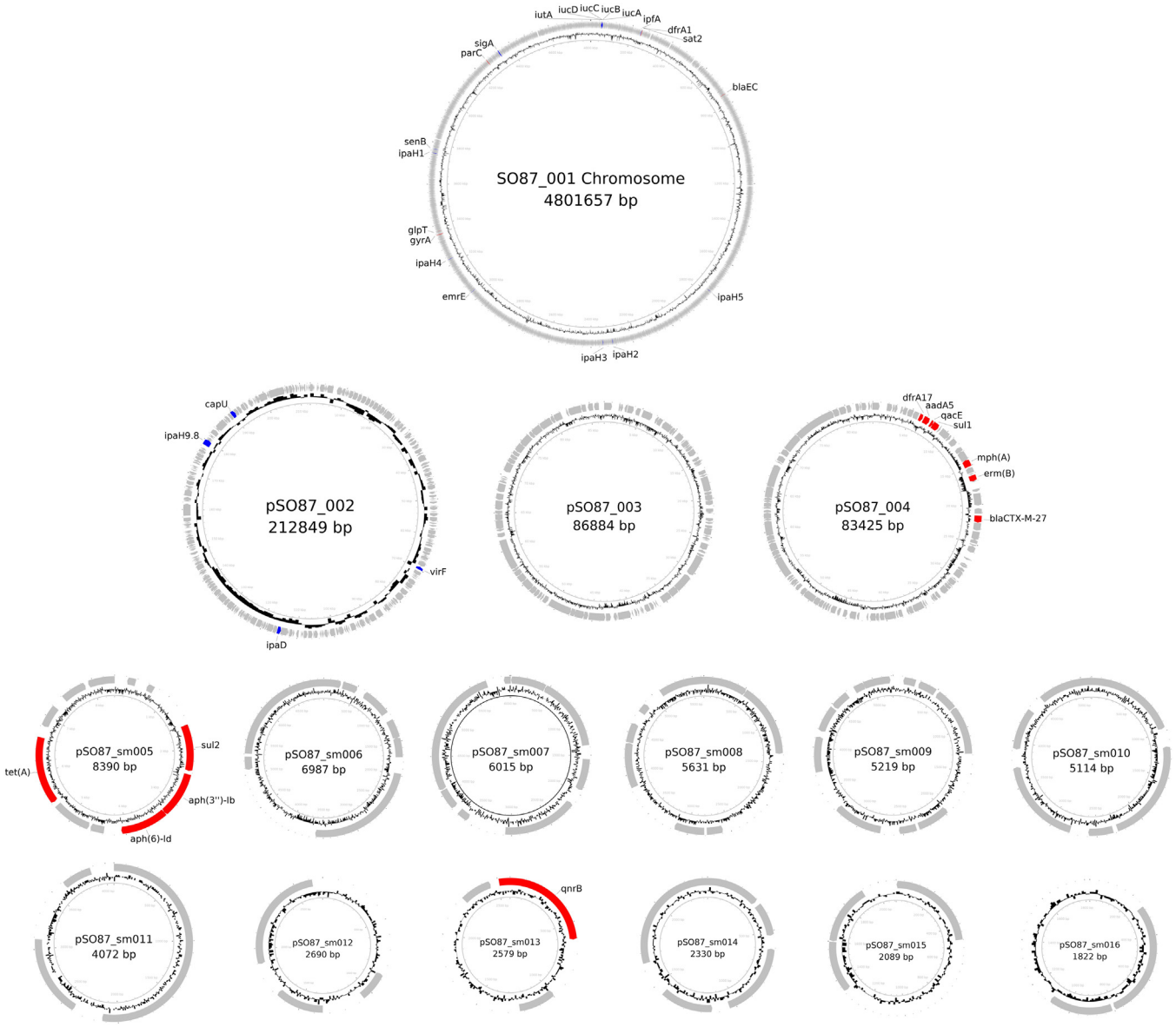

Fig. 1. Graphic representation of SO87 chromosome and plasmids. Coding sequences are depicted as arrows. Antimicrobial resistance genes are shown in red. Virulence genes are shown in blue.

tration (MIC) results were interpreted following the latest EUCAST Clinical Breakpoints v12.0 (available at https://www.eucast.org/clinical_breakpoints). The DNeasy Blood & Tissue Kit (Qiagen, Basel, Switzerland) was used to extract genomic DNA from a pure bacterial culture. Whole-genome sequencing was performed using the Illumina iSeq 100 platform (Illumina, USA) and the Oxford Nanopore MinION device (Oxford Nanopore Technologies, UK). The Nextera DNA Flex Library Preparation Kit was used to prepare the library for Illumina sequencing, while the Rapid Sequencing Kit (SQK-RAD004) was used for Nanopore sequencing. Reads underwent quality control with FastQC v0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and were used for hybrid genome assembly with Unicycler v0.5.0 (https://github.com/rrwick/Unicycler). The assembly quality was assessed with Bandage v0.8.1 (https://github.com/rrwick/Bandage). Additionally, the correctness of plasmid nucleotide sequences was verified by BLAST alignment against the non-redundant NCBI nucleotide database. Sequence type identification was performed using MLST v2.11 (https://github.com/tseemann/mlst) by scanning the assembly file against PubMLST schemes (https://pubmlst.org/). Coding sequences were annotated using RASTtk v2.0 (https://rast.nmpdr.org/). AMRFinderPlus v3.10.45 (https://github.com/ncbi/amr) was used for identification of ARGs and virulence genes (Identity >98%, Query Cover >70%). Plasmid replicons were identified using PlasmidFinder v2.1 (https://cge.food.dtu.dk/services/PlasmidFinder/).

3. Results

Antimicrobial susceptibility tests showed that the *S. sonnei* strain SO87 was resistant to most β-lactams, including third- and fourth-generation cephalosporins (cefotaxime, ceftazidime, and cefepime), as well as ciprofloxacin and sulfamethoxazole/trimethoprim. The strain remained susceptible to piperacillin/tazobactam, amoxicillin/clavulanic acid, and carbapenems (Table S1 in the Supplementary Material).

Whole-genome sequencing was performed to identify resistance determinants and characterise the complete genome of the multidrug resistance phenotype. A total of 2 668 782 paired end

reads were produced by Illumina sequencing, while Nanopore sequencing yielded 211 506 sequences, ranging from 110–313 400 bp (mean, 4568 bp). Hybrid genome assembly generated a circular genome of 5 237 753 bp with a coverage depth of 261 × and a 50.82% GC content. MLST analysis demonstrated that *S. sonnei* strain SO87 belonged to ST152. The strain was composed of 1 chromosome of 4 801 657 bp; 3 major plasmids of 212 849 bp, 86 884 bp, and 83 425 bp, respectively; and 12 small cryptic plasmids (SCPs) ranging from 8390 bp to 1822 bp. BLAST alignment indicated that the 212 849 bp plasmid (pSO87_002) had a high degree of homology to pSS (CP000039.1), a large virulence plasmid necessary for the invasion of epithelial cells (query cover 100%, identity 99.94%) [4]. In addition, all SCPs exhibited high homology to previously deposited plasmids (query cover 100%, identity > 98%). Genome annotation predicted 5522 coding regions, 460 (8.33%) of which encoded for hypothetical proteins. Nineteen ARGs were identified that confer resistance to aminoglycosides (*aadA5, aph(3")-Ib, aph(6)-Id*), β-lactams ($bla_{CTX\text{-}M\text{-}27}$, bla_{EC}), fosfomycin (*glpT*), macrolides (*erm(B), mph(A)*), quaternary ammonium compounds (*qaceEδ1*), quinolones (*gyrA, parC, qnrB*), streptothricin (*sat2*), sulphonamides (*sul1, sul2*), tetracyclines (*tet(A)*), trimethoprim (*dfrA1, dfrA17*), and the multidrug efflux pump *emrE*. Three of these genes [*aph(3")-Ib, aph(6)-Id, sul2, tet(A)*] were located in plasmid pSO87_sm005, and one, (*qnrB*) in pSO87_sm013. A total of 17 genes with roles in virulence were also identified, 4 (*capU, ipaD, ipaH9.8, virF*) of which were situated in the pSO87_002 large virulence plasmid. We also detected 91 genes encoding for tRNAs and 22 genes encoding for rRNAs (Figure 1 and Table S2).

4. Discussion

Herein, we characterised the complete genome of an ESBL-producing *S. sonnei* clinical strain harbouring several SCPs. SCPs, known to be commonly transferred between strains of microbiota, have been proposed to act as carriers of ARGs [5]. This work provides a complete genomic analysis of an *S. sonnei* strain carrying several SCPs and increases our knowledge of these plasmids. More research is needed to fully understand their role in the spread of antimicrobial resistance.

Data availability: The SO87 complete genome was deposited in the NCBI database (accession number SAMN32329321) and is freely accessible.

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Competing interests: None declared.

Ethical approval: Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2023.02.015.

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