Journal of Global Antimicrobial Resistance 33 (2023) 218–220

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Genome Note

The first description of the complete genome sequence of multidrug-resistant *Salmonella enterica* serovar monophasic Typhimurium (1,4,[5],12:i:-) isolate with the *mcr-1.1* gene on IncHI2 found in pig in Poland

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ARTICLE INFO

Article history:
Received 15 February 2023
Revised 26 March 2023
Accepted 16 April 2023
Available online 20 April 2023

Editor: Stefania Stefani

Keywords:
colistin resistance
mcr-1
Salmonella
pig
IncHI2

ABSTRACT

Monophasic *Salmonella* Typhimurium (1,4,[5],12:i:-) is one of the leading *Salmonella* serovars causing human salmonellosis in Europe. It has been observed in Poland since 2008. This serovar is considered the one with the highest rate of *mcr* prevalence. This report presents a sequence characteristic of the multidrug-resistant (MDR) monophasic *S.* Typhimurium isolated from a pig faecal sample with the confirmed presence of the *mcr-1.1* gene. The genome was assembled into the complete chromosome and 4 plasmids: IncHI2 (232 119 bp), IncFIB/IncFIC (133 901 bp), ColRNAI (6659 bp), and Col8282 (4066bp). The strain identified as ST34 carried multiple antimicrobial resistance genes located both on chromosome (tet(B)) and plasmids: *mcr-1.1* and *bla*TEM-1B on ST4-IncHI2, and *mef*(B), *bla*TEM-1B, *aadA1, qacL, dfrA12, aadA2, cmlA1, sul3, tet(*M*)* on IncFIB/FIC. The *mcr-1.1* gene was previously identified in *E. coli* deriving mainly from poultry, but this is the first case of the occurrence of *mcr*-positive *Salmonella* in Poland. The obtained results of analysis of the genome content draw attention to the problem of multidrug-resistant pathogens, especially in the context of resistance to colistin which is a last-resort antimicrobial.

1. Introduction

Monophasic *Salmonella* (*S.*) Typhimurium (1,4,[5],12:i:-), a worldwide emerging pathogen, is one of the leading *Salmonella* serovars causing human salmonellosis and the most common serovar among pig and pork isolates in Europe[1]. This variant has been occurring in Poland since 2008 mainly in pigs[2,3]. Resistance to the last resort antimicrobials, including colistin, has become a challenging problem that poses a threat to public health. That challenge has become even greater after discovering the mobile colistin resistance (*mcr*) genes. The *mcr* genes are now also identified in different *Salmonella* serovars, with monophasic *S.* Typhimurium considered as the serovar with the highest rate of the *mcr* prevalence[4]. This phenomenon is noted globally including European countries[4]. Many studies have focused on the detection and characterization of the new variants of this gene and ways of transmission in different bacteria species. Here we report sequence characteristics of multidrug-resistant (MDR) monophasic *S.* Typhimurium isolate with the *mcr-1.1* gene located on a specific transmissible plasmid.

2. Materials and Methods

The strain was isolated in 2014 from a fecal sample collected during the national *Salmonella* monitoring program in pigs according to PN-EN ISO 6579:2003/A1:2007. The pure culture was confirmed to genus level on Matrix-Assisted Laser Desorption Ionization-Time Of Flight (MALDI-TOF) using the extraction method following the producer guidelines (Bruker Daltonik GmbH) and serotyped according to the White-Kaufmann-Le Minor scheme.

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https://doi.org/10.1016/j.jgar.2023.04.008

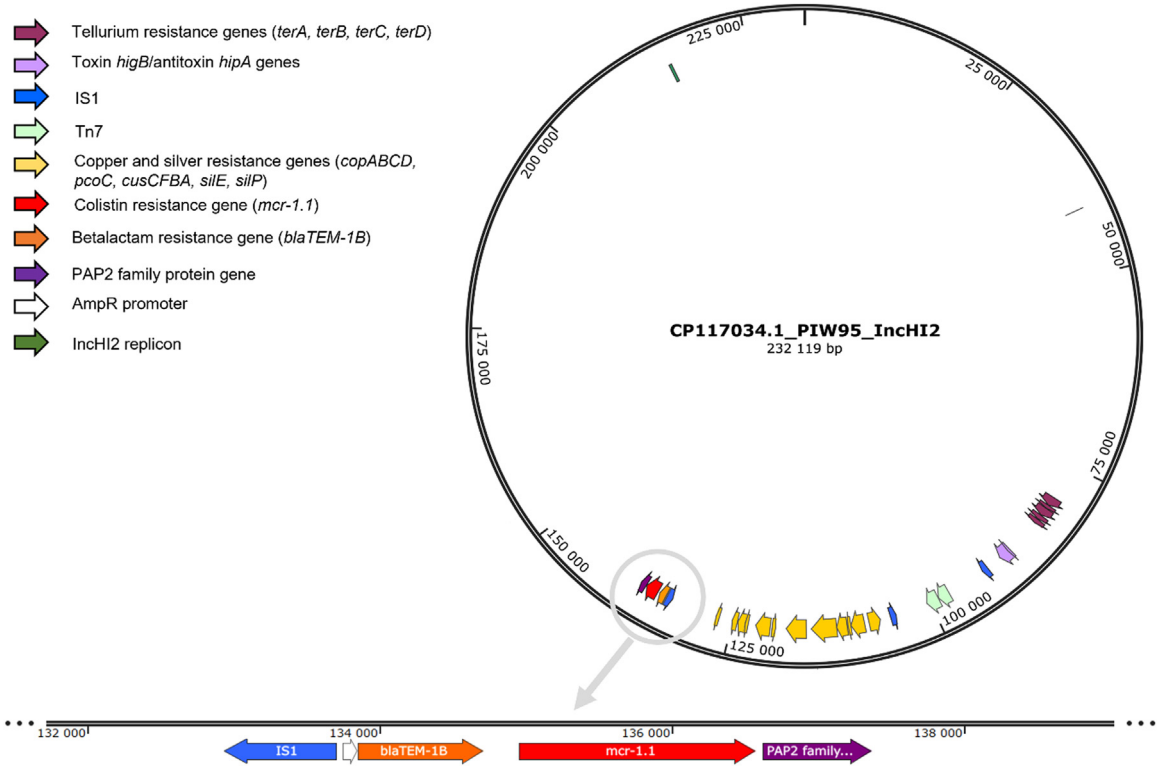

Figure 1. Characterization of the IncHI2 plasmid with the genetic environment of the *mcr-1.1* gene

Antimicrobial susceptibility testing was performed with the microbroth dilution method (Sensititre EUVSEC plates; TREK Diagnostic Systems, Thermo Fisher Scientific, USA). Epidemiological cut-off values (ECOFFs) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for minimal inhibitory concentration (MICs) obtained for 14 compounds representing 9 antimicrobial classes: beta-lactams, quinolones, phenicols, aminoglycosides, folate-path inhibitors, tetracyclines, polymyxins, macrolides, and glycylcyclines.

DNA was extracted from overnight pure nutrient agar culture at 37°C for 24 h using Maxwell Rapid Sample Concentrator (RSC) cultured cells DNA Kit (Promega). Quantity and quality of DNA were assessed by Qubit 3.0 (Thermo Fisher Scientific) and capillary electrophoresis using Fragment Analyzer (Agilent). Short- and long-fragment libraries were constructed using the KAPA HyperPlus Kit (Roche) and Ligation sequencing kits (SQK-LSK109; Oxford Nanopore), respectively. Whole genome sequencing was performed on the MiSeq (v3 2 × 300bp, Illumina) and MinION (Oxford Nanopore) in parallel. QC of short reads was checked using fastp 0.20.0 (https://github.com/OpenGene/fastp), of long reads with Porechop 0.2.4 (https://github.com/rrwick/Porechop). The genome was assembled by the Unicycler v0.4.8 (https://github.com/rrwick/Unicycler), polished with Pilon v1.24 (https://github.com/broadinstitute/pilon), mapped with bowtie2 v2.4.4 (https://github.com/BenLangmead/bowtie2), and annotated using the RAST Server (https://rast.nmpdr.org/). Bioinformatic tools from the Center of Genomic Epidemiology (CGE) (http://www.genomicepidemiology.org/services) have been used to determine MLST type (MLST 2.0), detect plasmids replicons (PlasmidFinder 2.1; thresholds: 80% identity and 80% coverage), pDLST (pMLST 2.0; database version: 2023-02-27), the resistance genes and the mobile genetic elements (MGE v1.0.3; database version: v1.0.2; 95% identity and 90% coverage). The SnapGene 6.1.2 software (https://www.snapgene.com) was used for the visualization of the gene features with a manual curation for the obtained annotation.

3. Results and discussion

Monophasic *S.* Typhimurium PIW95 exhibited MDR phenotype including ampicillin, chloramphenicol, colistin, sulfamethoxazole, tetracycline, and trimethoprim. The total assembly size was 5 375 002 bp with the N50 value of 499 8257 bp and a GC content of 51.8%. The genome of the strain PIW95 was assembled into the complete chromosome and 4 plasmids: ST4-IncHI2 (232 119 bp), IncFIB/IncFIC (133 901 bp), ColRNAI (6659 bp), and Col8282 (4066bp). The MLST analysis showed that the strain represented ST34. Multiple resistance genes were identified as followed: *mcr-1.1, blaTEM-1B, mef(B), aadA1, qacL, dfrA12, aadA2, cmlA1, sul3, tet*(M), and *tet*(B) which were in agreement with the observed resistance phenotype. BLAST analysis showed the presence of the gene cassette containing IS1, *blaTEM-1B*, and *mcr-1.1* gene followed by PAP2 family protein which was located on the IncHI2 plasmid (Figure 1). Moreover, multiple resistance genes to copper and silver were identified. IncHI2 is a common type of large *mcr-1*-carrying plasmid that have been found worldwide[4]. Plasmid IncFIB/FIC(AP001918) carried the following resistance genes: *aadA1, aadA2, blaTEM-1B, cmlA1,* dfrA12, *mef(B), sul3,* and *tet*(M). The *tet*(B) gene was located on the chromosome.

Although the *mcr-1.1* gene was identified in *E. coli* from poultry in Poland[5], this is the first case of the occurrence of *mcr*-positive *Salmonella* in the country. Notably, identified plasmid carried genes coding tolerance to metals which were not found in IncHI2 of previously studied *E. coli* (data not published). The analyzed genome content draws attention to the problem of multidrug-resistant pathogens isolated from livestock and a potential threat to human health.

GenBank accession

The complete genome of monophasic *Salmonella* Typhimurium strain PIW95 was assigned GenBank project number **PRJNA928386**

and accession numbers **CP117033-CP117037**. The strain was included in the reference collection created in the CARE project. The information about the isolate and its availability is located on a website: https://cirmbp.bio-aware.com (strain ID CARE_00166).

Ethical approval

Not required.

Funding

This work was supported by funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 773830: One Health European Joint Programme and from the Ministry of Education and Science funds for science in 2018-2022 allocated to the implementation of an international co-financed project, agreement No. 3932/H2020/2018/2.

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