COMPLETE GENOME SEQUENCE OF COLISTIN-SUSCEPTIBLE SALMONELLA ENTERICA SEROVAR MINNESOTA STRAIN HARBOURING MCR-9 ON AN INCHI2/INCHI2A PLASMID ISOLATED FROM CHICKEN MEAT IN RIYADH, SAUDI ARABIA.

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INTRODUCTION

The extensive use and misuse of the antibiotic colistin in agriculture and poultry farming played an important role in the development and dissemination of plasmid-mediated mobile colistin resistance (mcr) genes via horizontal gene transfer. Although Salmonella is one of the major pathogens responsible for foodborne illnesses worldwide, there is minimal information regarding the presence of mcr genes in Salmonella strains. To date, 10 mcr genes have been described (mcr-1 to mcr-10) in different bacteria isolated from different animals, humans, foods and environments. The mobile colistin-resistance (mcr-9) gene was first detected in Salmonella Typhimurium, which highlighted the need for rigorous monitoring of the potential spread of this new gene. Here, we determined the complete genome sequence of SA18578, the mcr-9-positive Salmonella enterica serotype Minnesota, which was isolated from commercial chicken meat in Saudi Arabia in 2020. To our knowledge, there has been no previous identification of the presence of the mcr-9 gene in this strain.

METHODOLOGY

The strain was isolated and confirmed to belong to Salmonella serotype Minnesota using the traditional cultural and molecular methodology. Minimum inhibitory concentrations (MICs) were determined using standard broth microdilution (BMD) according to Clinical and Laboratory Standards Institute (CLSI) standards. The SA18578 isolate was cultured and genomic DNA was extracted from a single starting colony using the QIAamp DNA Mini kit following the manufacturer's instructions. Short-read sequencing was generated using MiSeq Illumina technology with the MiSeq V3 kit using 2 × 300 base pair paired-end chemistry (Illumina, San Diego, CA, United States). Long-read sequences were generated using Oxford Nanopore Technology using the ligation sequencing kit SQK-LSK109 on the MinION sequencer (Oxford Nanopore Technologies, Ltd., United Kingdom). The short and long sequence reads of the SA18578 isolate were combined following the EToKi pipeline, generating five contigs with an average sequencing coverage depth of 40×. The NCBI

Prokaryotic Genome Annotation Pipeline was used for the annotation of the SA18578 chromosome and plasmid sequences.

RESULTS

The results showed that SA18578 was resistant to Amoxicillin/Clavulanic Acid (MIC, ≥32 mg/L), ampicillin (MIC, ≥32 mg/L), gentamicin (MIC, ≥16 mg/L), Sulfisoxazole (MIC, ≥256 mg/L) and Tetracycline (MIC, 32 mg/L). The BMD method was also performed to determine the MIC of colistin (MIC ≤ 1 mg/L) and, interestingly, exhibited susceptibility to colistin (MIC ≤ 1 mg/L).

The genome of SA18578 consisted of one 4,826,311 bp chromosome with a GC content of 52 %, and four plasmids designated as pSA18578_MCR9_1 (IncHI2/IncHI2A: 352,141 bp, 48 % GC content), pSA18578_2 (IncA/C2: 129,660 bp, 51 % GC content), pSA18578_3 (Col440I: 3,228 bp, 48 % GC content), pSA18578_4 (3,383 bp, 55 % GC content). The in silico MLST analysis indicated that the SA18578 strain belonged to sequence type (ST) 548. Plasmid pSA18578_MCR-9_1 carried the colistin-resistant mcr-9 gene and belonged to the incompatibility group IncHI2/IncHI2A and plasmid multilocus sequence typing (pMLST) subtype ST1. Plasmid pSA18578_MCR-9_1 coharboured genes encoding resistance to aminoglycosides and β-Lactams (blaTEM-1B and aac (3)-IId, respectively). Further resistant genes were detected located either on the chromosome or the plasmids.

DISCUSSION

Based on our results, it appears that the presence of the mcr-9 gene is actually not associated with colistin resistance. A few studies have investigated the role of the mcr-9 gene in colistin resistance and concluded that this gene is neither inducible nor expressed under normal conditions. Therefore, further studies are necessary to determine the impact of the mcr-9 gene on colistin susceptibility. In summary, this is the first report of the S. Minnesota strain harbouring the colistin-resistant mcr-9 gene isolated from commercial chicken meat, which will be useful for future epidemiological studies concerning investigations into the spread of plasmid-mediated colistin resistance worldwide.