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First whole genome sequencing of *Escherichia coli* carrying *mcr-1* from pig in Argentina

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A B S T R A C T

The objective of this study is to communicate the findings of the first whole genome sequencing of a colistin-resistant *Escherichia coli* isolate harboring *mcr-1* gene obtained from a pig in Argentina. Genomic DNA was sequenced using the MinION Oxford Nanopore platform. The libraries were prepared using a SQK-RBK110-96 protocol. The sequencing process was conducted on a MinION Mk1C MIN 101-C, utilizing a FLO-MIN106 flow cell. The quality of the reads was evaluated using NanoPlot. *De novo* assembly was conducted using Canu 1.6 and the quality of contigs was evaluated using QUAST. Annotation was performed using Prokka. The CBC20 strain exhibited a colistin MIC of 4 µg/mL. The genome size was 5178653 bp with a GC content of 50,31%. The N50 value was 133,250, while the L50 value was 21. A total of 11,620 genes, 11,518 coding sequences, 77 transfer RNAs and 24 ribosomal RNAs were identified. A serotype O9:H37 with sequence type ST-297 was observed. A total of seven antimicrobial resistance genes were identified, including *mcr-1.5*, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{EC-18}}$, *bla*$_{\text{TEM-70}}$, *aph(3')-Ia*, *mph(A)* and *sul3*. The presence of punctual mutations was observed in the genes encoding the proteins *GyrA* (S83L, D87N) and *ParC* (S80I). Five distinct plasmid replicon types were identified, including IncFII, IncY, IncFIB, IncX1 and Col440II. Our findings may assist in the comprehension of the mechanisms of antimicrobial resistance, genomic epidemiology and dissemination of *mcr-1* gene among animals and environment, which could potentially impact human health.

Keywords: Colistin, antibiotic resistance, one health, next-generation sequencing, (*Source: MeSH*).

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Primera secuenciación de genoma completo de *Escherichia coli* portadora de *mcr-1* en un cerdo en Argentina

R E S U M E N

El objetivo de este estudio es comunicar la primera secuenciación de genoma completo de un aislamiento de *Escherichia coli* resistente a colistina mediada por el gen *mcr-1* obtenido de un cerdo en Argentina. El ADN genómico se secuenció utilizando la plataforma MinION Oxford Nanopore. Las bibliotecas se prepararon utilizando un protocolo SQK-RBK110-96. El proceso de secuenciación se realizó en un MinION Mk1C MIN 101-C, utilizando una flow cell FLO-MIN106. La calidad de las lecturas se evaluó mediante NanoPlot. El ensamblaje *de novo* se realizó utilizando Canu 1.6 y la calidad de los contigs se evaluó utilizando QUAST. La anotación se realizó utilizando Prokka. CBC20 exhibió una CIM de colistina de 4 µg/mL. El tamaño del genoma fue de 5.178.653 pb con un contenido de GC del 50.31 %. El valor N50 fue 133.250, mientras que el valor L50 fue 21. Se identificaron un total de 11.620 genes, 11.518 secuencias codificantes, 77 ARN de transferencia y 24 ARN ribosómicos. Se observó el serotipo O9:H37 con un secuenciotipo ST-297. Se identificaron siete genes de resistencia, incluyendo *mcr-1.5*, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{EC-18}}$, *bla*$_{\text{TEM-70}}$, *aph*(3')-*Ia*, *mph(A)* y *sul3*. Se observó la presencia de mutaciones puntuales en los genes que codifican las proteínas *GyrA* (S83L, D87N) y *ParC* (S80I). Se identificaron cinco tipos distintos de plásmidos, incluidos IncFII, IncY, IncFIB, IncX1 y Col440II. Nuestros hallazgos podrían ayudar a comprender los mecanismos de resistencia antimicrobiana, la epidemiología genómica y la diseminación del gen *mcr-1* entre animales y el medio ambiente, lo que potencialmente podría afectar la salud humana.

Palabras clave: colistina, resistencia antibiótica; una salud, secuenciación de próxima generación (*Fuente: MeSH*).

INTRODUCTION

The "One Health" initiative represents a transdisciplinary approach to the treatment of human health, animal health, and ecosystem health[1]. Antimicrobial resistance represents an increasing public health concern worldwide. The extensive utilization of antibiotics in animals has selected for the emergence of multidrug-resistant (MDR) bacteria, which can cause the occurrence of severe infections within human medicine[2].

Colistin is a polycationic polypeptide that is employed in the treatment of human infections as a last resort antimicrobial agent. In veterinary medicine, it has been used extensively as a growth stimulant in both porcine and poultry production[3]. Furthermore, colistin has been employed for the prophylaxis, metaphylaxis and treatment of enteric diarrhea in pigs[4]. A novel mobile colistin resistance gene (*mcr-1*) was recently reported in food, humans, and pigs from China. Subsequently, the *mcr-1* gene was identified in humans and animals in various countries around the world[5].

Bacterial genome sequencing is an appropriate tool for epidemiological surveillance and the genomic characterization of antibiotic resistance. This study presents the first whole genome sequencing (WGS) of an *Escherichia coli* strain *mcr-1* positive in a pig from Argentina. The objective of this study was to perform a molecular characterization of a colistin-resistant *E. coli* isolate carrying the *mcr-1* gene, in order to detect resistance genes, plasmids and virulence genes.

MATERIALS AND METHODS

Bacterial isolate and study site. One *mcr-1*-producing *E. coli* isolate (CBC20) obtained from a bacterial collection, was analyzed. This strain was selected for its multidrug resistance and was recovered from a rectal swab of a healthy fattening pig in Chaco Province, Argentina during 2021.

Antimicrobial susceptibility testing. The minimal inhibitory concentration (MIC) values were determined by broth microdilution method using the Sensititre® (Thermo Fisher, USA) system, in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI)[6]. The following antibiotics were tested: ampicillin, ampicillin/sulbactam, cephalotin, cefotaxime, ceftazidime, cefepime, piperacillin/tazobactam, ciprofloxacin, gentamicin, amikacin, chloramphenicol, trimethoprim/sulfamethoxazole, tetracycline, imipenem, meropenem, nitrofurantoin, colistin and tigecycline.

Whole genome sequence analysis. Genomic DNA was extracted using the INBIO Highway® ADN PuriPrep-B kit in accordance with the manufacturer's instructions. The quality of the DNA was analyzed by measuring the absorbance ratio A260/280 and A260/230 using the Nanodrop ND-1000 spectrophotometer (Thermo Fisher, USA). Prior to sequencing, the quantity of DNA was determined using a Qubit 2.0 (Thermo Fisher, USA) and the DNA molecules were concentrated and purified with magnetic beads. Library preparation commenced with 480 ng genomic DNA, in accordance with the Nanopore protocol (SQK-RBK110-96). The sequencing process was conducted on a MinION Mk1C MIN 101-C, utilizing a FLO-MIN106 flow cell (Oxford Nanopore Technologies, UK) for a period of 24 hours.

Analysis of DNA sequence data. The high-accuracy basecalling process was conducted using Guppy v6.1.3. NanoFilt v2.8 was employed to eliminate sequences of length less than 1,000 bp and with a Q-value less than 10[7]. The quality of the reads was evaluated using NanoPlot v1.20.0[7]. De novo assembly was conducted using Canu v1.6[8] with statistics obtained using QUAST v5.0.2[9] and annotation performed with Prokka[10]. The serotype was determined using SerotypeFinder 2.0. The clonal typing of CBC20 was performed using the MLST 2.0 database. To identify resistance genes and plasmids, ResFinder 3.0 and PlasmidFinder 2.1 were employed, respectively. The VirulenceFinder software was employed to identify virulence factor genes (VFGs). The chromosomal point mutations responsible for quinolone resistance were identified through the use of PointFinder 2.2. The genome sequences of the CBC20 have been deposited in the NCBI database under the accession number JAPDFU000000000.1.

RESULTS

Antibacterial susceptibility profile. CBC20 exhibited resistance to ampicillin (MIC≥16 µg/mL), ampicillin/sulbactam (MIC≥16/8 µg/mL), cephalotin (MIC= 32 µg/mL), ciprofloxacin. (CIM>2 µg/mL), trimethoprim–sulfamethoxazole (CIM>2/38 µg/mL), tetracycline (CIM >16 µg/mL) and colistin (CIM= 4 µg/mL). Furthermore, susceptibility to chloramphenicol, cefotaxime, ceftazidime, meropenem, imipenem, gentamicin, amikacin, nitrofurantoin, fosfomycin, and tigecycline was observed (Table 1). CBC20 was categorized with a multidrug-resistance (MDR) profile, defined as a resistance to one or more antibiotics belonging to three or more distinct drug groups.

Table 1. Antibacterial susceptibility of CBC20 isolate.

Antimicrobial agent	MIC value (µg/mL)	Phenotype
Colistin	4	R
Ampicilin	≥16	R
Ampicillin- Sulbactam	≥16/8	R
Cefalotine	32	R
Cefotaxime	≤1	S
Ceftazidime	≤2	S
Cefepime	≤2	S
Meropenem	≤1	S
Imipenem	≤0,5	S
Ciprofloxacin	≥2	R
Gentamicin	≤2	S
Amikacin	≤8	S
Chloramphenicol	>16	R
Tetracycline	>16	R
Trimetroprim- Sulfamethoxazole	≤2/38	S

MIC: Minimal inhibitory concentration, S: Susceptible, R: Resistant.

WGS analysis of CBC20 isolate. The purity of the DNA was demonstrated by the absorbance ratios 260/280 nm of 1.8 and 260/230 nm of 2.1, respectively. A total of 752,923 reads were obtained, with an average length of 4,123 bp. The average Q value was 13.1, with Q10 and Q20 reads quality values of 84.5% and 43.2%, respectively. The complete genome was assembled into 5,178,653 bp, comprising 266 contigs and a GC content of 50.3%. The longest contig was 23,982 bp. The sequencing depth was 42X, while the N50 value was 133,250 and the L50 value was 21. A total of 11,620 genes, 11518 coding sequences, 77 transfer RNAs and 24 ribosomal RNAs were identified. *In silico* typing and MLST indicate that CBC20 belongs to the O9:H37 serotype with sequence type (ST) 297. ResFinder analysis revealed the presence of seven resistance genes: *mcr-1.5*, *bla*$_{\text{TEM-1B}}$, *bla*$_{\text{TEM-70,}}$ aph(3')-Ia), mph(A) and *sul3* (Table 2). Single point mutations in the quinolone-resistant determining regions (QRDR) were identified, resulting in the amino acid substitutions S83L and D87N in the GyrA protein (98,86% identity) and S80I in the ParC protein (99,07% identity). Plasmid Finder revealed that the CBC20 strain harboured five distinct plasmids, including IncFII, IncY, IncFIB, IncX1 and Col440II (Table 2). The *mcr-1* gene was identified in contig 185, with no evidence of plasmid replicons present in the region surrounding the gene. In contrast, the genes *bla*$_{\text{TEM-1B}}$, aph(3')-Ia and *sul3* were located in the same contig as the IncFIB replicon type. A virulence profile analysis of the strain revealed the presence of 48 VFGs, including type 1 fimbriae regulatory protein (fimB), chaperone protein precursor (fimC), FimH protein precursor (fimH) and isochorismate synthase 1 (entC), with >98,0% identity.

Table 2. Resistance determinants and plasmids profile of *E. coli* isolate.

Sequences	Contig	Alignment Coverage	Identity (%)	Accession Number
mcr-1.5	contig_185	1-1626/1626	99.75	KY283125
bla$_{TEM-1B}$	contig_203	1-861/861	99.30	AY458016
bla$_{TEM-70}$	contig_202	1-861/861	99.19	AF188199
aph(3')-Ia	contig_203	1-816/816	99.63	V00359
mph(A)	contig_61	1-1233/1233	97.73	Y08743
sul3	contig_203	1-792/792	99.37	AJ459418
IncFII	contig_228	1-499/499	98.20	AP001918
IncY	contig_201	1-765/765	98.43	K02380
IncFIB	contig_203	1-682/682	98.97	AP001918
IncX1	contig_19	1-374/374	98.40	EU370913
Col440II	contig_129	1-282/282	99.65	CP023921.1

DISCUSSIONS

In this study, we conducted a WGS analysis of a colistin-resistant *mcr-1*-positive *E. coli* strain isolated from a pig. To the best of our knowledge, this would be the first report of a whole genome sequencing of an isolate of *E. coli* carrying the *mcr-1* gene from swine in Argentina. WGS allows for the rapid sequencing of millions of DNA fragments simultaneously, thereby providing comprehensive insights into genome structure, genetic variations, clonality profiles and coding regions present[11]. The MinION technology offers several advantages, including portability, real-time analysis, a reduced cost in comparison to alternative sequencing technologies and the capability to obtain both long and ultra-long reads, which simplify assembly and enable more comprehensive analysis of the genome. In comparison to other less expensive methodologies, this approach enables a significant reduction in the analysis time and the integration of several processes that are carried out separately and cumbersomely into a single methodology.

CBC20 exhibited a phenotypic resistance to five distinct categories of antibiotics and was classified as MDR. A correlation was observed between the *mcr-1* genes and resistance mechanisms to beta-lactams (*bla*$_{TEM-1B}$, *bla*$_{TEM-70}$), which is consistent with previous studies that have reported the co-occurrence of *mcr-1* with this beta-lactams genes in pigs from South America[12,16]. Furthermore, the ciprofloxacin resistance phenotype was consistent with the genotypic profile, which demonstrated point mutations in *gyrA* (S83L, D87N) and *parC* (S80I). These results are in agreement with previous publications that describe the presence of *mcr-1*-carrying *E. coli* with a MDR phenotype and multiple chromosomal resistance genes[13]. The analysis of the microbial isolation sources revealed the presence of ST-297 in *E. coli* from humans, animals, and the environment[14]. However, this clone has not previously been reported as a source of the *mcr-1* in pigs. Consequently, our study represents the first documented case of an *E. coli* ST-297 strain carrying the *mcr-1* gene isolated from swine.

On the other hand, the scientific literature contains only limited information about the epidemiology and virulence of *E. coli* O9:H37. This serotype has been isolated from goose samples in China but not from healthy pigs[15]. Currently, the presence of the *mcr-1* gene associated with this serotype has not been reported. Concurrently, a significant number of VFGs were identified, and the virulence pattern demonstrated the potential presence of potential Extraintestinal Pathogenic *Escherichia coli* (ExPEC) pathotypes with a high level of pathogenicity. This could be a significant cause for concern, as this pathogen is the primary etiological agent of urinary infections and sepsis worldwide, both in human and veterinary medicine[16].

A recent report of a *mcr-1.1* variant in a Salmonella enterica isolate originating from Brazilian pig farming[17] is in contrast to the findings of our study, which revealed the presence of the *mcr-1.5* variant. In turn, these results are consistent

with those of previous studies in both animal and human populations in Argentina, where the *mcr-1.5* variant was detected in 33% of fattening pigs[18] and in 26 of 192 clinical isolates (13.5%)[19]. In Argentina, IncI2 types of plasmids are frequently reported in *mcr-1*-carrying *E. coli* isolates, both in human and veterinary medicine[18,19]. The analysis of the CBC20 isolate indicated the presence of five different incompatibility groups, while IncI2 was absent. Consequently, further studies utilizing WGS technology would be beneficial in order to ascertain whether there have been any alterations to the circulation and dissemination patterns of these mobile genetic elements in the region.

CONCLUSIONS

This study represents the first WGS-based investigation of a colistin-resistant *E. coli* isolate carrying the *mcr-1* gene sourced from a swine sample in Argentina. These findings are of significant import for a more comprehensive understanding of antibiotic resistance mechanisms, genomic epidemiology and the dissemination of *mcr-1* among animals and natural ecosystems, with a possible impact on human health in this region.

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CONFLICT OF INTEREST

There is no conflict of interest between authors during the planning, implementation, writing and presentation of the document to the journal.

AUTHORS' CONTRIBUTIONS

- Pellegrini Juan Leandro contributed to the design, data analysis, interpretation and writing of the paper.
- Lorenzini Campos Melina, contributed to data analysis and interpretation the results of study.
- Lucero Raul Horacio, contributed to revising critically of the paper with an important intellectual contribution.
- Amadio Ariel, contributed to interpretation the results of study and revising critically of the paper with an important intellectual contribution.
- Lösch Liliana Silvina, contributed to analysis the data and collaborated on drafting the manuscript.
- Di Conza Jose Alejandro, contributed to final approval of the version to be published.
- Merino Luis Antonio, contributed to final approval of the version to be published.

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