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Genome Note

Draft genome sequence of an *mcr-1*/IncI2-carrying multidrug-resistant *Escherichia coli* B1:ST101 isolated from meat and meat products in Egypt

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ABSTRACT

Objectives: The aim of this study was to investigate the occurrence of plasmid-encoded colistin resistance among Gram-negative bacteria isolated from meat and meat products in Egypt and to report the draft genome sequence of an*mcr-1*/IncI2-carrying multidrug-resistant (MDR) *Escherichia coli* B1:ST101 isolate.
Methods: A total of 128 colistin-resistant strains were isolated from various meat and meat product samples in different cities in Egypt. Multiplex PCR screening for plasmid-mediated colistin resistance genes was performed. Whole-genome sequencing was performed using an Illumina NextSeq platform and the genome was assembled using CLC Genomics Workbench 7.5.1.
Results: A single*mcr-1*-positive MDR *E. coli* strain was isolated from beef sausages. The genome size of the *E. coli* strain was calculated at 5 044 715 bp, with a total of 226 contigs and a G + C content of 50.5%. The strain belonged to ST101 (phylogroup B1). The *mcr-1* gene was located on an IncI2-type self-conjugative plasmid of 64.6 kb in size. The strain showed a MDR phenotype, with a colistin MIC of 4 mg/L. A large number of acquired antimicrobial resistance genes was identified, including genes encoding resistance to colistin (*mcr-1*), β-lactams (bla_{TEM-1}), phenicols (*floR*), trimethoprim (*dfrA12*), aminoglycosides [*aac(3)-IIa*, *aph(3")-Ib* and *aadA2*], macrolides (*mphA* and *mdfA*), tetracyclines (*tetA*), sulfonamides (*sul1* and *sul2*) and quinolones (*qnrS1*).
Conclusion: Here we report the first draft genome sequence of an*mcr-1*/IncI2-carrying MDR *E. coli* B1:ST101 isolated from beef sausage in Egypt. This study highlights the potential role played by food products in the spread of colistin resistance to humans.

The continuing spread of multidrug-resistant (MDR) Gram-negative bacteria, especially carbapenem-resistant bacteria, is a global health concern and has led to the re-use of old antibiotics, particularly colistin, for treating critical infections [1,2]. Resistance to colistin was initially linked to mutational changes identified within chromosomal genes that contribute to the biosynthesis of lipopolysaccharide. Recently, a plasmid-mediated colistin resistance gene (*mcr-1*) was reported from humans, animals, food and the environment in China [3]. This gene encodes a phosphoethanolamine transferase responsible for acquired resistance to colistin and has now been reported worldwide.

The aim of this study was to evaluate the occurrence of plasmid-mediated colistin resistance among Gram-negative bacteria isolated from meat and meat products in Egypt. A total of 128 colistin-resistant strains [minimum inhibitory concentration (MIC) > 2 mg/L] were isolated from various meat and meat product samples (fresh beef, frozen beef, mutton, minced meat, burger, sausage, luncheon, kofta and pastırma) randomly collected from different supermarkets, slaughterhouses and butcher's shops in different cities in Egypt. Isolates were recovered by direct

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spreading on MacConkey agar supplemented with 2 mg/L colistin. PCR screening for plasmid-mediated colistin resistance genes (*mcr-1* to *-8*) was performed but resulted negative [4], except for a single *Escherichia coli* isolate in which the *mcr-1* gene was detected. This strain (MC13) was recovered from a beef sausage sample.

Antimicrobial susceptibility testing of *E. coli* strain MC13 was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, except for colistin. The colistin MIC was determined by broth microdilution in cation-adjusted Mueller–Hinton broth (Bio-Rad, Marnes-la-Coquette, France) and the result was interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)/CLSI joint guidelines (www.eucast.org). *Escherichia coli* strain MC13 was resistant to ampicillin, ciprofloxacin, nalidixic acid, kanamycin, gentamicin, chloramphenicol and tetracycline but remained susceptible to broad-spectrum cephalosporins, carbapenems and aztreonam. The colistin MIC was 4 mg/L, being in the resistant range.

Whole genomic DNA was obtained from an overnight culture of strain MC13 using a PureLink® Genome DNA Mini Kit (Invitrogen, Darmstadt, Germany) and a genomic library was prepared using an Illumina Nextera XT DNA Library with 2 × 300-bp paired-end reads on an Illumina MiSeq instrument (Illumina Inc., San Diego, CA, USA). De novo genome assembly was performed using CLC Genomics Workbench 7.5.1. The assembled draft genome sequence of *E. coli* MC13 consisted of 226 contigs comprising 5 044 715 bp with a G + C content of 50.5%. Multilocus sequence typing (MLST) analysis (https://cge.cbs.dtu.dk/services/MLST/) showed that strain MC13 belonged to ST101, pathogenic phylogroup B1, a background that has previously been associated with the production of MCR-1 in a patient with a urinary tract infection in South Africa [5].

Only a single *mcr-1*-positive *E. coli* human clinical isolate has been identified in Egypt so far, from a patient with bacteraemia [6]. Regarding animals, the *mcr-1* gene was identified in an *E. coli* isolate from a cow suffering from subclinical mastitis [4]. Furthermore, a single *mcr-1*-positive *E. coli* strain was isolated from raw milk cheese in 2019 [7].

Serotyping and *fimH* subtyping were performed using SerotypeFinder 2.0 (https://cge.cbs.dtu.dk/services/SerotypeFinder/) and FimTyper 1.0 (https://cge.cbs.dtu.dk/services/FimTyper/), respectively, and revealed the O15:H10-*fimH86* profile. Virulence genes, including *cma* (colicin M), *ireA* (siderophore receptor), *gad* (glutamate decarboxylase), *lpfA* (long polar fimbriae) and *iss* (increased serum survival), were detected by VirulenceFinder 2.0 (https://cge.cbs.dtu.dk/services/VirulenceFinder/). Various antimicrobial resistance genes were predicted in the genome by ResFinder 3.2 (https://cge.cbs.dtu.dk/services/ResFinder/), including genes encoding resistance to ampicillin (bla_{TEM-1}), phenicols (*floR*), trimethoprim (*dfrA12*), aminoglycosides [*aac(3)-IIa*, *aph(3")-Ib and aadA2*], macrolides (*mphA* and *mdfA*), tetracyclines (*tetA*), sulfonamides (*sul1* and *sul2*) and quinolones (*qnrS1*).

The plasmid harbouring *mcr-1* (designated pEGYMCR-1) was found to be 64 600 bp in size and belonged to the IncI2 group (broad host range). Of note, the first reported *mcr-1* gene found in animal and human isolates in China was also identified on an IncI2-type plasmid [3]. The sequence of this plasmid was identical to that of the *mcr-1*-positive plasmid pMCR-GN775 (accession no. KY471307) identified from an *E. coli* ST624 isolate recovered from a Canadian patient who previously received health care in Egypt [8].

Generally, the *mcr-1* gene is identified as being in association with insertion sequence IS*Apl1*, which may play a major role in its mobilisation [2]. Of note, the pEGYMCR-1 plasmid did not display an IS*Apl1* element in the vicinity of the *mcr-1* gene. However, the *mcr-1–pap2* element was identified in a similar context, between the *top* (encoding a DNA topoisomerase III) and *nikB* (relaxase) genes, like in pMCR-GN775 and other IncI2 plasmids as previously reported [8].

Transfer of the *mcr-1*-harbouring IncI2 plasmid (pEGYMCR-1) was demonstrated by a filter-mating assay using azide-resistant *E. coli* strain J53 as recipient. Transconjugants were selected on LB agar supplemented with sodium azide (150 mg/L) and colistin (2 mg/L). The transconjugant was resistant only to colistin (MIC = 4 mg/L), and no other resistance determinant was co-transferred, indicating that only the *mcr-1* gene was located on the conjugative plasmid.

In conclusion, here we report the occurrence of a colistin-resistant *mcr-1* gene in a MDR *E. coli* strain recovered from a meat product in Egypt. With the emergence of *mcr-1*-bearing bacteria in meat and meat products, this highlights the potential reservoir for colistin resistance, thereby leading to a higher possibility of transmission of resistant bacteria from meat to humans. Strict monitoring and surveillance of resistant bacteria among foods of animal origin, particularly meat and meat products, are strongly needed to prevent their dissemination to humans.

The draft genome sequencing project of *E. coli* strain MC13 had been deposited at GenBank under accession no. **PRJNA587479**.

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Competing interests

None declared.

Ethical approval

Not required.

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