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Research Article

Escherichia coli Harboring Mcr-1 on a Novel Plasmid in a Mink in Eastern China

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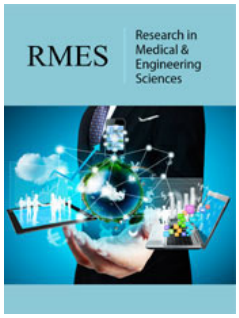

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Abstract

In the current study, we described the characterization of an mcr-1-positive *E. coli* isolate from a dead mink in Dongping Lake, China. The molecular characterization of plasmids was also investigated. The strain was resistant to colistin, but it remained susceptible to several other agents, including amikacin, piperacillin-tazobactam, all carbapenems, and ESBLs. *E. coli* Mink_1 belonged to sequence type 140. Whole Genome Sequencing (WGS) showed that two plasmids, pM1mcr and pM1ctx, coexisted in the same bacterial. Blast and phylogenetic tree imply that Epidemic plasmids are vehicles for the dissemination of colistin resistance genes among the *Enterobacterales.* Restrictive use of antibiotics in animal husbandry should be taken to reduce the dissemination of mcr-1.

Keywords: Mcr-1; Mink; Plasmid; *Escherichia coli*

Introduction

The discovery of a colistin resistance gene, mcr-1, in China may herald the emergence of pandrug-resistant bacteria [1]. The gene has been found primarily in *Escherichia coli* (*E. coli*) but has also been identified in other members of the *Enterobacterales*, in human, animal, food, and environmental samples in over 50 countries on every continent except Antarctica [2-5]. However, the prevalence and characteristics of *E.coli* from mink was rarely reported. Here, we report the presence of mcr-1 in an *E. coli* strain cultured from a mink in Dongping Lake, China.

Methodology

E. coli Mink_1 was cultured from a dead mink which presented to a veterinary station in Dongping, China on 12th, July 2019. The strain of *E. coli* was isolated from liver, where bleeding spots were found. Since our research did not hurt living animals, ethical approval was not required for the study. The isolate was forwarded to SDAU for susceptibility testing. Antimicrobial Susceptibility Testing (AST) of the isolate was performed using broth dilution method. The Minimum Inhibitory Concentrations (MICs) of colistin for the *E. coli* isolate was also tested by the broth dilution method. The result interpretations were based on Clinical and Laboratory Standards Institute guidelines [6], except for colistin, where European Committee on Antimicrobial Susceptibility Testing guidelines breakpoints(≥2μg/mL) were used [7].

Whole Genome Sequencing (WGS) of *E. coli* Mink_1 was performed using a Nanopore technology and a sequence platform GridION. Complete Genome Sequencing was used to determine the location of the resistance genes. When we obtained the genome or plasmid sequences, we compared the sequences with resistance gene sequences. If the Alignment showed above 95% identity, we speculated that the resistance genes located on the responding sequence. The bioinformatics tools included Ediseq, Blastn, and Snapgene. By Blastn, we analysis the identity and coverage of our plasmid with pHNSHP45. The replicon types of plasmids were determined by PCR-Based Replicon Typing (PBRT) [8-11]. The bioinformatics tools uesd include Megalign, Blastn, plasmid finder, Snapgene, etc. The sequences of mcr-1-postive plasmids was downloaded from GenBank. Then the similar sequence were cut for comparison. The construction of the phylogenetic tree was performed by the maximum likelihood method using MegAlign 8.1.4 (DNAstar Co., Ltd).

Results

Table 1: Antibiotic resistance profile of E. coli Mink_1.

Antibiotic(s)	MIC(s) (μg/ml)
Amikacin	8, S
Amoxicillin/clavulanate	16/8, I
Ampicillin	>16, R
Aztreonam	>16, R
Cefazolin	>16, R
Cefepime	>16, R
Ceftazidime	>16, R
Ceftriaxone	>32, R
Ciprofloxacin	>2, R
Colistin	8, R
Ertapenem	0.25, S
Gentamicin	>8, R
Imipenem	0.25, S
Levofloxacin	>4, R
Meropenem	0.25, S
Nitrofurantoin	16, S
Piperacillin-tazobactam	4/4, S
Tetracycline	>8, R
Tobramycin	>8, R
Trimethoprim-sulfamethoxazole	>2/38, R
Sulfanilamide	>4,R

R: resistant, I: intermediate, and S: susceptible

E. coli Mink_1 expressed a level of high resistance to seven different antimicrobial compounds in addition to colistin (MIC = 8mg/L) (Table 1). To note, it has a MIC of Sulfanilamide of >4μg/mL. Susceptibility testing using double-disk test indicated an ESBL phenotype. *E. coli* Mink_1 belonged to sequence type 140 (ST140), a rare *E. coli* ST. The two plasmids, pM1mcr and pM1ctx, coexisted in the same bacterial. pM1mcr, was 238kb in size and BLAST analysis and referring to GenBank indicated that pM1mcr was identified and found to be of an IncF type that encoded a mcr-1 gene along with six additional antimicrobial resistance genes, including the ESBL gene bla$_{CTX-M-14}$, and tetA, Sul2, bla$_{TEM}$. The main reploins we found was FIA and FIB.

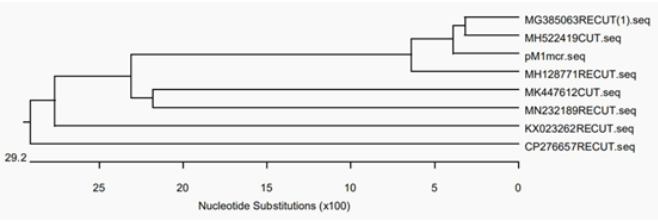

Figure 1: Phylogenetic tree of pM1mcr and similar plasmids.

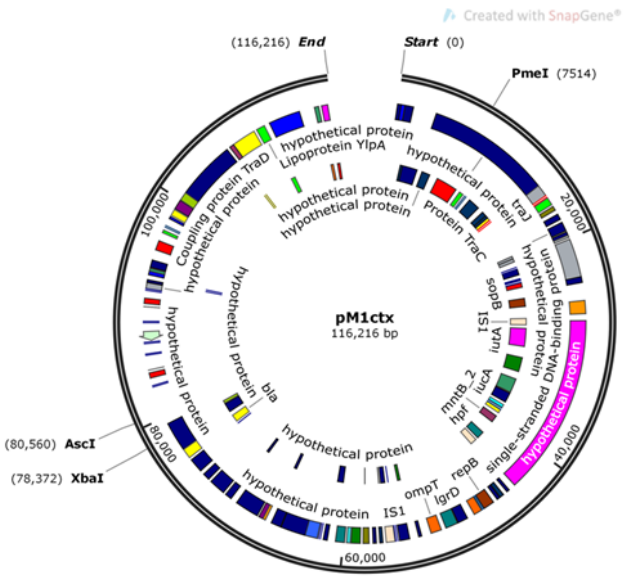

Figure 2: Structure of pM1ctx from Escherichia coli Mink 1.

The second plasmid, pM1ctx,was 116kb in size and was assigned to IncN, which carried antibiotic resistance genes aph(3')-Ia and bla$_{CTX-M-1}$ (Figure 1). Blastn analysis showed that pM1mcr had a query coverage of 94% and maximal 99% identity to pHNSHP45, the first plasmid reported to harbor mcr-1. Results of phylogenetic tree of pM1mcr construction was shown in (Figure 2). MH522419, the most similar sequence to pM1mcr, was a strain of mcr-1-harboring Salmonella from diarrhoeal outpatients in Shanghai, China. Nucleotide sequence accession numbers. The whole genome sequence data reported in this paper have been deposited in the Genome Warehouse in National Genomics Data Center, Beijing Institute of Genomics (BIG), Chinese Academy of Sci-

ences, under accession number GWHACBG00000000 that is publicly accessible at https://bigd.big.ac.cn/gwh.

Discussion

According to the present data from GenBank, the mcr-1-positive plasmids were mainly from China, with a percentage of 52.3%, and *E.coli* is the primary host of mcr-1 in China. mcr-1 mainly exists in livestock breeding and animal derived food in China which varied from 1.5~30.9% in different years. He Jie detected 611 strains of *Escherichia coli* from 23 hospitals in 11 cities in China, and the prevalence of mcr-1 in hospital was 0.98~1.5% in hospital, which is significantly lower than that in animal husbandry, but it is worthy of people's attention because it shows that drug-resistant genes are prevalent and spread among people. The *E.coli* was isolated from liver of a mink. Before the death of the mink, it showed depression and liver bleeding spots were found after autopsy. So, we speculated that the strain was pathogen, and further test should be done to determine whether the strain is pathogenic or not. The lethal pathogen may be EPEC from the environment. Meanwhile, it may also be conditional pathogen in vivo. The association between mcr-1 and epidemic plasmids is concerning, as epidemic plasmids like IncF are vehicles for the dissemination of colisitn resistance genes among the *Enterobacterales*. Continued surveillance to determine the true frequency for mcr-1 is critical and measures including restrictive use of antibiotics in animal husbandry should be taken to reduce the dissemination of mcr-1.

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