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Humans as a Source of Colistin Resistance: In Silico Analysis of Public Metagenomes for the *mcr*-1 Gene in the Gut Microbiome

ORIGINAL INVESTIGATION

Aycan Gündoğdu[1,2], Özkan Ufuk Nalbantoğlu[2, 3]

ABSTRACT

Objective: Colistin is our last line of defense in the treatment of infections caused by multidrug-resistant gram-negative pathogens. However, resistance against colistin has been observed worldwide. In a recent study, a plasmid-mediated colistin resistance gene (mcr-1) was identified for the first time. The purpose of the present study was to conduct an in silico search for the mcr-1 gene and the plasmid harboring it in a cohort of human gut microbiomes, which have already been deposited in public metagenome databases in different geographies.

Materials and Methods: The gut metagenomes of 344 Chinese and 145 European individuals were investigated. Each metagenome sample consisted of sequencing reads obtained from next-generation sequencing. Each DNA read was aligned against the entire mcr-1 and pHNSHP45 plasmid sequences using the BLASTn program. A nucleotide identity threshold of 95% similarity was set, and the reads aligned under that similarity were filtered out.

Results: According to our investigation, 6 out of the 344 individuals in the Chinese cohort harbored the mcr-1 gene and close homologs of pHNSHP45 plasmid in their gut microbiota, whereas no related genetic elements were found in the European cohort.

Conclusion: As the human gut microbiome is one of the key reservoirs of the resistome, the presence of the mcr-1 gene in human microbiota is alarming. It can be said that the dissemination of colistin resistance genes will be more prevalent in the clinic. Further investigations, such as the surveillance of dissemination of related genes, in countries where colistin resistance has become a serious problem, like Turkey, should be considered.

Keywords: *Mcr*-1 gene, colistin resistance, gut microbiome

[1]Department of Microbiology and Clinical Microbiology, Erciyes University School of Medicine, Kayseri, Turkey

[2]Genome and Stem Cell Center, Erciyes University, Kayseri, Turkey

[3]Department of Computer Engineering, Erciyes University School of Engineering, Kayseri, Turkey

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Correspondence
Dr. Aycan Gündoğdu, Erciyes Üniversitesi Tıp Fakültesi, Mikrobiyoloji Anabilim Dalı ve Kliniği, Türkiye
Phone: +90 533 405 83 63
e.mail: agundogdu@erciyes.edu.tr

INTRODUCTION

After their discovery in 1947, polymyxins have been parenterally used in the treatment of infections caused by gram-negative bacteria. During the 1980s, due to their high nephrotoxicity, the parenteral use of polymyxins was abandoned, limiting their use only to topical and oral intake. Since then, colistin had been out of favor except in few treatment options (1). As multidrug-resistant strains continue to be a major problem in the treatment of infectious diseases, in recent years, critical measures have started to be taken for the mitigation of this problem. One popular solution has been the re-introduction of previously abandoned classes of antibiotics, such as polymyxins (2). Despite its unfavorable specifications, colistin has been used in the treatment of infections caused by gram-negative pathogens. However, in the last few years, several cases of colistin-resistant strains have been reported in many countries, including Turkey (3-6).

Since its inclusion to the repertoire of clinically employed antibiotics, drug-resistance case reports associated with colistin have been due to the vertical evolution of the pathogens (i.e., via gene mutations related to a lipopolysaccharide modification) (7, 8). Although this is worrisome as it results in infections that are very difficult if not impossible, to treat, because the resistance mechanism is not transferrable, it has remained as an isolated health threat. However, unfortunately, recently a plasmid-mediated colistin resistance gene (*mcr-I*) was described (9). Being mediated by a stable mobile genetic element, this newly discovered gene raises an alarming health concern. While this is evidence that the dissemination of colistin resistance via horizontal gene transfer is possible and the emergence of pandrug-resistant pathogens might be faced in a very near future, a natural question immediately rises, namely "*What is the current span of the mcr-I gene in the resistome ecosystem?*" In this direction, shortly after its discovery, the existence of *mcr-1* in Enterobacteriaceae was recently reported in isolates from chicken meat and from a patient with urinary tract infection in Denmark (10) and also in the fecal samples of Dutch travelers (11). It is well known that one of the main reservoirs of the resistome in its ecosystem is the human microbiome (12). Detect-

ing colonized colistin resistance in the human microbiome would be an indication that in case of an infection, infectious agents are likely to rapidly acquire resistance genes under antibiotic stress. Taking this into consideration, we conducted an *in silico* search of the *mcr-1* gene on large metagenomics datasets of the human gut microbiome.

MATERIALS and METHODS

In order to capture a picture of the dissemination of the *mcr-1* gene and its mediator pHNSHP45 plasmid in the human gut microbiome, we conducted a retrospective *in silico* study on a cohort of public metagenome data. We selected two sample sets from different geographies. The first set was originally collected for a study conducted on the relationship between type 2 diabetes and the gut microbiome in China (13). For this purpose, the gut metagenome of 344 Chinese individuals with type 2 diabetes and healthy controls (170 cases, 174 controls) were sampled and sequenced using high-throughput DNA sequencing in that study. Here the second dataset used consisted of data about the gut metagenome of 145 European individuals (102 with type 2 diabetes, 43 controls) (14). These two cohorts were among the best available datasets with the largest amount of individuals from different geographies, and this is the underlying reason for employing them in the search.

The basic methodology for mining large metagenome data for the *mcr-1* gene and pHNSHP45 plasmid involved using the sequence alignment program BLASTn. Each metagenome sample consisted of the sequencing reads obtained from next-generation sequencing, and we aligned each DNA read against the entire *mcr-1* and pHNSHP45 sequences. A nucleotide identity threshold of 95% identity was set and the reads aligned under that similarity scores were filtered out. According to the gene annotation of the pHNSHP45 plasmid (GenBank accession number: KP347127), each open reading frame was determined and the metagenomic fragments mapped on each gene were recorded. Also the percentage coverage of the plasmid for each metagenome after alignment was calculated by reference-based assembly of the plasmid fragments.

Statistical analysis

National Center for Biotechnology Information Blast+ (version 2.2.28-2, NCBI, USA) was employed in the sequence similarity calculations, using the options: "-word_size 28 -gapopen 5 -gapextend 2 -reward 2 -penalty 2." 95% identity threshold filtering was performed using in-house Python (version 2.7.11, Python Software Foundation) scripts.

RESULTS

Our findings indicate that the colistin-resistance gene and the plasmids carrying these genes can be detected in the human gut microbiome. The results showed that 6 out of 344 individuals in our Chinese cohort harbor the *mcr-1* gene and close homologs of pHNSHP45 plasmid in their gut microbiota (Table 1). Although the entire plasmid was not able to be covered with the metagenome data, all the sequencing reads were mapped onto the plasmid with high identity. Collectively, around 78% of the entire plasmid (>50,000bp, >96% identity) was covered by the metagenome reads, including the reads from all 82 open reading frames annotated on the plasmid. We conducted a similar analysis with the gut microbiome of 145 European individuals, but none of the European metagenomes were found to be carrying either the *mcr-1* gene or pHNSHP45 plasmid.

Table 1. The *mcr*-1 gene-carrying individuals and their metagenome hit results

Subject	Plasmid coverage (%)	Nucleotide identity (%)	#ORFs hit
Male (36 years old)	10.49	96.60	30
Female (35 years old)	2.14	96.14	10
Female (51 years old)	75.10	98.86	80
Female (41 years old)	1.54	98.47	8
Male (68 years old)	5.58	96.34	11
Male (43 years old)	1.66	95.46	7
Total	78.20	96.34	82

ORF: open reading frame

DISCUSSION

Emerging resistance against colistin, which is a drug of last resort, has become a major problem around the world (1, 5). The recent discovery of the *mcr-1* gene by Liu et al. (9) on plasmids implies the mobility of colistin resistance throughout a resistome ecosystem. Yi-Yun Liu and et al. (9) pointed out that the agricultural use of colistin in China is among the highest in the world. Accordingly, they reported the first cases of colistin resistance genes in food animals. Therefore, it can be expected that resistant plasmid carrying strains are in circulation and they might have adopted human beings as hosts. Indeed, our findings on the Chinese dataset indicate that the dissemination of colistin resistance among the human population can be observed and colonization of it in the gut microbiome can be hypothesized. In our *in silico* search, we detected no *mcr-1* genes or pHNSHP45 plasmids in the gut microbiome samples of European individuals. Geographical separation and more strict antibiotic use regulations might be behind this result. It should be noted that the experiments generating the data used in our bioinformatic search were not designed for detecting colistin resistance genes, and that the detected elements constitute a very small portion of the data, indeed only sparsely hitting the target elements. However, these results are encouraging to conduct surveillance studies to determine the distribution, dissemination, and prevalence of mobile genetic-element-mediated colistin-resistance genes in the human microbiome. In an unpublished study, we did not detect any *mcr-1* genes in a set of colistin-resistant clinical pathogens isolated from blood, urine, and abscess cultures of hospitalized patients in Erciyes University Hospitals. This might not mean that the mcr-1 gene does not exist in Turkey. On the contrary, large cohorts or environmental investigations including metagenome studies should be conducted to reveal if the *mcr-1* gene is disseminated in the biogeography of the country.

The discovery of genetic elements related with colistin resistance colonized in human microbiota in certain geographies around the world would encourage the design of similar investigations in coun-

tries where high colistin resistance prevalence has been reported. Since exploring the status of the acquirable drug-resistance dissemination would give an idea on how close we are to the emergence of superpathogens, such studies would shed light on the possibility of a prospective antibiotic crisis.

CONCLUSION

The presence of the *mcr-1* gene in the human microbiome may pose a risk for the dissemination of this gene and its dissemination raises a global risk for pandrug resistant strains. From an anthropocentric point of view, this dissemination means the last key genetic element before the emergence of the superpathogens has made its way to our bodies and it is then only a matter of time that the dissemination of colistin resistance genes will be prevalent in the clinic, bringing the world closer to an antibiotic crisis.

Informed Consent: The informed consent was not required because the study was performed on animals.

Peer-review: Externally peer-reviewed.

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