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RESEARCH ARTICLE

In Silico Analysis of Resistance Gene Identifiers on Plasmids of blaNDM-5 Producing Carbapenem-Resistant *Escherichia coli* Strains Isolated in Humans and Animals

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ABSTRACT

Due to their ability to resist endpoint antimicrobials such as carbapenem, it is very important to detect and monitor multi-drug resistant Gram negative strains with plasmids containing genes such as blaNDM-5 by new molecular methods. This study aimed to perform in silico analysis of resistance gene identifiers on human and animal-derived blaNDM-5 plasmids found in open databases, which were analyzed by new whole genome sequencing techniques and to compare these resistance genes. The plasmid genomic sequences of 4 human and 2 animal *E. coli* strains containing blaNDM-5 genes included in our study were analyzed in Silico using the Resistance Gene Identifier (RGI) option of the comprehensive antibiotic resistance gene database using default values. Human and animal strains included in our study were found to have different antimicrobial resistance genes in addition to blaNDM-5. All plasmids were found to have at least 8 perfect antimicrobial resistance gene sequences matches. When the resistance gene identifiers in all plasmids were examined, 35 resistance gene identifiers were found. Besides blaNDM-5, mphA, qacEdelta1 and sul1 were found in all plasmids. As a conclusion, it was determined by our study results that, regardless of the source, there may be different antimicrobial resistance gene identifiers besides the blaNDM-5 resistance gene in plasmids. We are of the opinion that routine molecular surveillance studies should be carried out considering the one health approach of Gram-negative pathogens such as *E. coli,* which can contain plasmids that cause multi-drug resistance and can be isolated from all sources.

Key words: blaNDM-5, Carbapenem resistant *E.coli*, in silico analysis, resistance gene identifier.

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İnsanlardan ve Hayvanlardan İzole Edilen blaNDM-5 Üreten Karbapenem Dirençli *Escherichia coli* Kökenlerinin Plazmidleri Üstünde Bulunan Direnç Gen Tanımlayıcılarının İn Silico Analizi

ÖZ

Karbapenem gibi son nokta antimikrobiyallere direnç yetenekleri dolayısıyla, blaNDM-5 gibi genler ihtiva eden plazmidlere sahip çoklu ilaç direnci gösteren Gram negatif kökenlerin yeni moleküler yöntemlerle tespiti önemlidir. Biz de çalışmamızda bu yeni tekniklerle tüm genom sekans analizi yapılan ve açık veritabanlarında bulunan insan ve hayvan kaynaklı blaNDM-5 plazmidleri üstünde bulunan direnç gen tanımlayıcılarının in silico analizini yapmayı ve bu direnç genlerini karşılaştırmayı amaçladık. Çalışmamıza dahil edilen 4 insan ve 2 hayvan kaynaklı, blaNDM-5 geni içeren *E.coli* kökenlerine ait plazmid genomik dizileri, varsayılan değerler kullanılarak kapsamlı antibiyotik direnç gen veritabanının, direnç geni tanımlayıcı (RGI) seçeneği kullanılarak bilgisayar ortamında analiz edildi. Çalışmamıza dahil edilen insan ve hayvan kaynaklı kökenlerde blaNDM-5 yanında farklı antimikrobiyal direnç genlerinin de olduğu tespit edildi. Tüm plazmidlerin en az 8 mükemmel antimikrobiyal direnç gen dizisi eşleşmesi gösterdiği saptandı. Tüm plazmidlerde bulunan direnç gen tanımlayıcıları incelendiğinde 35 direnç gen tanımlayıcısı saptandı. blaNDM-5 yanında, mphA, qacEdelta1 ve sul1'in bütün plazmidlerde olduğu tespit edildi. Sonuç olarak, kaynak fark etmeksizin, plazmidlerde, blaNDM-5 direnç geni yanında farklı antimikrobiyallere direnç gen tanımlayıcılarının da olabildiği çalışma sonucunda tespit edilmiştir. Çoklu ilaç direncine neden olan plazmidleri ihtiva edebilen ve tüm kaynaklardan izole edilebilen *E. coli* gibi Gram negatif patojenler üzerinde tek sağlık yaklaşımı düşünülerek rutin moleküler surveyans çalışmalarının yapılması gerektiği kanaatindeyiz.

Anahtar Kelimeler: blaNDM-5, direnç gen tanımlayıcıları, in silico analiz, Karbapenem dirençli *E.coli*

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INTRODUCTION

Antimicrobial resistance is a huge threat to public health around the world. Carbapenems; are last-resort treatment options against infections with Cephalosporinase and/or extended spectrum-lactamase producing Gram-negative pathogens showing multi-drug resistance, including Enterobacteriaceae (Huang et al. 2021). These bacteria can produce different carbapenemases to inactivate carbapenems ; Of these, New Delhi Metallo-β-lactamase (NDM) is one of the newly emerging and of serious clinical importance (Zhu et al. 2016). NDM-1 was first identified from a Klebsiella pneumoniae strains in India in 2008 (Young et al. 2009). 29 NDM variants have been reported in a short time (Basu, 2020). NDM-5 was discovered in a multi-resistant *Escherichia coli* (*E.coli*) strains in the United Kingdom in 2011 (Hornsey et al. 2011). It differs two amino acid (Val88Leu and Met154Leu) changes with NDM-1 and appears to confer increased resistance to extended-spectrum cephalosporins and carbapenems. The coding of the blaNDM-5 gene in isolates is defined (Zhu et al. 2016). It has been reported that blaNDM-5 is carried in different incompatible type plasmids for transfer, such as IncF, IncN, and IncX3. These plasmids can facilitate the spread of blaNDM-5 among Enterobacteriaceae members through horizontal gene transfer (Li et al. 2018). *E. coli* strains showing multi-drug resistance and producing blaNDM-5 plasmid were identified in human, animal, and food isolates, and plasmids in these strains were sequenced using the whole genome sequencing style (He et al. 2017, Feng et al. 2018, Liu et al. 2018, Xie et al. 2018, Tyson et al. 2019, Marchetti et al. 2020). It is important to determine the survelliance of the resistance profiles of the strains obtained from different disciplines in a one health approach (Aenishaenslin et al. 2021). In our study, we aimed to examine the in silico analysis of resistance gene identifiers with human and animal blaNDM-5 plasmids in the open database and to compare these resistance genes.

MATERIALS and METHODS

Plasmid database

In our study, the plasmid genomic sequences of *E.coli* strains of the blaNDM-5 gene whose sequencing has been completed with the whole genome analysis in the NCBI open database, the valid values were used in the comprehensive antibiotic resistance gene database (CARD - https://card.mcmaster.ca/analyze/rgi) in resistance gene identifier (RGI) option and in silico analysis was performed. The Comprehensive Antibiotic Resistance Database ("CARD") that provides molecular data and algorithms on key gene markers of antimicrobial resistance was used. The CARD online software provides reference gene sequences and analysis of SNPs in genetic sequences through Antibiotic Resistance Ontology ("ARO"). Resistance gene identifiers (RGI) obtained through the models obtained from the sequencing analysis data can be used for resistome prediction (Alcock et al, 2020). The identified resistance gene were compared in human and animal isolates.

RESULTS

Information on human and animal sources included in our study are summarized in Table1. After RGI analysis of all genome sequence sequences, it was observed that there are different resistance genes in addition to blaNDM-5 in human and animal sources. It was determined that at least 8 of the resistance gene identifiers in all plasmids showed a perfect (100%) sequence match. Minimum inhibitory concentration (MIC) values for these carbapenem-resistant isolates were also examined and presented in Table1 and Figure1.

When the resistance gene identifiers in all plasmids were examined, 35 resistance gene identifiers were found. Except for blaNDM-5, mphA, qacEdelta1 and sul1 were found in all strains. All genes identified in Table2 are presented comparatively according to human and animal sources. Ten genes causing aminoglycoside resistance were detected on the NDM-5 gene carrying plasmid. It was determined that 50% of the plasmids of both human and animal strains carried an important antimicrobial resistance gene such as blaCTX-M-15.

Table1. RGI analysis of whole genome analyzed plasmids containing the blaNDM-5 gene.

NCBI accession number	The living thing from which the origin is isolated	Insulation Material	Carbapenem MIC	Perfect Sequence Match	Strict Sequence Match	Loose Sequence Match	References
CP041393	DOG		4	10	3	5	7
KY990887	COW	Endotracheal Washing Fluid	64	10	5	5	10
MN218686	HUMAN	Cow stool material	16	8	7	6	8
MN197360	HUMAN	Stool material obtained from the baby	16	9	2	2	8
CP023871	HUMAN	Stool material obtained from the baby	64	15	2	8	9
MF679147	HUMAN	Urine material	16	8	3	8	11

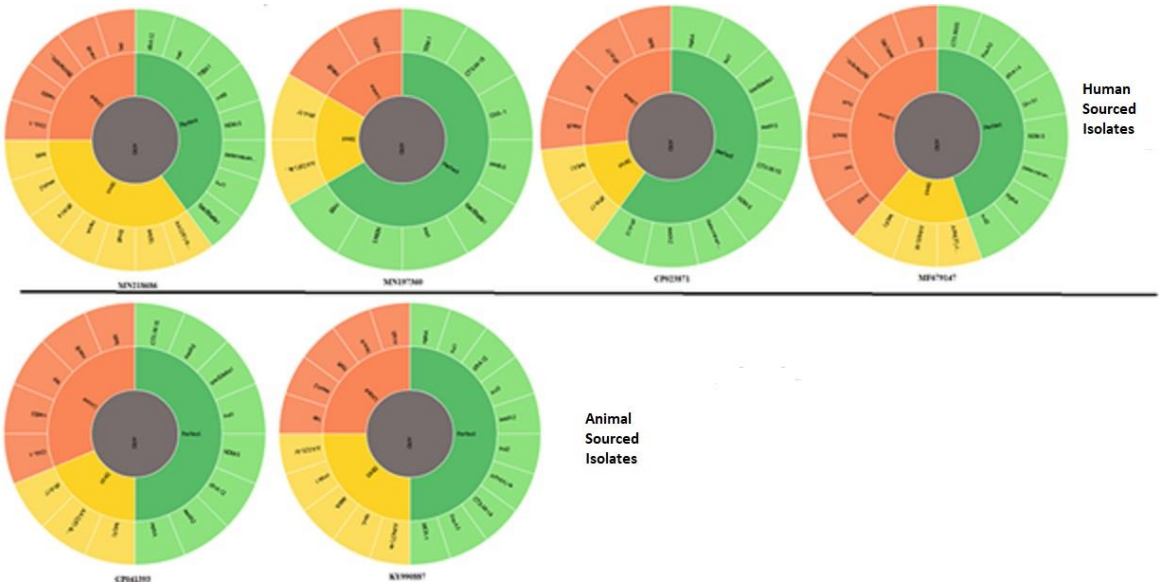

Figure1: Distribution of resistance gene identifiers obtained from whole genome sequencing data of plasmids carrying with carbapenem resistant blaNDM-5 gene. (Green:Perfect Sequence Match, Red: Strict Sequence Match, and Yellow:Loose Sequence Match).

Table2. Genus of gene identifiers found in plasmids detected in human and animal isolates.

Gene name detected on the plasmid	Antimicrobial (s) on which it acts	human source (n: 4)	Percentage (%) of matching region in human-derived plasmids	Animal source (n: 2)	Percentage (%) matched region in animal derived plasmids
AAC(3)-IV	aminoglycoside	0 (%0)		1 (%50)	99.61
AAC(6')-Ib-cr	fluoroquinolone, aminoglycoside	2 (%50)	98.99	1 (%50)	98.99
aadA	aminoglycoside	0 (%0)		1 (%50)	99.61
aadA2	aminoglycoside	2 (%50)	99.8	2 (%100)	100
aadA5	aminoglycoside	3 (%75)	100.0	1 (%50)	100.0
APH(3')-Ia	aminoglycoside	0 (%0)		1 (%50)	98.52
APH(3'')-Ib	aminoglycoside	1 (%25)	99.63	0 (%0)	
APH(4)-Ia	aminoglycoside	0 (%0)		1 (%50)	100
APH(6)-Id	aminoglycoside	1 (%25)	99.28	0 (%0)	
blaCTX-M-14	Cephalosporin	0 (%0)		1 (%50)	100
blaCTX-M-15	Cephalosporin	2 (%50)	100	1 (%50)	100
blaCTX-M-65	Cephalosporin	1 (%25)	100	0 (%0)	
blaNDM-5	carbapenem, cephalosporin, cephamycin, penam	4 (%100)	100	2 (%100)	100
blaOXA-1	carbapenem, cephalosporin, penam	1 (%25)	100	0 (%0)	
blaTEM-1	monobactam, cephalosporin, penam, penem	2 (%50)	100.0	0 (%0)	
BRP(MBL)	Glycopeptide	3 (%75)	100	0 (%0)	
catI	Phenycol	1 (%25)	100	0 (%0)	
cmlA1	Phenycol	0 (%0)		1 (%50)	99.76
dfrA12	Diaminopyrimidine	2 (%50)	100	2 (%100)	100
dfrA14	Diaminopyrimidine	2 (%50)	99.68	0 (%0)	
dfrA17	Diaminopyrimidine	2 (%50)	99.36	1 (%50)	99.36
ErmB	macrolide,lincosamide, streptogramin	1 (%25)	97.96	0 (%0)	
FosA3	fosfomycin	1 (%25)	100	1 (%50)	100
MCR-1.1	peptide antibiotic	0 (%0)		1 (%50)	100
mphA	macrolide	4 (%100)	100	2 (%100)	100
qacEdelta1	acridine paint	4 (%100)	100	2 (%100)	100
qacL	quaternary ammonium compound	0 (%0)		1 (%50)	93.64
QnrS1	Fluoroquinolone	1 (%25)	100	0 (%0)	
rmtB	aminoglycoside	2 (%50)	100	0 (%0)	
sul1	sulfonamide	4 (%100)	100	2 (%100)	100
sul2	sulfonamide	1 (%25)	100	1 (%50)	100
sul3	sulfonamide	0 (%0)		1 (%50)	100
tet(A)	Tetracycline	2 (%50)	99.74	1 (%50)	99.74
tet(B)	Tetracycline	1 (%25)	99.25	0 (%0)	
tetR	Tetracycline	1 (%25)	100	0 (%0)	

DISCUSSION

Carbapenems are a class of beta-lactam antibiotics that are active against many aerobic and anaerobic Gram-positive and Gram-negative organisms. They are critically important antibiotics used to treat serious bacterial infections. Carbapenem resistance is rare and typically results from the production of a carbapenemase enzyme that can hydrolyze penicillins, cephalosporins, monobactams and carbapenems (Tyson et al. 2019). The presence of plasmids that carry the NDM-5 gene can create Pan-resistant *E. coli* strains (He et al. 2017). Moreover, these plasmids can be transferred by conjugation, which has shown that they can be self-infectious (Feng et al. 2018). When the studies on carbapenem resistant *E. coli* strains producing blaNDM-5 were examined; Sun et al. reported in their study in 2019 that they detected blaCTX-M-15 and blaCTX-M-14 genes as well as rmtB and aac (6')-Ib-cr genes in human sources, in addition to blaNDM-5 (Sun et al. 2019). Similarly, among the plasmids we examined in our study, we found that rmtB and aac (6')-Ib-cr genes were detected more frequently, especially in human sources. While blaCTX-M-15 was detected in human and animal sources, blaCTX-M-14 gene was not detected in human sources. Sun et al. reported that they detected the blaCTX-M-64 gene in some of their strains [14]. We could not detect the blaCTX-M-64 gene in our study. Brown et al. (2018) reported that they detected the blaCTX-M-65 gene in Salmonella strains in chicken meat. In our study, we found that blaCTX-M-65 gene was carried in a blaNDM-5 plasmid of *E.coli* strains obtained from a human sample. This has shown us that these genes can switch between bacterial species. Tian et al. (2020) reported that they detected aadA2 and dfrA12 resistance gene cassettes in plasmids containing blaNDM-5, which they detected in children in their study in 2020. In our study, we found that both gene identifiers were found in sources similarly. While these genes were detected in 50% of our human sources, they were found in all of our animal sources. Ramadan et al., (2020) detected blaNDM-5 producing strains in humans and dogs. In the same study, they reported that they detected multiple resistance genes related to β-lactams, aminoglycosides, tetracyclines, quinolones, phenicols, macrolides and folate pathway inhibitors in all isolates. However, they did not find mcr genes in human or dog isolates in this study. In our in silico analysis, we detected the resistance genes for all these antimicrobials in strains from both human and animals. In addition, KY990887 (NCBI accession number), which was obtained from the cow feces samples was carried mcr gene (He et al. 2017). *E. coli* strains carried NDM-5-plasmids with multi-drug resistance, including carbapenem, also reported from environmental sources (Zhao et al. 2021), nutritional sources (Liu et al. 2018) and waters (Liu et al. 2019) except humans and animals.

CONCLUSION

As a result, multi-drug resistant *E. coli* strains carried blaNDM-5 plasmids can be identified, regardless of the source. Our study results revealed that plasmids belonging to these strains may also have resistance gene identifiers against different antimicrobials in addition to the blaNDM-5 resistance gene. We conluded that routine molecular surveillance studies should be carried out, considering the onehealth approach for Gram-negative pathogens such as *E. coli,* which can carry different plasmids that cause multi-drug resistance

Etik Kurul Bilgileri : Çalışma kapsamında etik kurul onay belgesine ihtiyaç bulunmamaktadır.

Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan eder.

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