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Case Report

Emergence of an NDM-5-producing clinical *Escherichia coli* isolate in Egypt

Ahmed M. Soliman [a,b], Hazim O. Khalifa [a,c], Ashraf M. Ahmed [a,d], Toshi Shimamoto [a], Tadashi Shimamoto [a,*]

[a] *Laboratory of Food Microbiology and Hygiene, Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima 739-8528, Japan*
[b] *Department of Microbiology and Immunology, Faculty of Pharmacy and Drug Industries, Kafrelsheikh University, Kafr El-Sheikh, Egypt*
[c] *Department of Pharmacology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt*
[d] *Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh, Egypt*

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SUMMARY

The first occurrence of New Delhi metallo-β-lactamase 5 (NDM-5), carried on an IncI1-Iγ-type plasmid of >93 kb in a multidrug-resistant *Escherichia coli* strain in Kafr El-Sheikh, Egypt, is reported. The strain was isolated from a wound pus swab from a patient diagnosed with a fracture of the right femur. This *E. coli* strain was found to belong to sequence type (ST) 5018 and also to carry other resistance genes, including $bla_{CTX\text{-}M\text{-}15}$, $bla_{CMY\text{-}42}$, $bla_{OXA\text{-}1}$, and *aac(6′)-Ib-cr*.

1. Introduction

New Delhi metallo-β-lactamase 1 (NDM-1) has received global attention due first to its high level of resistance to many different β-lactams, except aztreonam, second to its dissemination worldwide to different countries, third to its association with hospital-acquired infections, and fourth to its acquisition by many common Gram-negative bacteria.[1] NDM-1 was first discovered in *Klebsiella pneumoniae* in a Swedish patient previously hospitalized in India.

The discovery of NDM-5 was similar; it was first reported in 2011 in an *Escherichia coli* isolate recovered from a patient in the UK after a recent hospitalization in India.[2] It has since been reported from many other countries, including India, Algeria,[3] Spain, Japan, Australia, the USA, and China.[4] NDM-5 differs from NDM-1 by two amino acid substitutions (Val88Leu and Met154Leu), which cause a reduced susceptibility of *E. coli* TOP10 transformants to extended-spectrum cephalosporins and carbapenems.[2] The first NDM-5-producing carbapenem-resistant *E. coli* isolate identified in Egypt is described herein.

2. Case report

A 65-year-old man was admitted to the emergency department of a hospital in the city of Kafr El-Sheikh, Egypt, on May 30, 2014, with a diagnosis of right femur fracture resulting in the formation of a deep wound. After surgery on May 31, 2014, the patient was transferred to the department of orthopedics for the completion of treatment, and empiric intravenous ceftriaxone (1 g twice daily for 13 days) was given to prevent bacterial infection. Unfortunately, the wound began to exude yellow pus on June 13, 2014, and the patient was started on intravenous levofloxacin (0.5 g twice daily for 6 days). On June 16, 2014, a multidrug-resistant *E. coli* strain, designated EC169, was isolated from the wound pus specimen. In accordance with the drug resistance pattern of EC169, the patient's antibiotic treatment was changed to intramuscular amikacin (1 g twice daily). The patient recovered after 9 days, and a wound pus swab was negative for *E. coli*.

* Corresponding author. Tel.: +81 82 424 7897; fax: +81 82 424 7916.
E-mail address: tadashis@hiroshima-u.ac.jp (T. Shimamoto).

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EC169 was identified biochemically using the API 20E System (bioMérieux, Marcy l'Etoile, France). The broth microdilution method was used to determine the minimum inhibitory concentrations (MICs) of selected antimicrobials; the results were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Amoxicillin, cefotaxime, ceftazidime, and ceftriaxone powders were supplied by Sigma-Aldrich (Tokyo, Japan), meropenem, gentamicin, nalidixic acid, norfloxacin, and colistin by Wako Pure Chemical Ind. Ltd (Osaka, Japan), imipenem, doripenem, and cefoperazone by LKT Laboratories, Inc. (Minnesota, USA), and tetracycline powder was purchased from Nacalai Tesque (Kyoto, Japan). For all experiments, the purified powder of each antibiotic was diluted following the CLSI recommendations. The reference strain *Escherichia coli* ATCC 25922 was included as a quality control.

EC169 showed resistance to imipenem, meropenem, doripenem, aztreonam, amoxicillin, cefotaxime, ceftazidime, cefoperazone, ceftriaxone, gentamicin, nalidixic acid, tetracycline, and norfloxacin, while it was sensitive to amikacin and colistin (Table 1). EC169 was also positive on the modified Hodge test, by carbapenem inactivation method (CIM), and when tested with the MBL method, using two disks containing 30 μg of ceftazidime and one disk containing 3 mg of sodium mercaptoacetic acid (SMA) (Eiken Chemical Co. Ltd, Tokyo, Japan), indicating the production of metallo-β-lactamase. Of note, The CIM consists of two steps: (1) incubation of a meropenem disk with the isolate tested, and (2) incubation of this meropenem disk with the *Escherichia coli* ATCC 25922 strain. Carbapenemase activity can be detected easily after the second incubation step by the absence of an inhibition zone, which indicates enzymatic hydrolysis of meropenem during the first incubation step.

Following this, PCR and DNA sequencing were used to screen for carbapenemase-encoding genes,[5] extended-spectrum β-lactamases (ESBLs), plasmid-mediated quinolone resistance genes, integrons, and 16S rRNA methylases. PCR fragments were purified using a FastGene Gel/PCR Extraction Kit (Nippon Genetics Co., Tokyo, Japan). Both DNA strands of the PCR product were sequenced using a 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

The results demonstrated that EC169 carried not only bla_{NDM-5}, but also $bla_{CTX-M-15}$, bla_{CMY-42}, bla_{OXA-1}, *aac(6′)-Ib-cr*, and a class 1 integron with two gene cassettes (*dfrA17–aadA5*). Multilocus sequence typing (MLST) was performed using seven housekeeping genes (*adk*, *fumC*, *icd*, *purA*, *gyrB*, *recA*, and *mdh*), according to the *Escherichia coli* MLST Database (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). The results indicated that EC169 belongs to sequence type (ST) 5018.

Table 1
Minimum inhibitory concentrations for the NDM-5-producing *Escherichia coli*

Antimicrobial	Minimum inhibitory concentration (μg/ml)
IMP	≥32
MEM	≥32
DOR	≥32
AZM	≥512
AMX	≥512
CTX	≥512
CAZ	≥512
CFP	≥512
CRO	≥512
GEN	≥256
NAL	≥512
TET	≥256
NOR	≥128
CST	1

IMP, imipenem; MEM, meropenem; DOR, doripenem; AZM, aztreonam; AMX, amoxicillin; CTX, cefotaxime; CAZ, ceftazidime; CFP, cefoperazone; CRO, ceftriaxone; GEN, gentamicin, NAL, nalidixic acid; TET, tetracycline; NOR, norfloxacin; CST, colistin.

PCR mapping and DNA sequencing were used to analyze the immediate genetic environment of the bla_{NDM-5} gene using primers specific for the genetic environment of previously published bla_{NDM} genes. The insertion sequence IS*Aba125* was identified upstream from the bla_{NDM-5} gene, and the bleomycin resistance gene ble_{MBL} was identified downstream from the bla_{NDM-5} gene. Southern blot hybridization showed that bla_{NDM-5} was located on a plasmid of >93 kb. Probe labeling and membrane hybridization were performed according to the protocol of the Amersham ECL Direct Nucleic Acid Labeling and Detection System (GE Healthcare Japan, Tokyo, Japan). Transferability of the bla_{NDM-5}-carrying plasmid was performed by conjugation experiment between the clinical donor isolate (EC169) and azide-resistant *E. coli* strain J53 as a recipient. The transconjugants were selected on MacConkey agar plates containing 4 μg/ml meropenem and 150 μg/ml sodium azide. Unfortunately, conjugation and mating experiments were unsuccessful after several attempts, indicating that this plasmid is unconjugable.

PCR-based replicon typing (inc/rep PCR) was applied to determine the Inc type of the NDM-5-carrying plasmid. This plasmid was cut and purified from a low melting point agarose gel using standard agarase treatment (Nippon Gene Co., Ltd, Toyama, Japan) and used as a DNA template for inc/rep PCR. The result revealed that the NDM-5-carrying plasmid belonged to the IncI1-Iγ type.

3. Discussion

To date, 16 variants of NDM-type β-lactamases (NDM-1 to NDM-16) have been detected and assigned according to the gene bank available at the Lahey Clinic website (see http://www.lahey.org/studies/other.asp#table1). However, only NDM-1[1] and NDM-2[5] have been reported previously in Egypt. The discovery of bla_{NDM-5} in a clinical *E. coli* isolate from a patient with no history of travel beyond the Egyptian border probably suggests that bla_{NDM-5} is an autochthonous genetic determinant in Egypt. The overuse and/or misuse of antimicrobials in Egyptian hospitals and the community may be responsible for the development of high levels of antimicrobial resistance. Due to the poor hygiene conditions in developing countries like Egypt, the food chain and water may comprise one of the possible sources that help the spread of these resistance genes. Moreover, NDM-5 was recently reported in *Escherichia coli* ST1284 from a rectal swab of a domestic dog in Algeria. Also, cases of NDM-5 have arisen in the community in China without hospitalization (*E. coli* ST5131) or a travel history (*Klebsiella pneumoniae* ST14).

The MLST analysis revealed that *E. coli* EC169 belongs to ST5018, which is completely unlike the ST types of NDM-5-producing *E. coli* detected in the UK (ST648),[2] India (ST648), Algeria (ST2659),[3] Spain (ST448), Japan (ST540), Australia (ST648), the USA (ST167), and China (ST167).[4] This appears to be the first report of an ST5018 *E. coli* strain expressing NDM-5 β-lactamase. The genetic environment of bla_{NDM-5} is very similar to that previously described for most NDM-1-producing *Enterobacteriaceae*.[2,4] Other genetic determinants, such as $bla_{CTX-M-15}$[2,4] and class 1 integron (*dfrA17–aadA5*),[2] have also been reported in NDM-5-producers.

In conclusion, the worldwide dissemination of NDM-producing Gram-negative bacteria is of great concern. Medical authorities must implement antimicrobial programs and infection control policies to effectively prevent the rapid spread of these genetic determinants. The increasing bacterial drug resistance is a forceful reminder that our world is very close to the situation in the pre-antibiotic era, especially because there are no new antibiotics in the pipeline.

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