Letter to the Editor

First identification of clinical isolate of a Novel “NDM-4” producing Escherichia coli ST405 from urine sample in Pakistan

Dear Sir,

New Delhi metallo-β-lactamase producing Enterobacteriaceae are a serious threat to the public health sectors worldwide. NDM producing pathogens generally display resistance against several different classes of antibiotics including carbapenems; considered last resort to treat infections caused by such pathogens.1,2 Until now 19 NDM variants (NDM-1 to NDM-19) have been identified from different parts of the world http://www.lahey.org/Studies/other.asp. NDM-4 differs from NDM-1 by a single point mutation at position 154 (M-L) and is associated with high carbapenemase activity.3 We are first time reporting the description of NDM-4 producing Escherichia coli isolated from urine sample in Pakistan.

A 4-year-old female patient was hospitalized in a tertiary care hospital Lahore, Pakistan in March 2014. After admission, her urine culture revealed the presence of E. coli which was confirmed by VITEK® 2 system (bioMerieux, France) and MALDI-TOF (Bruker, Germany). Isolate was also carbapenemase and metallo-β-lactamase producer identified by modified Hodge’s test and double disk synergy method respectively.4 Minimum inhibitory concentration (µg/mL) of antibiotics using GN XN05 card in VITEK® 2 compact system (bioMerieux, France) displayed pan-drug resistance to commonly used antibiotics including meropenem and only effective drug was colistin. Furthermore, colistin susceptibility was determined by broth microdilution assay in 96 microtiter Plate5 (Table 1). A previous study reported that NDM-4 producing bacteria has higher MIC (µg/mL) and hydrolytic activity as compared to NDM-1 producing bacteria.3 Genotyping of blaNDM-4 was accomplished by PCR (NDM-F-TGGCTTTTGAACTGTGCCAC, NDM-R- CTGTCA-CATCAGAATC GGCGGA) and DNA sequence analysis. Plasmid characterization was performed as previously reported.4 S1 nuclease pulse field gel electrophoresis and in gel DNA hybridization revealed the presence of blaNDM-4 on 120 kb of plasmid (data not shown). As per Carratoli’s procedure of plasmid typing, the isolate contained the Incompatible II group of plasmids. Multilocus sequence typing (MLST) was performed as described earlier and E. coli belonged to the sequence type (ST) 405.7 ST405 NDM producing E. coli has also been isolated from two inpatients in Italy.8 It is documented that the Indian sub-continent is the main reservoir and source of NDM producing bacteria globally. Present study also supported this fact in that the patient had no previous travel history. Moreover, NDM-4 producing E. coli has also been reported from different parts of the world including India and Italy.3,8 This study indicates, the emergence of NDM-4 producing E. coli ST405 which can lead to therapeutic failure and deaths particularly in children. This isolate could be transferred through risk factors such as bed sharing, substandard infection control practices and most importantly urinary catheters.

The blaNDM containing IncFII plasmids might have become the common vehicle for the spread of various NDM alleles

<table>
<thead>
<tr>
<th>Isolates</th>
<th>SAM</th>
<th>PIP</th>
<th>CXM</th>
<th>CXA</th>
<th>CFM</th>
<th>CRO</th>
<th>FEP</th>
<th>ATM</th>
<th>MEM</th>
<th>LEV</th>
<th>MXF</th>
<th>MNO</th>
<th>TE</th>
<th>TMP</th>
<th>C</th>
<th>TGC</th>
<th>CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDM-4 producing E. coli</td>
<td>≥32</td>
<td>≥128</td>
<td>≥64</td>
<td>≥64</td>
<td>≥64</td>
<td>≥64</td>
<td>≥16</td>
<td>≥16</td>
<td>≥8</td>
<td>≥16</td>
<td>≥16</td>
<td>≥16</td>
<td>≥64</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

among Enterobacteriaceae. Furthermore, they could also play a
relevant role in the spread of such strains and other resistance
genes, especially if the isolate is present as part of the dom-
inant microbiota and the hygienic conditions are suboptimal
particularly in low-resource settings.

Nucleotide sequence accession number: The nucleotide
sequence of blaNDM-4 producing E. coli strain has been
deposited in the BankIt/GenBank/NCBI data base under acces-
sion number KY912035.

Funding

We are thankful to Higher Education Commission (HEC),
Pakistan for approving research grant (Number: 20-
3742/NRPU/R&D/HEC/14/430) and International Research
Support Initiative Program (IRSIP) fellowship for School of
Medicine, Cardiff University, Cardiff, United Kingdom.

Conflicts of interest

All authors declared that there is no conflict of interest.

REFERENCES

metallo-beta-lactamase gene, bla(NDM-1), and a novel
erthyromycin esterase gene carried on a unique genetic
structure in Klebsiella pneumoniae sequence type 14 from India.
2. Qamar M, Nahid F, Walsh T, Komran R, Zahra R. Prevalence
and clinical burden of NDM-1 positive infections in pediatric
and neonatal patients in Pakistan. Pediatr Infect Dis J.
2015;34:452–454.
3. Nordmann P, Boulanger A, Poirel L. NDM-4
metallo-beta-lactamase with increased carbapenemase
2012;56:2184–2186.
activity of Manuka honey against NDM-1-producing Klebsiella
5. Chew K, La M, Lina R, Teo J. Colistin and polymyxin B
susceptibility testing for carbapenem-resistant and
mcr-positive Enterobacteriaceae: comparison of Sensititre,
Microscan, Vitek 2, and Etest with broth microdilution. J Clin
7. Liu X, Thungrat K, Bothe D. Multilocus sequence typing and
virulence profiles in uropathogenic Escherichia coli isolated
from cats in the united states. PLOS ONE. 2015;10:e0143335.
Delhi metallo-beta-lactamase-4 (NDM-4)-producing

Associate Editor: Afonso Barth
Muhammad Usman Qamar a,b,c, Timothy R. Walsh c,
Mark A. Toleman c, Sidrah Saleem a, Shah Jahan a
a University of Health Sciences, Department of Microbiology,
Lahore, Pakistan
b Government College University, Faculty of Life Sciences,
Department of Microbiology, Faisalabad, Pakistan
c Cardiff University, Department of Infection and Immunity, School
d of Medicine, Cardiff, United Kingdom
d University of Health Sciences, Department of Immunology, Lahore,
Pakistan

* Corresponding author.
E-mails: usman9785@gmail.com, musmanqamar@gcuf.edu.pk
(M.U. Qamar).

© 2018 Sociedade Brasileira de Microbiologia. Published by
Elsevier Editora Ltda. This is an open access article under the
CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
https://doi.org/10.1016/j.bjm.2018.02.009
Available online 12 April 2018