An ST405 NDM-4-producing Escherichia coli isolated from a Danish patient previously hospitalized in Vietnam

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Sir,

Recently, a novel New Delhi metallo-β-lactamase (NDM) variant was described by Nordmann et al.¹ This variant, designated NDM-4, differs from NDM-1 by a single amino substitution and an increased carbapenemase activity. NDM-4 was first detected in an Escherichia coli isolated from a urinary sample from a patient previously hospitalized in India. A second finding of the NDM-4 variant was reported in an E. coli rectal isolate from a patient transferred from Cameroon to France.² This patient reported no previous travel to India. Here, we describe the first NDM-4-producing E. coli isolate in Denmark.

The isolate was obtained from a male patient in his 50s transferred from Vietnam to Denmark. The patient was hospitalized in two Vietnamese hospitals in late 2012 before being transferred to Aalborg University Hospital, Denmark. Nasal, throat and perineal swabs were obtained for routine screening for multiresistant bacteria (extended-spectrum β-lactamase- and carbapenemase-producing Gram-negative bacteria, methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus spp.). A carbapenem-resistant E. coli from the perineal swab was detected. Consequently, the patient stayed in isolation during the entire hospital stay.

The isolate was sent to Statens Serum Institut and tested for antimicrobial susceptibility to 27 antimicrobial agents by microbroth dilution (Sensititre, Trek Diagnostics System, East Grinstead, UK) as devised by the CLSI,³ with the exception of tigecycline and ertapenem, which were tested using MIC Test Strips (Liofilchem, Roseto degli Abruzzi, Italy) (Table 1). E. coli ATCC 25922 was used for quality control. The MIC results were interpreted using CLSI guidelines.³ The E. coli isolate was resistant to all β-lactam antimicrobial agents tested, including carbapenems (MICs of imipenem and ertapenem were >16 and >32 mg/L, respectively) (Table 1). Additionally, the isolate was resistant to gentamicin and fluoroquinolones, but remained susceptible to neomycin, colistin, sulfamethoxazole, trimethoprim and tigecycline. The resistance profile of the NDM-4 E. coli was similar to that of the NDM-4 isolates from India and Cameroon.²

Table 1. Antimicrobial resistance profile of an ST405 blaNDM-4-producing E. coli isolated from a Danish patient and the blaNDM-4-positive transconjugant E. coli MG1655

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>ST45 E. coli</th>
<th>transconjugant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Cefalotin</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Cefotaxime/clavulanic acid</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Ceftaziidime/clavulanic acid</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Ceftarixone</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ceftaroline</td>
<td>&gt;4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;8</td>
<td>&gt;8</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;32</td>
<td>—</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>&gt;64</td>
<td>—</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;4</td>
<td>—</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

PCR and sequencing were performed in search of different carbapenemase and extended-spectrum β-lactamase genes.⁵ Results showed that the E. coli isolate harboured the blaNDM-4, blaCTX-M-15 and blaTEM-1 genes. The isolate was PCR negative for the 16S rRNA methylase genes armA and rmtC conferring high-level resistance to aminoglycosides.⁵ The blaNDM gene, a novel gene reported to encode a bleomycin resistance protein coexpressed with the blaNDM-1 gene, was also detected using PCR.² Bleomycin is a glycopeptide antibiotic used as an anticancer agent to induce DNA strand breaks. It has been speculated that bleomycin chemotherapy may drive the emergence of NDM producers; even so, the patient had no history of such treatment. However, during the hospital stay in Vietnam the patient was treated with ceftazidime and sulbactam, which may have contributed to the selection of the NDM-4-producing E. coli later isolated. Interestingly, blaNDM-1-producing E. coli and Klebsiella pneumoniae have previously been reported from two Vietnamese surgical patients without history of travel outside Vietnam and as well as from two sites (3 km apart) in the Kim Nguu river running through Hanoi.⁶ The reservoir of such bacteria may not be limited to the hospital environment.

To investigate the mobility of blaNDM-4 conjugation experiments were carried out in Luria–Bertani (LB) broth with streptomycin-resistant E. coli MG1655 as the recipient, as described previously.⁷ Transconjugants were selected on LB agar plates supplemented with streptomycin (50 mg/L) and ertapenem (4 mg/L). PCR amplification and sequence analysis confirmed successful transfer of...
blaNDM-4 and blaTEM-1 (but not blaCTX-M-15) to E. coli MG1655. The
transfer frequency was $1.14 \times 10^{-6}$ per donor.

Phylogenetic typing and multilocus sequence typing using
the Achtmann scheme showed that the isolate belonged to phy-
logroup D and sequence type ST405, along with ST38, ST131 and ST648, has been identified as a contributor to the world-
wide emergence of human clinical CTX-M-15-producing E. coli.14
NDM-1-producing E. coli obtained from patients hospitalized in
the UK, Italy and Canada (with reported links to India) and the
NDM-4-producing E. coli from the patient transferred from Camer-
ono to France have likewise been assigned to the D-ST405
lineage.2,6,15 It is possible that this sequence type contributes
to the global dissemination of NDM producers in addition to
CTX-M-producing E. coli.

In December, ~2 months after the patient was first transferred
from Vietnam to the Danish hospital, the patient was readmitted.
Again, the patient was routinely swabbed for multiresistant bac-
teria and culture yielded an NDM-4-producing E. coli from the
peri- neum, indicating persistent carriage of NDM-4 producers for this
patient. Since the isolate was not stored, we could not investigate
whether the NDM-4-producing E. coli was a persistent clone or a dif-
ferent E. coli strain that had acquired the blaNDM-4 gene via horizon-
tal gene transfer.

To our knowledge, we report here the third NDM-4-producing
E. coli and the first to be detected in Denmark. Travel-associated
 dissemination of NDM-4 producers, as in the case of NDM-4,
spreading from South-East Asia in addition to the Indian subcon-
tinent and Africa may be anticipated. Our findings, along with other
reports, of the ST405 sequence type stress the potential of this
lineage to assist in the global spread of NDM producers.

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Transparency declarations
None to declare.

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INQ-1, a chromosome-encoded AmpC
β-lactamase from Inquilinus limosus

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