Transfer of $bla_{CTX-M14}$ into the ST131 B2 E. coli recipient of porcine origin was possible for three of the seven CTX-M-14-producing isolates; likewise $bla_{CTX-M15}$ was transferred from one of the two CTX-M-15 isolates (Table 1).

Our study shows that pigs and pork can be a reservoir of ExPEC CTX-M-14-producing E. coli. CTX-M-14-producing E. coli has been detected previously in Danish healthy army recruits, indicating a non-hospital reservoir of ESBL-producing E. coli in Denmark.10

The isolates in the present study did not belong to ST131, but four of the isolates belong to STs that had previously given rise to infections in humans. Furthermore, the $bla_{CTX-M14}$ and $bla_{CTX-M15}$ genes were transferable to a ST131 E. coli recipient of pork origin. Our study therefore shows that pigs and pork can be a reservoir of human pathogenic ESBL-producing E. coli and a reservoir of transferable $bla_{CTX-M14}$ and $bla_{CTX-M15}$ genes. Further studies are needed to quantify this risk for the $bla_{CTX-M14}$ and $bla_{CTX-M15}$ present in E. coli of porcine origin in relation to human health.

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Transparency declarations
None to declare.

References


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An NDM-1-producing Escherichia coli obtained in Denmark has a genetic profile similar to an NDM-1-producing E. coli isolate from the UK

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Sir,
We read with interest the recent article by Mushtaq et al.1 on the phylogenetic diversity of 18 NDM-1 carbapenemase-producing Escherichia coli strains from England, Pakistan and India. The E. coli isolates producing NDM-1 belonged to six sequence types (ST101, ST405, ST648, ST90, ST410 and ST156) and three phylogroups (B1, D and A).1 Nevertheless, isolates of B1-ST101 accounted for half the collection, and included isolates from both England and Pakistan. Sixteen of 18 isolates had a group 1 CTX-M gene, 13 had a C12-type acquired AmpC and 16 had either one or both of the armA and rmtC 16S rRNA methylase genes.1

Recently we have detected the first NDM-1-producing E. coli in Denmark. As with many of the NDM-1-producing isolates from...
Table 1. Description of the first NDM-1-producing E. coli isolate in Denmark, the recipient strain and two transformants

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>clinical isolate</th>
<th>recipient strain</th>
<th>transformant NDM-1</th>
<th>transformant CTX-M-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;32</td>
<td>4</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Amoxicillin/</td>
<td>&gt;32/16</td>
<td>4/2</td>
<td>&gt;32/16</td>
<td>16/8</td>
</tr>
<tr>
<td>clavulanic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>&gt;64/4</td>
<td>≤4/4</td>
<td>&gt;64/4</td>
<td>64/4</td>
</tr>
<tr>
<td>Mecillinam</td>
<td>16</td>
<td>0.5</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;64</td>
<td>≤4</td>
<td>&gt;64</td>
<td>8</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;16</td>
<td>≤8</td>
<td>&gt;16</td>
<td>≥16</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;64</td>
<td>≤0.25</td>
<td>&gt;64</td>
<td>≥64</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;128</td>
<td>≥0.25</td>
<td>&gt;128</td>
<td>32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;128</td>
<td>&lt;1</td>
<td>&gt;128</td>
<td>≥128</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&gt;16</td>
<td>&lt;1</td>
<td>&gt;16</td>
<td>≥16</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>&gt;64</td>
<td>≤0.064</td>
<td>64</td>
<td>≤64</td>
</tr>
<tr>
<td>Imipenem</td>
<td>8</td>
<td>≤0.5</td>
<td>&gt;16</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;8</td>
<td>&lt;1</td>
<td>&gt;8</td>
<td>≤1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;4</td>
<td>≤0.016</td>
<td>≤0.016</td>
<td>≤0.016</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>&gt;64</td>
<td>≤4</td>
<td>≤4</td>
<td>≤4</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>128</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;16</td>
<td>≤4</td>
<td>&gt;16</td>
<td>8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4</td>
<td>≤2</td>
<td>4</td>
<td>≤2</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>&gt;32</td>
<td>≤1</td>
<td>&gt;32</td>
<td>≥1</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>&gt;1024</td>
<td>≤64</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>Colistin</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>&gt;64</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

The MICs of the antimicrobial agents shown in Table 1 were determined by use of the microbroth dilution method as defined by the CLSI, with the exception of the MICs of tigecycline and mecillinam, which were determined using MIC test strips (Liofichem, Roseto degli Abruzzi, Italy). Results were interpreted according to EUCAST (www.EUCAST.org), except for the results for nalidixic acid, tetracycline, streptomycin and sulfamethoxazole, which were interpreted according to CLSI. The reference strain E. coli ATCC 25922 was used for quality control.

The blaNDM gene was identified by PCR using primers NDM-F (5′-GAAGCTTACGCCCTGTTT-G-3′) and NDM-R (5′-TGCGCCTGACGACTATGGTT-G-3′) to amplify an internal fragment of 761 bp. Furthermore, the isolate was screened for acquired AmpC genes (MOX, CIT, DHA, ACC, ENT and FOX) and for blaCTX-M, blaOXA and blaTEM by PCR using primers and conditions described previously. The isolate was screened for the 16S rRNA methylase genes armA and rmtC according to Galimand et al. and Mushtaq et al., respectively.

Our NDM-1-producing E. coli isolate was assigned to one of the major E. coli phylogenetic groups (A, B1, B2 and D) by multiplex PCR. Multilocus sequence typing (‘MLST’) was performed using seven conserved housekeeping genes (http://mlst.ucc.ie/mlst/dbs/Ecoli).

Plasmid purification was performed using the Plasmid Mini AX kit (A&A Biotechnology, Poland). Plasmid profiling and restriction analysis using EcoRI revealed the presence of two large plasmids and one small plasmid. Plasmids were electroporated into electrocompetent E. coli O157:H7 (OneshotGen-Ehogs, Life Technology, Denmark) and transformants were selected on Luria-Bertani agar containing 2 mg/L cefotaxime. Screening of transformants identified strains transformed by either of the two large plasmids. PCR replicon typing of the original isolate and the two single plasmid transformants was performed.

The isolate was resistant to all antimicrobial agents except colistin and tigecycline (Table 1). It tested positive by PCR for metallo-β-lactamases of the NDM group, ESBL enzymes belonging to group 1 CTX-M, OXA and TEM, and the CIT-type AmpC enzymes. PCR and further sequencing showed the following profile for the isolate: blaNDM-1, blaCTX-M-15, blaOXA-2, blaTEM-1 and armA. The isolate was ST101 and belonged to phylgroup B1 and showed a similar genetic profile to one of the B1-ST101 isolates from the UK, which had a CIT AmpC enzyme, had a group 1 CTX-M-type enzyme and was positive for armA. NDM-1-producing B1-ST101 E. coli isolates have also been obtained from patients from Australia, Germany and Canada. Our study adds information to the global spread of NDM-1-producing B1-ST101 E. coli isolates.

Characterization of the two large plasmids revealed blaNDM-1 to be located on an IncA/C plasmid together with blaOXY-4 and armA. The other large plasmid contained blaCTX-M-15 and blaTEM-1, and was not typeable by replicon typing.

Recently Sekizuka et al. published a completely sequenced blaNDM-1 plasmid from an ST38 E. coli isolate from Japan. Similar to our NDM-1 plasmid, the NDM-1 plasmid in the Japanese isolate contained blaOXY-4 and armA in addition to blaNDM-1 and was IncA/C. This may suggest dissemination of similar plasmids into several different sequence types of E. coli.

Further studies of NDM-1 isolates and their plasmids are needed to follow the global dissemination of this gene.

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We thank Karin Sixhøj Pedersen for technical assistance.

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Transparency declarations
None to declare.
Sir,

The current emergence of the carbapenem-hydrolysing class D β-lactamase OXA-48 in Enterobacteriaceae is of great concern, since the corresponding gene is encountered in various enterobacterial species that are often multidrug resistant.1 OXA-48 hydrolysates penicillins at high level and carbapenems at low level; however, its impact on carbapenems is significant, especially when combined with additional mechanisms and, in particular, permeability defects.2 The first identification of OXA-48 producers was from Turkey, where it is now considered endemic.1,2 A rapid dissemination of OXA-48 producers has been observed, and North Africa is now being considered as an additional reservoir.1 The spread of the blaOXA-48 gene is linked to the dissemination of an epidemic 62 kb IncM/M-type and self-conjugative plasmid that has been identified in many clonally unrelated strains and different enterobacterial species from distantly located geographic areas.3

Recently the blaOXA-48 gene has been identified in Israel and Lebanon, whereas the blaOXA-181 gene (a blaOXA-48 derivative) has been identified in France, India, The Netherlands and Sultanate of Oman, and the blaOXA-163 (another derivative encoding resistance to broad-spectrum cephalosporins) has been identified in Argentina.4 In Kuwait, the only carbapenemase-producing Enterobacteriaceae so far correspond to two recently identified NDM-1-producing Klebsiella pneumoniae.4

Our study was initiated by the isolation of carbapenem-resistant K. pneumoniae from a diabetic patient who had been hospitalized in July 2011 in Kuwait, where she developed gangrene in her left foot. In August, she was transferred to Paris, France, where her left leg was amputated and she underwent prosthetics surgery. Upon admission at the Paris hospital, a rectal swab was performed to screen for multidrug-resistant bacteria. Selection was performed onto a Drigalski plate on which an imipenem-containing disc had been placed, and onto a ChromID ESBL plate (bioMérieux, La Balme-les-Grottes, France). It grew K. pneumoniae strain ALI showing resistance to carbapenems. No secondary transmission occurred at the Paris hospital following the rapid implementation of strict infection control measures.

The antibiogram determined by the disc diffusion method and MICs determined by Etest (Ab bioMérieux, Solna, Sweden) and interpreted according to the CLSI guidelines5 revealed that K. pneumoniae isolate ALI was highly resistant to penicillins and carbapenems, with MICs of ertapenem, imipenem, meropenem and doripenem being >32, 32, 32 and 8 mg/L, respectively. Isolate ALI was fully susceptible to expanded-spectrum cephalosporins, with MICs of ceftazidime and ceftriaxone being 0.5 mg/L. This isolate was susceptible to all quinolones and aminoglycosides, being resistant only to fosfomycin and nitrofurantoin. Multilocus sequence typing

References