In summary, this study showed that the blaNDM-1 is present in bacteria of food animal origin, although currently at a very low prevalence. Nevertheless, further monitoring of the presence of the blaNDM-1 gene and other carbapenemase genes in bacteria of animal origin is warranted.

Acknowledgements
We thank the Commissariat à l’Energie Atomique/Direction des Sciences du Vivant for kindly providing the \textit{A. baylyi} ADP1 strain.

Funding
This study was supported by grants from the National ‘973’ programme (no. 2012CB518801) and the National Science Foundation of China (no. NSFC31201862).

Transparency declarations
None to declare.

Supplementary data
Table S1 and Figure S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References
11 Michael GB, Eidam C, Kadlec K \textit{et al}. Increased MICs of gamithromycin and tildipirosin in the presence of the genes \textit{erm}(42) and \textit{msr(E)-mph(E)} for bovine \textit{Pasteurella multocida} and \textit{Mannheimia haemolytica}. \textit{J Antimicrob Chemother} 2012; \textbf{67}: 1555–7.

Identification of Enterobacteriaceae isolates with OXA-48 and coproduction of OXA-181 and NDM-1 in Norway

Ørjan Samuelsen\textsuperscript{1,}\textsuperscript{*}, Umaer Naseer\textsuperscript{1,2}, Nabil Karah\textsuperscript{1,2}, Paul Christoffer Lindemann\textsuperscript{3}, Anita Kanestrøm\textsuperscript{4}, Truls M. Leegaard\textsuperscript{5,6} and Arnfinn Sundsfjord\textsuperscript{1,2}

\textsuperscript{1}Reference Centre for Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway; \textsuperscript{2}Research Group for Host–Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway; \textsuperscript{3}Department of Microbiology, Haukeland University Hospital, Bergen, Norway; \textsuperscript{4}Center for Laboratory Medicine, Østfold Hospital Trust, Fredrikstad, Norway; \textsuperscript{5}Department of Microbiology and
Sir,

The global emergence and dissemination of acquired carbapenemases among Gram-negative bacteria are considered a major public health problem. The dominant carbapenemases include the class A serine β-lactamasases (e.g. KPC), the class B metallo-β-lactamasases (e.g. NDM, VIM and IMP) and the class D OXA group. The carbapenemase-encoding genes are often located on plasmids along with other resistance genes, resulting in multidrug-resistant, extremely drug-resistant or pan-drug-resistant bacteria. Among the class D β-lactamasases, OXA-48-like carbapenemases have increasingly been identified in Enterobacteriaceae with a wide geographical distribution. After the first identification in Turkey, the dissemination of OXA-48-like carbapenemases was mainly identified in North African and Middle Eastern countries. However, a growing number of reports show a global dissemination of OXA-48-like carbapenemases and, in some European countries, OXA-48-like has become the predominant carbapenemase. The OXA-48-like enzymes have limited activity against extended-spectrum cephalosporins, in contrast to other carbapenemases, and the MIC values of carbapenems are often below the clinical breakpoints. This has implications for detection procedures in the laboratory. In the Nordic countries, the emergence of carbapenemase producers has mainly been associated with patient transfer from other countries. In this study, we characterized the first three OXA-48-like producing Enterobacteriaceae from Norway, of which one isolate coproduced NDM-1. The bacterial isolates, two Klebsiella pneumoniae and one Escherichia coli, were submitted by three different laboratories to the National Reference Centre for Detection of Antimicrobial Resistance in Norway, based on reduced susceptibility to carbapenems (Table 1). All three isolates were isolated from patients who had recently been hospitalized or in contact with a hospital abroad in three separate countries (Romania, Morocco and Thailand) (Table 1). National guidelines in Norway recommend that patients who have been hospitalized in countries with a known high prevalence of multidrug-resistant Gram-negative bacteria are screened; two of the isolates were obtained from rectal screening samples. The third isolate was associated with a urinary tract infection. Reasons for hospitalization abroad included a traffic accident or heart problems.

Analysis of the β-lactamase content of the isolates using PCRs followed by sequencing, as previously described, or a nucleic acid-based microarray (Check-Points, Wageningen, The Netherlands) revealed that two isolates harboured blaOXA-48 while one isolate contained both the blaOXA-181 variant and blaNDM-1 (Table 1). The coproduction of OXA-181 and NDM-1 has previously been described in K. pneumoniae from the Sultanate of Oman. Further, all isolates also possessed a CTX-M group 1 extended-spectrum β-lactamase (ESBL), as is often found in isolates with blaOXA-48. All isolates were positive for the OXA-1 group, but negative for the OXA-2 group and OXA-9. The isolate that coproduced OXA-181 and NDM-1 showed high-level resistance to all carbapenems, while the two OXA-48-producing isolates were susceptible to both meropenem and imipenem, but resistant to ertapenem according to EUCAST clinical breakpoints (Table 1). High-level resistance to extended-spectrum cephalosporins was observed in all isolates due to the presence of blaNDM-1 and/or blaTX-K. Other acquired antimicrobial resistance markers identified by PCR assays included the aac(6’)-Ib-cr variant found in all isolates, as well as qnrB in the OXA-48-positive K. pneumoniae isolate. The OXA-181- and NDM-1-producing isolate was also resistant to colistin, making this isolate an extremely drug-resistant variant. The two K. pneumoniae isolates were sequence typed as sequence type (ST) 405 and ST525, respectively, by multilocus sequence typing (MLST; Table 1). ST405 has recently been associated with OXA-48 in Spain. The E. coli isolate was assigned to ST405, a representative ST of the virulent phylogroup D lineage often associated with blaCTX-M-15.

The blaOXA-48 gene has previously been found to be part of the composite transposon Tn1997 and variants thereof. Mapping of the genetic structure surrounding blaOXA-48-like showed that it was present on our isolates using previously described primers. In both OXA-48-producing isolates, IS1997 was found on both ends of blaOXA-48, where the IS1997 element upstream of blaOXA-48 was interrupted by an IS1997, as described for the Tn1997 variant. In the OXA-181-positive isolate, ISEcP1 was identified upstream of blaOXA-181, as previously described. Conjugation experiments to E. coli J53-2 were successful for both blaOXA-48 isolates (Table 1). For the OXA-181-carrying isolate, only transconjugants positive for blaOXA-181 were obtained. The blaOXA-48 gene has mainly been associated with an ~62 kb IncL/M plasmid harbouring conserved backbone genes (repA, traU and parA). Both blaOXA-48-positive isolates and the corresponding transconjugants were positive for repA, traU and parA by PCR. repA, traU and parA were not detected in the OXA-181 isolate. Plasmid analysis using S1 nuclease digestion of total genomic DNA followed by PFGE and Southern blot hybridization with the blaOXA-48 and parA probes revealed that blaOXA-48 was localized on an ~50–60 kb plasmid that cohybridized with the parA probe (data not shown). The OXA-181 gene was localized on an ~200 kb plasmid.

In conclusion, this study further documents the ongoing global dissemination of blaOXA-48-like, as well as the coexistence of blaNDM-1 and blaOXA-181 in a single K. pneumoniae strain. The acquisition of blaOXA-48-like was related to colonization during foreign travel. These observations emphasize the necessity of rigorous screening of relevant patients upon entry after foreign travel, to prevent the further spread of these strains in low-prevalence countries.

Acknowledgements
We acknowledge the excellent technical assistance of Bjørg Haldorsen, Bettina Aasnaes, Ruma Walden and Cathrine Ramberg.
Table 1. Relevant characteristics of OXA-48-like isolates and respective transconjugants

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species</th>
<th>Laboratory</th>
<th>Month/year</th>
<th>Specimen</th>
<th>Travel history</th>
<th>STa</th>
<th>Carbapenemase(s)</th>
<th>Other resistance markers</th>
<th>Antimicrobial susceptibility, MIC (mg/L)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>50531633</td>
<td>K. pneumoniae</td>
<td>Haukeland</td>
<td>08/2011</td>
<td>rectal screening</td>
<td>Romania</td>
<td>525</td>
<td>OXA-181, NDM-1</td>
<td>CTX-M group 1, SHV-WT, TEM-WT, OXA-1 group, AAC(6’)-Ib-cr</td>
<td>&gt;32 &gt;32 &gt;256 &gt;256 &gt;1024 32 1 16</td>
</tr>
<tr>
<td>50572569</td>
<td>K. pneumoniae</td>
<td>Akershus</td>
<td>12/2011</td>
<td>rectal screening</td>
<td>Morocco</td>
<td>405</td>
<td>OXA-48</td>
<td>CTX-M group 1, SHV-WT, TEM-WT, OXA-1 group, AAC(6’)-Ib-cr, QnrB</td>
<td>1 4 256 256 &gt;256 32 4 2 0.5</td>
</tr>
<tr>
<td>50579417</td>
<td>E. coli</td>
<td>Østfold</td>
<td>01/2012</td>
<td>urine</td>
<td>Thailand</td>
<td>405</td>
<td>OXA-48</td>
<td>CTX-M group 1, OXA-1 group, AAC(6’)-Ib-cr</td>
<td>0.5 1 8 &gt;256 &gt;256 &gt;256 16 &gt;32 1 1</td>
</tr>
<tr>
<td>50572569 TC</td>
<td>E. coli</td>
<td>— — — — — — — OXA-48</td>
<td>none</td>
<td>0.5 1 2 0.5 2 256 0.125 0.25 0.5 0.125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50579417 TC</td>
<td>E. coli</td>
<td>— — — — — — OXA-48</td>
<td>none</td>
<td>0.5 1 1 0.5 2 256 0.125 0.25 0.5 0.125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J53-2</td>
<td>E. coli</td>
<td>— — — — — — — — OXA-48</td>
<td>none</td>
<td>0.06 0.25 0.016 0.5 0.125 2 0.125 0.25 0.5 0.125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MEM, meropenem; IPM, imipenem; ETP, ertapenem; CAZ, ceftazidime; CTX, cefotaxime; TZP, piperacillin/tazobactam; GEN, gentamicin; CIP, ciprofloxacin; TGC, tigecycline; CST, colistin; WT, wild-type; TC, transconjugant.

aMLST was performed according to the protocols described at the MLST web sites for K. pneumoniae (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) and E. coli (http://mlst.ucc.ie/mlst/dbs/Ecoli).

bThe antimicrobial susceptibility profiles of the isolates were determined using Etests according to the manufacturer’s instructions (bioMérieux) and interpreted according to the clinical breakpoints set by EUCAST (http://www.eucast.org/clinical_breakpoints/).
Funding
Part of this study was funded by a research grant from the Northern Norway Regional Health Authority.

Transparency declarations
None to declare.

References

Comparison of the Phoenix automated system, the Etest method and broth microdilution in determining temocillin susceptibility of Enterobacteriaceae

Trupti A. Patel*, Rachel Dilley, Alan Williams, Gemma L. Vanstone and Indran Balakrishnan

Department of Microbiology, Royal Free London NHS Foundation Trust, Pond Street, London NW3 2QG, UK

*Corresponding author. Tel: +44-207-794-0500; Fax: +44-207-472-6240; E-mail: truptipatel1@nhs.net

Keywords: antimicrobial resistance, extended-spectrum β-lactamases, Gram-negative bacteria

Sir,

Temocillin, a semi-synthetic 6-α-methoxy derivative of ticarcillin, has been shown to be clinically efficacious in infections caused by extended-spectrum β-lactamase (ESBL)- and AmpC-producing Enterobacteriaceae.1,2 It is licensed in the UK for bacteraemia, urinary tract infections (UTIs) and lower respiratory tract infections where susceptible Gram-negative bacteria are suspected or confirmed.

Currently, only the BSAC has defined temocillin breakpoints for Enterobacteriaceae (susceptible if MIC ≤8 mg/L for systemic infections and ≤32 mg/L for UTIs).3

Here, we report a comparison of three temocillin susceptibility testing methodologies [BD Phoenix™ Automated Microbiology System (instrument version 5.15A, software version 6.01AV5.15A) (Becton Dickinson, Oxford, UK), Etest (AB Biodisk, Solna, Sweden) and broth microdilution (BMD)]. Our data indicate that, whereas the level of agreement for ‘susceptible’ results is excellent, the Phoenix system overcalls temocillin non-susceptibility.

A total of 281 consecutive clinical Enterobacteriaceae isolates from distinct patients were collected from urine, blood culture, fluid, respiratory and tissue specimens. Isolates comprised Escherichia coli (194), Klebsiella pneumoniae (19), Proteus mirabilis (16), Enterobacter cloacae (9), Serratia marcescens (7), Citrobacter koseri (6), Enterobacter aerogenes (5), Citrobacter freundii (4), Morganella morgani (4), Klebsiella oxytoca (3), Proteus vulgaris (3), Citrobacter freundii (3), Pasteurella agglomerans (2), Pantoea agglomerans (2), Serratia liquefaciens (2), Citrobacter braakii (1), Enterobacter gergoviae (1), Klyuyera sp. (1), Providencia rettgeri (1) and Raoultella ornithinolytica (1). Twenty-seven (9.6%) were ESBL producers, 16 (5.7%) were inducible AmpC β-lactamase producers and 8 (2.8%) were derepressed AmpC β-lactamase producers.

Identification and susceptibility testing were performed on overnight cultures using the BD Phoenix™ AP instrument (automated nephelometry) and the BD Phoenix™ Automated Microbiology System (instrument version 5.15A, software version 6.01A/V5.15A) with Gram-negative Phoenix panels (NMIC-84). Temocillin MICs were also determined by BMD in cation-adjusted Mueller–