Identification of NDM-1-producing Enterobacteriaceae in Norway

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Sir,

Carbapenemase-producing Enterobacteriaceae are still rare in Scandinavia and the majority of reported cases have been clearly linked to hospitalization abroad.¹⁻⁴ New Delhi metallo-β-lactamase-1 (NDM-1) was first identified in 2008 in Klebsiella pneumoniae and Escherichia coli isolates from a Swedish patient following hospitalization in India.⁵ In 2009 the HPA in the UK issued an addendum to National Resistance Alert 3 (http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1248854045473) due to a worrying increase in NDM-1-producing clinical strains of Enterobacteriaceae. Many of the UK cases could be linked to recent medical exposure in India or Pakistan.⁶ Recent epidemiological studies describe the prevalence of NDM-1-producing Enterobacteriaceae in India and warn of the potential for worldwide spread.⁶,⁷ NDM-1-producing Enterobacteriaceae have recently been reported from the USA,⁷ the Netherlands,⁸ Australia⁶,⁹ and Canada.¹⁰

Here we report two cases of NDM-1-producing Enterobacteriaceae identified in Norway. Case 1 was an elderly male from India who required hospitalization for urosepsis on two occasions during a visit to Norway. The patient had undergone a transurethral resection of the prostate in India in early 2009. The post-operative period was complicated by partial urinary incontinence and recurrent urinary tract infections. The patient had been treated with several courses of antibiotics, including faropenem, a penem available for oral administration in India. Two weeks after arrival in Norway in late 2009 (5 months post-operative) the patient was admitted to hospital for urosepsis. A CTX-M-producing Klebsiella pneumoniae was isolated from urine and blood cultures. The isolate was resistant to ertapenem, intermediate to meropenem and susceptible to imipenem using Etest (bioMérieux) and EUCAST clinical breakpoints, but no carbapenemase activity was detected by biochemical and molecular assays. The infection was cleared after initial treatment with meropenem followed by colistin in combination with rifampicin and urinary catheter insertion. Subsequent urological investigation revealed metastatic cancer of the prostate. The patient remained in Norway, but 3 months later, in early 2010, was re-admitted due to urinary retention and urosepsis. An NDM-1-producing Escherichia coli (K71-77) was isolated from urine and blood cultures. Antimicrobial susceptibility testing showed that the E. coli isolate was resistant to penicillins and cephalosporins and intermediate to aztreonam (Table 1). No synergy was observed with β-lactamase inhibitors. The isolate was resistant to ertapenem, but susceptible to both meropenem and imipenem. In addition, the isolate showed high-level resistance to aminoglycosides and ciprofloxacin, but was susceptible to tigecycline, colistin and trimethoprim/sulfamethoxazole. Carbapenemase activity was detected in bacterial cell extracts, which was inhibited with EDTA. PCR and DNA sequence analysis revealed blaNDM-1 primers used for detection of resistance genes are listed in Table S1, available as Supplementary data at JAC Online). The isolate harbouried the plasmid-mediated AmpC gene blaCMY-6, but was negative for extended-spectrum β-lactamase (ESBL) genes (blaTEM, blaSHV and blaCTX-M) and 16S rRNA methylases. Multilocus sequence typing (MLST)¹¹ revealed sequence type (ST) 410, which belongs to ST complex 23. The infection was cleared after initial treatment with meropenem followed by trimethoprim/sulfamethoxazole.

Case 2 was a Norwegian female patient who was hospitalized in India in 2010 following a traffic accident. The patient underwent orthopaedic surgery and prophylactic antibiotic treatment (linezolid) in India prior to her repatriation. On arrival in Norway the patient was admitted directly to hospital. A multidrug-resistant K. pneumoniae (K66-45) isolate was cultured from a catheter urine sample. The catheter had been inserted in India. The urine isolate was judged on clinical grounds to represent catheter colonization and did not require antibiotic treatment. The isolate was resistant to penicillins, cephalosporins and aztreonam (Table 1). Synergy was observed with ceftazidime and clavulanic acid using combination discs. The isolate expressed resistance to meropenem, ertapenem and intermediate resistance to imipenem. Moreover, the isolate showed high-level resistance to aminoglycosides, ciprofloxacin and trimethoprim/sulfamethoxazole, and resistance to tigecycline. The isolate was susceptible to colistin only. Carbapenemase
activity was detected in bacterial cell extracts, which was inhibited by EDTA. PCR and sequence analysis revealed blaNDM-1. In addition, blaTEM-1, blaSHV-11, blaCTX-M-15 and the 16S rRNA methylase gene armA were detected. MLST analysis showed ST11, a single-locus variant of ST258, which is associated with the global dissemination of *K. pneumoniae* carbapenemase (KPC)–producing *K. pneumoniae*, suggesting a potential for further spread and dissemination. To the best of our knowledge this is the first description of blaNDM-1–producing Enterobacteriaceae isolates in Norway and contributes to current knowledge of the global dissemination of this carbapenemase resistance gene. The relatively long time period between hospitalization in India and the identification of the NDM-1 isolate in Case 1 (8 months) suggests faecal colonization over long time periods.

The NDM-1 enzyme is capable of inactivating virtually all β-lactam antibiotics and is often associated with other resistance mechanisms, resulting in extremely drug-resistant Gram-negative bacteria. Of concern is the fact that the NDM-1 gene is located on transferable plasmids, and dissemination into multiple genera—including *K. pneumoniae*, *E. coli*, *Enterobacter* spp., *Citrobacter freundii*, *Morganella morganii*, *Proteus* spp., *Providencia* spp. and *Acinetobacter baumannii*—has already occurred. Microbiological laboratories must maintain a high level of alertness during the interpretation of antibiotic results. The detection of NDM-1–producing Enterobacteriaceae cannot be based solely on the current clinical breakpoints for carbapenems. This is also the case for strains expressing other carbapenemases. The relatively low carbapenem MIC values observed for isolate K71–77 highlights this problem.

The global dissemination of carbapenemase–producing Gram-negative bacteria has to be controlled. Of importance is the prudent use of antibiotics, good and rapid microbiological laboratory services and targeted infection control measures. The epidemiological situation should be carefully monitored by including the characterization of clinical strains of Enterobacteriaceae with reduced susceptibilities to carbapenems in surveillance systems. Further, patients who have been hospitalized in countries with a high prevalence of carbapenemase–producing Enterobacteriaceae should be screened upon hospital admission.

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### Table 1. Antimicrobial susceptibility profile of NDM-1–producing isolates identified in Norway

<table>
<thead>
<tr>
<th>Isolate</th>
<th>AMP</th>
<th>TTP</th>
<th>FOX</th>
<th>CTX</th>
<th>CAZ</th>
<th>AZT</th>
<th>MEM</th>
<th>IPM</th>
<th>ETP</th>
<th>CIP</th>
<th>AMK</th>
<th>GEN</th>
<th>TOB</th>
<th>CST</th>
<th>TGC</th>
<th>SXT</th>
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<tbody>
<tr>
<td>K71-77</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>8</td>
<td>0.5</td>
<td>1</td>
<td>4</td>
<td>&gt;32</td>
<td>&gt;256</td>
<td>&gt;1024</td>
<td>&gt;256</td>
<td>1</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>K66-45</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>16</td>
<td>4</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;256</td>
<td>&gt;1024</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>1</td>
<td>8</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

AMP, ampicillin; TTP, piperacillin/tazobactam; FOX, cefoxitin; CTX, cefotaxime; CAZ, ceftazidime; AZT, aztreonam; MEM, meropenem; IPM, imipenem; ETP, erlotapenem; CIP, ciprofloxacin; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; CST, colistin; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole.

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### Transparency declarations
None to declare.

### Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

### References


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Plasmid-encoded OXA-48 carbapenemase in Escherichia coli from Israel

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Sir,
Carbapenem resistance among Enterobacteriaceae in Israel emerged in 2004 and was observed mainly in Klebsiella pneumoniae, but also in Enterobacter species and Escherichia coli. Since its emergence, carbapenem resistance in these species, both in clinical and colonizing isolates, has been rendered by the production of plasmid-mediated K. pneumoniae carbapenemase (KPC).

In late 2007, a woman in her early thirties previously diagnosed with acute lymphoblastic leukaemia, was admitted to the haematology ward in Tel Aviv Sourasky Medical Center. The patient had arrived in Israel from Jordan in order to undergo chemotherapy and, later, bone marrow transplantation. During the first month after transplantation the patient was intermittently febrile and was treated with various antimicrobials, including piperacillin/tazobactam, amikacin, ciprofloxacin, vancomycin, imipenem and voriconazole. Two months after admission, while being treated with imipenem and voriconazole, the patient suffered from fever, dyspnoea and renal failure, and was transferred to the intensive care unit. During that period a carbapenem-resistant E. coli strain (E. coli 1736) was isolated from a Hickman catheter, leading to removal of the catheter and further treatment with ceftazidime and colistin. Treatment with these antibacterial agents cleared the OXA-48-producing E. coli, yet unfortunately the patient died 3 months later from a systemic Pseudomonas infection.

E. coli 1736 was multidrug resistant, showing resistance to penicillins, piperacillin/tazobactam, aminoglycosides, quinolones and carbapenems, but susceptibility to all cephalosporins, aztreonam, tigecycline and colistin (Table 1). Analytical isoelectric focusing (IEF) performed on crude enzyme preparations revealed the presence of two β-lactamases with pIs of 5.4 and 7.2 (data not shown). PCR screening for the presence of β-lactamases in E. coli 1736 indicated the presence of blam TEM-1 and blam OXA-48 Genes corresponding to the pIs of the β-lactamases visualized by IEF. Plasmid analysis1 of E. coli 1736 revealed four plasmids, three of around ≤50 kb in size and a larger plasmid of around 100 kb. Plasmid DNA was purified and transformed into E. coli DH10B. Transformant colonies that were screened positive for blam OXA-48 by PCR harboured the 50 kb plasmid. Southern analysis of plasmid DNA derived from these transformants using a blam OXA-48-labelled probe demonstrated the presence of blam OXA-48 on the acquired 50 kb plasmid. Acquisition of this plasmid increased the MICs of imipenem, meropenem and ertapenem without conferring full resistance (Table 1).

PCR mapping of the genetic environment surrounding blam OXA-48 was performed in collaboration with the laboratory of Professor P. Nordmann (Hospital de Bicetre, Paris, France). blam OXA-48 was found to be located inside Tn1999.2, similar to the structure described for other enteric strains, such as the E. coli strain from Turkey and the K. pneumoniae strain from Lebanon.2

E. coli 1736 was not resistant to all β-lactam antibiotics as reported for other OXA-48-producing strains,3 yet it presented a high level of resistance to the commonly used carbapenems (MICs ≥16 mg/L), higher than usually seen in KPC-producing E. coli strains isolated in our hospital.3 These high carbapenem MICs suggested the presence of additional resistance mechanisms together with OXA-48 carbapenemase. Outer membrane protein (OMP) produced by E. coli 1736 was determined by PCR and sequencing of ompA, ompC and ompF genes. Further OMP analysis was performed by protein extraction and separation on Tris–Tricine gels using SDS-PAGE followed by mass spectrometry (performed in the Biological Mass Spectrometry Facility at the Weizmann Institute of Science). Both methods indicated the absence of at least one major porin, OmpC.

Until the isolation of E. coli 1736, carbapenem resistance in E. coli in our country was exclusively attributed to the Ambler class A carbapenemase KPC.3 This is the first identified Enterobacteriaceae isolate in our country possessing a carbapenem-hydrolysing oxacillinase. To seek the possible origin of this strain we performed multilocus sequence typing (MLST; http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html), which genotyped the strain as sequence type (ST) 2, an E. coli ST that has never been recorded previously in our