Emergence of clonally related *Klebsiella pneumoniae* isolates of sequence type 258 producing plasmid-mediated KPC carbapenemase in Norway and Sweden

Ørjan Samuelsen1*, Umaer Naseer1, Ståle Tofteland2, Dag Harald Skutlaberg3, Annette Onken4, Reidar Hjetland5, Arnfinn Sundsfjord1,6 and Christian G. Giske7

1Reference Centre for Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway; 2Department of Microbiology, Sørlandet Hospital, Kristiansand, Norway; 3Department of Microbiology and Immunology, The Gade Institute, Haukeland University Hospital, Bergen, Norway; 4Department of Microbiology, Central Laboratory, Hospital of Asker and Bærum, Oslo, Norway; 5Department of Microbiology, Førde Central Hospital, Førde, Norway; 6Department of Microbiology and Virology, University of Tromsø, Tromsø, Norway; 7Karolinska Institutet-MTC, Clinical Microbiology, Karolinska University Hospital, Solna, Stockholm, Sweden

Background: The class A carbapenemase KPC has disseminated rapidly worldwide, challenging the treatment of Gram-negative infections. This report describes the first KPC-producing *Klebsiella pneumoniae* isolates identified in Norway (n = 6) and the second isolate from Sweden.

Methods: Antimicrobial susceptibility profiles were determined using Etest. PCR and sequencing were used to determine the *bla*KPC variant, the surrounding genetic structure and the presence of AmpC and extended-spectrum β-lactamase genes. PFGE and multilocus sequence typing (MLST) were used for epidemiological comparisons. Localization of *bla*KPC was investigated by S1 nuclease digestion, followed by PFGE and Southern blot hybridization.

Results: All isolates expressed a multidrug-resistant phenotype with some variability in the carbapenem susceptibility profile. The Norwegian isolates carried *bla*KPC-2, while the Swedish isolate carried *bla*KPC-3. All isolates carried TEM-1, but were negative for *bla*CTX-M and *bla*AmpC genes. SHV-11 and SHV-12 were detected in the Norwegian isolates, while the Swedish isolate carried only SHV-11. Isolates from four patients were associated with import from Greece (n = 3) and Israel. The other isolates were probably associated with local transmissions. PFGE and MLST showed that the isolates were clonally related, with three isolates displaying ST258, a single locus variant of ST11 previously associated with the clonal spread of CTX-M-15-producing *K. pneumoniae* in Hungary. In all isolates, *bla*KPC was located on plasmids as part of isoform a of Tn4401.

Conclusions: The emergence of KPC-producing isolates of *K. pneumoniae* in Norway and Sweden is associated with multiple import events and probable local transmission of a successful multiresistant ST258 clone, closely related to the CTX-M-15-producing ST11 clone previously described in Hungary.

Keywords: Enterobacteriaceae, MLST, PFGE, class A β-lactamase, international clone, import, Scandinavia, Tn4401

Introduction

The *Klebsiella pneumoniae* carbapenemase (KPC) is a class A β-lactamase that confers resistance to virtually all β-lactam antibiotics including carbapenems.1,2 It was first reported in the USA in a *K. pneumoniae* isolate from 1996.3 Since then, KPC-producing *K. pneumoniae* have increased in prevalence and been responsible for outbreaks in parts of the USA and
KPC enzymes in Norway and Sweden

Israel. Reports are now appearing showing that KPC enzymes have disseminated worldwide with KPC-producing isolates identified in France, Greece, Colombia, Argentina, China and the UK. In Scandinavia, the first isolate producing KPC-2 was identified in 2007 in Sweden from a patient previously hospitalized in an intensive care unit in Greece.

Initially discovered in K. pneumoniae, KPC enzymes have now been detected in several other members of the Enterobacteriaceae family and Pseudomonas aeruginosa. The rapid dissemination and spread among different bacterial species of KPC enzymes are likely due to the localization of blaKPC genes on transferable broad host range plasmids and their association with transposons and IS elements. Clonal dissemination of KPC-producing K. pneumoniae isolates is often observed within countries but also between countries, indicating that there are major international clones circulating.

This study reports the first identification of KPC-producing K. pneumoniae in Norway along with epidemiological and genetic characterization of the Norwegian isolates and the second Swedish isolate identified.

Materials and methods

Bacterial isolates

The clinical isolates were referred to the Reference Centre for Detection of Antimicrobial Resistance in Norway and Karolinska University Hospital in Sweden based on non-susceptibility to carbapenems by disc diffusion and for the investigation of the resistance mechanisms (Table 1). Bacterial identification was performed using the Vitek2 ID-GNB system (bioMérieux, Marcy l’Étoile, France).

Antibiotic susceptibility testing

The antibiotic susceptibility profile of the isolates was determined using Etest according to the manufacturer’s instructions (AB BIODISK, Solna, Sweden) and interpreted according to clinical breakpoints from the European Committee for Antimicrobial Susceptibility Testing (EUCAST).

PCR amplification and DNA sequencing

Isolates were screened for the presence of blaKPC by PCR, and the genetic structure surrounding blaKPC genes was investigated using combinations of primers, as described by Naas et al. Consensus PCR for blaTEM, blaSHV, blaCTX-M and a multiplex PCR for bla AmpC genes were performed, as described previously. Sequencing of positive PCR products and partial sequencing of the genetic structure of Escherichia coli (Applied Biosystems, Foster City, CA, USA) were performed using BigDye 3.1 technology (Applied Biosystems) and BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and compared with sequences deposited in the GenBank database (www.ncbi.nlm.nih.gov).

PFGE

Isolates were typed by PFGE of XbaI-digested genomic DNA using the Chef-DR III System (Bio-Rad, Marnes-La-Coquette, France). Electrophoresis was run at 12°C with a pulse time of 1–20 s at 6 V/cm on a 120° angle in 0.5× Tris/borate/EDTA (TBE) buffer for 21 h. DNA relatedness was computationally analysed in GelCompar II (Applied Maths, Sint-Martens-Latem, Belgium) using the band-based dice similarity coefficient and the unweighted pairs geometric-matched analysis dendrogram with a position tolerance of 1% for optimization and band comparison.

Multilocus sequence typing (MLST)

MLST was performed according to the protocol described on the K. pneumoniae MLST web site (http://www.pasteur.fr/recherche/genopole/PGF/MLST/Kpneumoniae.html).

SI nuclease digestion and Southern blot hybridization

SI nuclease-digested (Promega, Southampton, UK) genomic DNA from all isolates was examined by PFGE, run in a Chef-DR III System (Bio-Rad), at 15°C, with a pulse time of 5–25 s for 6 h (block 1) and 30–45 s for 18 h (block 2), at 6 V/cm on a 120° angle in 0.5× TBE buffer. Each band was considered a unit length linear plasmid. Plasmids were blotted onto positively charged nylon membranes (Roche Applied Sciences, Penzberg, Germany) using the VacuGene™ XL Vacuum Blotting System (GE Healthcare Life Sciences, Buckinghamshire, UK) for transfer and hybridized with blaKPC gene probes generated by the PCR DIG Probe Synthesis Kit and detected using the DIG Luminescent Detection Kit (Roche Applied Sciences).

Results and discussion

The isolates were positive for blaKPC by PCR and showed a multidrug-resistant phenotype (Table 1). All isolates expressed high-level resistance to penicillins, cephalosporins and aztreonam, but showed varying levels of resistance to carbapenems. All isolates were resistant to ertapenem. Two isolates were resistant to imipenem, whereas three and two isolates were susceptible (MIC 2 mg/L) or intermediate susceptible (MIC 4 mg/L), respectively. Four isolates expressed resistance to meropenem, and three isolates were intermediate susceptible (MIC 4–8 mg/L). Moreover, the isolates were resistant or intermediate susceptible to amikacin, tobramycin and ciprofloxacin, but were all susceptible to gentamicin (MICs 1–2 mg/L). Further, two isolates were also resistant to colistin (MIC 8–16 mg/L), and six isolates were resistant or intermediate susceptible to tigecycline (MICs 2–4 mg/L). The resistance profile with susceptibility to gentamicin is similar to K. pneumoniae KPC-producing isolates from Israel and the UK.

All isolates were PCR-negative for bla AmpC and bla CTX-M genes. The presence of both blaSHV-11 and blaSHV-12 was found in isolates from Norway, while the Swedish isolate carried only blaSHV-11. All isolates carried bla TEM-1.

Previous studies regarding metallo-β-lactamase-producing P. aeruginosa and the identification of the first KPC-producing K. pneumoniae in Sweden have shown that the introduction of new mobile resistance mechanisms in Scandinavia has been associated with import. In this study, five of the isolates were likely import cases. Three isolates (K47-25, K52-10 and AO-8053) were derived from patients upon admission to the Norwegian or Swedish hospital after direct transfer from hospitals in Greece or Israel, respectively (Table 1). Coincidentally, the patients carrying K47-25 and K52-10 had been treated at the same hospital in Greece. The patient carrying isolates K52-36...
and K52-37 had been admitted to a hospital in Greece 3 months prior to the identification of the isolates. The other isolates (K48-58 and K52-43) were derived from patients with no history of hospitalization outside Norway. PFGE of XbaI-digested genomic DNA revealed that all isolates were clonally related (Dice coefficient ≥87%), but with minor differences (Figure 1a). The two isolates (K52-36 and K52-37) from the patient at Asker and Bærum Hospital (Eastern Norway) showed a three band difference, indicating co-infection or recent in vivo diversification. Isolates K47-25 and K52-10 likely imported from Greece were indistinguishable from isolate K52-43, which was not associated with import. K52-43 (Førde Central Hospital, Western Norway) is a possible local nosocomial transmission event, as the patient had been admitted to the Haukeland University Hospital (Western Norway) at the same time as the patient carrying K52-10. Although the patients were not simultaneously admitted to the same unit, contact with the same healthcare personnel cannot be ruled out. Isolate K52-37 was indistinguishable from K48-58 from Sørlandet Hospital, Kristiansand (Southern Norway). However, there were no discernable epidemiological connections between the cases or with the other cases. The Swedish isolate imported from Israel was most closely related to two of the isolates imported from Greece (K47-25 and K52-10). MLST confirmed the clonal relationship between the isolates. MLST typing of K47-25, K48-58 and AO-8053 showed that the isolates displayed ST258, which has previously been identified in isolates from Greece.5,15

**Table 1. Clinical details, epidemiological data, and antimicrobial susceptibility profiles of KPC-producing K. pneumoniae isolates from Norway and Sweden**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Clinical details</th>
<th>Epidemiological data</th>
<th>Antimicrobial Susceptibility Profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Specimen</td>
<td>Place of isolation</td>
<td>Antibiotic</td>
</tr>
<tr>
<td></td>
<td>Month/Year of isolation</td>
<td>Hospitalization abroad (country)</td>
<td>Piperacillin/tazobactam</td>
</tr>
<tr>
<td>K47-25</td>
<td>Expectorate</td>
<td>SHA</td>
<td>&gt;256</td>
</tr>
<tr>
<td>K48-58</td>
<td>Urine</td>
<td>SHK</td>
<td>&gt;256</td>
</tr>
<tr>
<td>K52-10</td>
<td>Perineal swab</td>
<td>SUH</td>
<td>&gt;256</td>
</tr>
<tr>
<td>K52-36</td>
<td>Blood culture</td>
<td>Greece</td>
<td>&gt;256</td>
</tr>
<tr>
<td>K52-37</td>
<td>Blood culture</td>
<td>Greece</td>
<td>&gt;256</td>
</tr>
<tr>
<td>K52-36</td>
<td>Blood culture</td>
<td>Greece</td>
<td>&gt;256</td>
</tr>
<tr>
<td>K52-37</td>
<td>Blood culture</td>
<td>Greece</td>
<td>&gt;256</td>
</tr>
<tr>
<td>K52-43</td>
<td>Wound secretion</td>
<td>Greece</td>
<td>&gt;256</td>
</tr>
<tr>
<td>AO-8053</td>
<td>Wound secretion</td>
<td>Greece</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

**ND. not determined.**

**MICs of antibiotics are given in terms of mg/L.**

**References:**

5. Samuelsen et al.

18. However, blaCTX-M PCR was negative for all the KPC isolates.
probe showed that bla<sub>KPC</sub> was plasmid located in all isolates (Figure 1c). bla<sub>KPC-3</sub> was located on a plasmid of 120 kb, while bla<sub>KPC-2</sub> was located on a plasmid of 100 kb. Isolate K47-25 also harboured bla<sub>KPC-2</sub> on an additional plasmid of 160 kb. Tn<sub>4401</sub> is a Tn<sub>3</sub>-like element that supports replicative transposition and has previously been described in different genetic environments in plasmids flanked by target site duplications. The observation of bla<sub>KPC</sub> on two plasmids in one isolate further supports the hypothesis that Tn<sub>4401</sub> contributes to the mobilization and dissemination of bla<sub>KPC</sub>.

In conclusion, KPC-producing <i>K. pneumoniae</i> have emerged in Norway and Sweden through four separate incident of import with a clonally related strain from Greece and Israel. Dissemination of this clone in Norway has occurred with one case of local transmission. Two additional KPC-producing <i>K. pneumoniae</i> isolates have recently been identified in Norway, of which one is another isolate imported from Greece while the other isolate probably represents further dissemination in that health region (data not shown). The dissemination of this clone illustrates the epidemic potential. The clonality between the isolates and the close phylogenetic relation to CTX-M-15-producing <i>K. pneumoniae</i> from Hungary indicates the dissemination of international biologically fit clones of <i>K. pneumoniae</i> with a high potential to acquire resistance mechanisms. The rapid dissemination of KPC enzymes in Enterobacteriaceae and <i>P. aeruginosa</i> worldwide and the consequences for treatment and infection control measures warrant a high degree of awareness and monitoring of these enzymes.

**Acknowledgements**

We are grateful to Bjørg Haldorsen for skilful technical assistance and Per Espen Akselsen for epidemiological data.

**Funding**

This study was funded through internal funding at the University Hospital of North Norway and University of Tromsø. Ø. S. is supported by a grant from the Northern Norway Regional Health Authority Medical Research Program.

**Transparency declarations**

None to declare.

**References**


Samuelsen et al.