suggested the persistence capacity of the mecC-positive CC130 clone over years, even though repeated introductions of mecC-positive isolates cannot be excluded. Finally, the description of divergent but closely related spa types and PFGE patterns among the mecC-positive isolates potentially reflects local microevolution or the circulation of different subclones. No epidemiological link was evidenced between the three colonized farms, neither at the animal level (exchanges and contiguous fields) nor at the farmer level (shared employees and relatives). Also, no human mecC-positive MRSA was reported in the nearby hospitals (F. Laurent, personal observation). Since the dynamics of expansion of this clone is not known, further studies are required to explore the capacity of mecC-positive isolates to persist and propagate on and between cattle farms and eventually disseminate from animals to humans.

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

References


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Emergence of Escherichia coli ST131 sub-clone H30 producing VIM-1 and KPC-3 carbapenemases, Italy

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Sir,
The spread of carbapenem-resistant Enterobacteriaceae is a serious public health concern. In Italy, a marked increase in the prevalence of carbapenem non-susceptible Klebsiella pneumoniae has recently been observed (http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/map_reports.aspx), mainly related to the spread of strains of clonal complex 258 producing KPC-type carbapenemases. Although the prevalence of carbapenemase-producing Escherichia coli isolates remains low compared with that of K. pneumoniae, the potential acquisition of this resistance mechanism by multidrug-resistant (MDR) E. coli is a matter of concern since it would strongly reduce the therapeutic options for the most prevalent enterobacterial pathogen. In particular, E. coli belonging to sequence type (ST) 131 has globally emerged as the major epidemic clone causing extraintestinal infections. Recently, a specific sub-clone (H30) containing allele 30 of the fimH type-1 fimbrial adhesin gene was found to be responsible for most MDR infections produced by ST131 isolates. Here we report the first detection of the KPC-3 enzyme among bloodstream E. coli isolates belonging to both ST131 and ST457 clones in Italy.

From October 2011 to March 2012 and from February to June 2013, E. coli isolates resistant or with reduced susceptibility to meropenem and/or imipenem (MIC ≥ 0.5 mg/L) were collected from bloodstream infections (BSIs) within two nationwide surveys promoted by the Antibiotico-Resistenza–Istituto Superiore di Sanità (AR-ISS) surveillance network, which included 23 hospital laboratories distributed across Italy. Phenotypic testing for carbapenemase was performed with the agar tablet/disc diffusion method using the KPC/MBL and OXA-48 Confirm Kit (ROSCO Diagnostica A/S, Sanita (AR-ISS) surveillance network, which included 23 hospital laboratories distributed across Italy. Phenotypic testing for carbapenemase was performed with the agar tablet/disc diffusion method using the KPC/MBL and OXA-48 Confirm Kit (ROSCO Diagnostica A/S, Taastrup, Denmark). Identification of carbapenemase-encoding genes (blaKPC, blaOXA-48, blaNDM, and blaIMP-type genes) and their variants was achieved by PCR and sequencing. Detection and sequencing of extended-spectrum β-lactamase (ESBL) and/or AmpC genes (blaCTX-M, blaSHV, blaTEM, and blaCMY-2-type genes), phylogenetic typing, multilocus sequence typing (MLST), PFGE genotyping and fimH-based subtyping of the ST131 isolates were performed as previously described. MICs were determined by reference broth microdilution (BMD) using TREK Sensititre custom panel ITGNEG (Thermo-Fisher TREK Diagnostic Systems, Inc., Cleveland, OH, USA). Imipenem and meropenem MICs were also determined by Etest and the Vitek 2 automated system (bioMérieux, Marcy-l’Etoile, France). The interpretative breakpoints were based on EUCAST criteria (http://www.eucast.org/clinical_breakpoints/). Conjugation experiments were performed in solid medium as previously described, using E. coli MKD-135 (argH, rpoB18, rpoB19, recA and rpsL) as recipient and medium containing rifampicin (250 mg/L) plus meropenem (0.25 mg/L) or ceftazidime (8 mg/L) for the selection of transconjugants.

Table 1. Clinical and epidemiological data on patients with BSIs caused by carbapenemase-producing E. coli and characterization of the isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Age/gender</th>
<th>Origin of patient</th>
<th>Acute care</th>
<th>Isolate</th>
<th>ST</th>
<th>Other</th>
<th>Carbapenem</th>
<th>Carbapenem</th>
<th>FIMH</th>
<th>Other</th>
<th>ESBL</th>
<th>KPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC022</td>
<td>39/M</td>
<td>Surgery</td>
<td>ICU</td>
<td>12/2011</td>
<td>131</td>
<td></td>
<td>≤ 0.125</td>
<td>≤ 0.125</td>
<td>0.5</td>
<td>≤ 0.125</td>
<td>≤ 0.125</td>
<td></td>
</tr>
<tr>
<td>EC021</td>
<td>81/F</td>
<td>Bacteraemia</td>
<td>ICU</td>
<td>11/2011</td>
<td>131</td>
<td></td>
<td>≤ 0.125</td>
<td>≤ 0.125</td>
<td>0.5</td>
<td>≤ 0.125</td>
<td>≤ 0.125</td>
<td></td>
</tr>
<tr>
<td>EC074</td>
<td>56/M</td>
<td>V AP surgery</td>
<td>PT</td>
<td>12/2011</td>
<td>457</td>
<td></td>
<td>≤ 0.125</td>
<td>≤ 0.125</td>
<td>0.5</td>
<td>≤ 0.125</td>
<td>≤ 0.125</td>
<td></td>
</tr>
<tr>
<td>EC013</td>
<td>76/M</td>
<td>Bacteraemia</td>
<td>ICU</td>
<td>04/2013</td>
<td>131</td>
<td></td>
<td>≤ 0.125</td>
<td>≤ 0.125</td>
<td>0.5</td>
<td>≤ 0.125</td>
<td>≤ 0.125</td>
<td></td>
</tr>
</tbody>
</table>

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Keywords: multidrug resistance, carbapenems, multilocus sequence typing
nature of the transferred carbenapenemase determinant was confirmed by PCR.2

A total of seven E. coli isolates with reduced susceptibility to carbenapenems were reported from 6 of the 23 hospitals during the study periods. Five of these isolates, from four hospitals (located in northern and central Italy), were confirmed to be carbapenemase producers. Considering the number of E. coli isolates from BSIs tested for susceptibility to meropenem and imipenem during the study period in each hospital as denominator data, the proportion of carbapenemase-producing E. coli isolates from the four hospitals was 0.4% (1 of 247), 0.5% (1 of 207), 2.7% (1 of 37) and 3.0% (2 of 67), respectively. Data on patients and microbiological features of the five carbapenemase-producing E. coli isolates are reported in Table 1. The isolates were found to produce either VIM-1 (n = 3) or KPC-3 (n = 2) carbapenemases. Results of phenotypic tests were consistent with the type of carbapenemase produced. ESBL genes (CTX-M- and/or SHV-type genes) were detected in four of the five isolates. All isolates also carried the blatEM-1 B-lactamase gene. No isolate carried blanc. All isolates belonged to phylogenetic group B2. The three VIM-1-producing isolates, of which one has already been partially characterized,3 and one KPC-3-producing isolate belonged to the H30 sublineage of ST131. The remaining KPC-3-producing isolate belonged to ST457. Conjugational transfer of the carbapenemase genes was only observed with the ST131 KPC-producing isolate EC013 (at a frequency of 3.8 × 10−5 transconjugants per recipient). By PFGE cluster analysis, three variants (coefficient of similarity <80%) were observed within the ST131 H30 sub-clone, with only two isolates (EC022 and EC074) displaying high genetic homology (similarity 95.2%). As expected, EC142 (belonging to ST457) appeared to be genetically unrelated to the ST131 isolates (data not shown).

According to the reference microdilution method, all isolates were resistant to extended-spectrum cephalosporins, ciprofloxacin and ertapenem, and intermediate or resistant to imipenem. Some isolates retained susceptibility to meropenem (n = 2), gentamicin (n = 1), amikacin (n = 4) and trimethoprim/sulfamethoxazole (n = 1). All isolates were susceptible to tigecycline and colistin. Interestingly, the carbapenem MICs determined by Etest showed frequency of 3.8 × 10−5 only observed with the ST131 KPC-producing isolate EC013 (at a frequency of 3.8 × 10−5 transconjugants per recipient). By PFGE cluster analysis, three variants (coefficient of similarity <80%) were observed within the ST131 H30 sub-clone, with only two isolates (EC022 and EC074) displaying high genetic homology (similarity 95.2%). As expected, EC142 (belonging to ST457) appeared to be genetically unrelated to the ST131 isolates (data not shown).

KPC carbapenemases, particularly KPC-3, remain very rare in E. coli and, to our knowledge, KPC-3 has been previously reported only in two papers.3,5 Our study demonstrates the occurrence of KPC-3 in bloodstream E. coli isolates belonging to different ST clones, and such occurrence deserves careful attention. Together with KPC-3, the ST457 isolate co-carried an uncommon CTX-M type (CTX-M-55) and SHV-11. The ST457 clone has not been found previously in Italy, but has been detected in the UK.3,11 In contrast, ST31 strongly predominates worldwide, including Italy.3,10 This is the first report describing the emergence of a KPC-3-producing E. coli ST313 isolate in Italy. Finally, although VIM-1-producing ST131 isolates have been reported in our country only recently, this study demonstrates that such isolates belonged to the MDR pandemic H30 sub-clone. The occurrence of both KPC-3 and VIM-1 in the successful H30 sub-clone is of great concern due to the ability of this sub-clone to disseminate in community settings.

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Transparency declarations
None to declare.

References
Plasma exchange significantly affects darunavir exposure

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Keywords: HIV protease inhibitors, pharmacokinetics, apheresis

Sir,

Plasma exchange (PE) is a therapeutic procedure aimed at reducing the amount of abnormal or toxic substances in the blood, e.g. immunoglobulins. It consists in removing a large volume of plasma from a patient and replacing it with some form of replacement fluid. PE is non-selective and may also remove circulating medications from blood (both protein-bound and unbound drug).1 Darunavir is a synthetic non-peptidic HIV protease inhibitor (PI) metabolized mainly by CYP3A4 isoenzymes and 95% bound by plasma proteins, primarily α1-acid glycoprotein.2 Increased clearance of atazanavir, another PI, during PE has already been reported,3 but the effect of PE on darunavir disposition is unknown. Since darunavir exhibits concentration–effect relationships,3 knowledge of this effect is critical for treatment efficacy.

We report here the case of a white woman in her 50s receiving highly active antiretroviral therapy (HAART) including darunavir and requiring PE treatment. Consent for publication was obtained from the patient. She was diagnosed HIV-1 positive in 2001 and started HAART in 2005. Viral load (VL) and CD4 count were 72 095 copies/mL and 13 cells/mm3, respectively, when she was hospitalized for visceral leishmaniasis with polyclonal hypergammaglobulinemia. She then started a new HAART combination consisting of darunavir/ritonavir (600/100 mg twice daily) with emtricitabine/tenofovir (200/245 mg once daily). Despite good virological response (VL < 20 copies/mL), immunological restoration was not achieved (CD4 count = 150 cells/mm3 and CD4/CD8 ratio = 0.08) and hypergammaglobulinemia increased. Because of hyperviscosity syndrome, she underwent PE sessions twice a week during which darunavir pharmacokinetics (PK) were explored.

Several blood samples were drawn over the dosing interval, i.e. just before, during and immediately after a 2 h PE session, in order to estimate the elimination rate constant during PE (keoff); a plasma sample collected during PE was also assayed in order to determine the amount of darunavir eliminated by PE. Three additional samples were drawn after PE in order to estimate the darunavir elimination rate constant after a PE session (keoff). Plasma darunavir concentrations were determined using a validated HPLC-UV method and ke values were estimated as the slope from the linear regression of log-concentrations on sample times. Corresponding t1/2 were estimated as log2/ke. In order to demonstrate the impact of PE on steady-state darunavir PK profiles, PK simulations were performed using Nonmem population PK software (Monte Carlo simulation, n = 1000 concentration–time profiles). Calculated keoff and mean estimated PK parameters obtained from the literature3 (CL/F = 10.7 L/h, V/F = 198 L and ka = 0.95 h−1) were used for that purpose.

PK analysis was performed during three PE sessions. The mean ± SD plasma volume and darunavir amount removed per session were 2.51 ± 0.03 L and 9.3 ± 8.3 mg, respectively. Actual darunavir concentrations measured before, during and after PE sessions were 8802 ± 4835, 4505 ± 2930 and 3314 ± 2103 ng/mL, respectively. Estimated keoff and ka were 0.10 ± 0.1 and 0.53 ± 0.13 h−1, respectively. These data

Figure 1. Simulated mean steady-state darunavir concentration. (a) 600 mg of darunavir/100 mg of ritonavir twice daily alone (broken line) and 600 mg of darunavir/100 mg of ritonavir twice daily + PE occurring between 10 and 12 h post-dose (continuous line). (b) 600 mg of darunavir/100 mg of ritonavir twice daily alone (broken line) and 600 mg of darunavir/100 mg of ritonavir twice daily + PE occurring between 2 and 4 h post-dose (continuous line).