Salmonella enterica subsp. enterica producing VIM-1 carbapenemase isolated from livestock farms

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Sir,

Third- and fourth-generation cephalosporins and carbapenems are ‘critically important’ antimicrobials as classified by the WHO (www.who.int). In fact, carbapenems are last-line clinical antibiotics against infections caused by multidrug-resistant Gram-negative bacteria.1,2 In contrast to cephalosporins, carbapenems are not hydrolysed by most β-lactamases, including AmpC β-lactamases and extended-spectrum β-lactamases (ESBLs). However, during the last decade the prevalence of carbapenem resistance in Enterobacteriaceae has increased worldwide.2,3 Whereas the increase in the prevalence of ESBL-producing Enterobacteriaceae isolated from livestock is becoming an important public health problem,4 the increasing prevalence of carbapenemases has only affected hospitals and the community.1,3 Recently, however, the occurrence of carbapenemase-carrying commensal Escherichia coli isolated from livestock and their environment has been reported,1 and this could be the beginning of a new era in the antibiotic resistance field.

Within the national RESET project (www.reset-verbund.de) several longitudinal and cross-sectional studies, collecting potential ESBL-carrying organisms from German farms, have been performed (using MacConkey agar with 1 mg/L cefotaxime as the selective medium). From the 221 isolates collected during 2011, 3 of them were ascribed to Salmonella enterica subsp. enterica (Table 1). The three Salmonella isolates (R3, R25 and R27) were obtained from two pig-fattening farms (R25 was collected outside the farm) and one broiler farm (Table 1). The three farms were distributed in different locations in the same German federal region, and although there was no apparent link between them, a common source cannot be excluded. The three isolates were tested for their susceptibility to 35 antimicrobials, including β-lactams/β-lactamase inhibitors (Table 1), phenicols, aminoglycosides, quinolones/fluoroquinolones, tetracycline, folate pathway antagonists, lipopeptides and fosfomycin, as previously described.1 For the present study, tigecycline (15 μg) and nitrofurantoin (300 μg) were included as well. The presence of ESBLs, AmpC β-lactamases and/or carbapenemase-encoding genes, class 1 and 2 integrons and other resistance genes was screened by PCR/sequencing, as previously described (Table S1, available as Supplementary data at JAC Online).1,5

The MIC values for some carbapenemase producers can be lower than the currently recommended breakpoints, and the results of the carbapenem susceptibility tests can be influenced by the genetic background.1,2,6 The Salmonella isolates R3, R25 and R27 showed decreased susceptibility to these antimicrobials [non-wild-type by the EUCAST epidemiological cut-off (ECOFF), but susceptible or intermediate according to the CLSI clinical breakpoint; Table 1]. This ‘decreased susceptibility’ could be transformed to a competent E. coli recipient, but conjugation or mobilization under the conditions used1,5 was unsuccessful. The three isolates carried both the AmpC-encoding gene blaACC-1 and the carbapenemase gene blaVIM-1, like in the previously reported E. coli isolates R178 and R29.3 When Salmonella R3 and the control strain E. coli R178 were grown in liquid medium with carbapenems (Luria-Bertani broth with 16 mg/L imipenem or 8 mg/L ertapenem inoculated with 1:1000 overnight culture), both isolates grew well, showing full carbapenem resistance (clinical breakpoints, CLSI versus EUCAST: imipenem ≥4 versus >8 mg/L; and ertapenem ≥1 versus >1 mg/L).

Several class 1 integrons (In31, In70, In71, In110 and In450), transposons (Tn3, Tn402 and Tn21) and plasmids (incompatibility groups IncHI2, IncN, IncI1 and IncW) carrying blaVIM genes have been described.1 Like in E. coli R178 and R29,3 in the three Salmonella isolates the blaVIM-1 gene was located on a class 1 integron (variable region with blaVIM-1-aacA4-aadA1 gene cassettes) harbouring by an ~300 kb IncHI2 plasmid (determined by S1-nuclease PFGE analysis, PCR-based replicon typing and Southern blot hybridization, as previously described),4,16 HI2 double plasmid sequence typing failed). The plasmid also carried blaACC-1, strA/B, catA1 and a trimethoprim resistance gene (not identified with the primers used; see Table S1, available as Supplementary data at JAC Online). The sequence of the complete integron of Salmonella R3 (5436 bp, including complete sul1 and orf5, obtained using as template the pRHR3 plasmid of this isolate; see Table S1, available as Supplementary data at JAC Online) was identical to the one from E. coli R178 (accession number HE663536) and was related to Tn402 (like in GQ422826).3 Salmonella R27 was isolated from the same farm as both E. coli R178 and R29 (Table 1). However, the IncHI2 plasmids harbouring these Salmonella and E. coli isolates were different in size and gene content (~300 versus 220 kb and presence versus absence of chloramphenicol- and trimethoprim-resistance genes), suggesting different plasmid evolutions.

The three isolates were classified as S. enterica group C, antigenic formula ‘6,7:–:–’ (www.pasteur.fr) at the National Salmonella Reference Laboratory (NRL-Salm, BFR). The sequence type ST32 (http://mist.uctt.eu/mist/abs/Senterica) and the PFGE patterns found (Figure S1, available as Supplementary data at JAC Online) are typical of Salmonella Infantis (6,7:r:1,5) and have also been detected in German isolates from humans, poultry/pig/pork meat and pig/pork meat.7 Salmonella Infantis is among the top 10 Salmonella serovars implicated in human salmonellosis worldwide (ranking in third place in 2011 in Europe; www.ecdc.europa.eu).1,2 Isolates from this serovar also caused disease with

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Table 1. Features of the isolates used in this work

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Species</th>
<th>Serovar</th>
<th>Origina</th>
<th>Date of isolation (dd/mm/yyyy)</th>
<th>Multilocus sequence typing</th>
<th>PFGE pattern</th>
<th>Resistance phenotypebusteristance genotype</th>
<th>Carbapenem susceptibility testb ETP/IMP/ MEM, mg/L; mm</th>
<th>Plasmid size (kb)</th>
<th>Plasmid Inc group</th>
</tr>
</thead>
<tbody>
<tr>
<td>R3</td>
<td>S. enterica</td>
<td>Infantis</td>
<td>poultry farm G1</td>
<td>18/03/2011</td>
<td>ST32</td>
<td>XS1a</td>
<td>β-lactams STR SMX CHL TMP/ blaACC-1 blaOXA-1 oocA4 aadA1 strA-strB sul1 catA1</td>
<td>0.125/1.0/0.25; 24/20/25</td>
<td>300</td>
<td>HI2</td>
</tr>
<tr>
<td>R25</td>
<td>S. enterica</td>
<td>Infantis</td>
<td>outside swine farm S1</td>
<td>01/03/2011</td>
<td>ST32</td>
<td>XS1b</td>
<td>β-lactams STR SMX CHL TMP/ blaACC-1 blaOXA-1 oocA4 aadA1 strA-strB sul1 catA1</td>
<td>0.125/1.0/0.125; 24/20/25</td>
<td>300</td>
<td>HI2</td>
</tr>
<tr>
<td>R27</td>
<td>S. enterica</td>
<td>Infantis</td>
<td>swine farm S2</td>
<td>16/02/2011</td>
<td>ST32</td>
<td>XS1a</td>
<td>β-lactams STR SMX CHL TMP/ blaACC-1 blaOXA-1 oocA4 aadA1 strA-strB sul1 catA1</td>
<td>0.125/0.5/0.125; 24/22/25</td>
<td>300</td>
<td>HI2</td>
</tr>
<tr>
<td>R291</td>
<td>E. coli</td>
<td>0118:NM</td>
<td>swine farm S2</td>
<td>18/04/2011</td>
<td>ST88</td>
<td>XE1a</td>
<td>β-lactams STR SMX TET/ blaACC-1 blaOXA-1 oocA4 aadA1 strA-strB sul1 tet(A)</td>
<td>0.125/1.0/0.125; 26/22/26</td>
<td>220</td>
<td>HI2</td>
</tr>
<tr>
<td>R1781</td>
<td>E. coli</td>
<td>0118:NM</td>
<td>swine farm S2</td>
<td>23/05/2011</td>
<td>ST88</td>
<td>XE1b</td>
<td>β-lactams STR SMX/blaACC-1 blaOXA-1 oocA4 aadA1 strA-strB sul1</td>
<td>0.125/1.0/0.25; 24/22/25</td>
<td>220</td>
<td>HI2</td>
</tr>
<tr>
<td>ATCC 25922</td>
<td>E. coli</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>≤0.008/0.125/ ≤0.008; 37/30/35</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>p1</td>
<td>S. enterica</td>
<td>Bareilly</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>blaACC-1</td>
<td>0.125/0.5/0.5; 29/29/33</td>
<td>—</td>
</tr>
</tbody>
</table>

CHL, chloramphenicol; ETP, ertapenem; IMP, imipenem; MEM, meropenem; NM, non-motile; SMX, sulfamethoxazole; STR, streptomycin; TET, tetracycline; TMP, trimethoprim. β-Lactams: penicillins (ampicillin, piperacillin and ticarcillin); first- to fourth-generation cephalosporins (cefalotin, cefuroxime, cefotaxim, ceftriaxone, ceftazidime, cefotaxime, cepodoxime and cefepime); cephamycins (cefoxitin); and β-lactamase inhibitors (amoxicillin/clavulanic acid).

aThe farms were located in the same German federal region. R3 was collected in G1, farm holding 360000 broilers. The sample taken was dust from a unit holding 22500 1-day-old chicks. R25 was taken using boot socks at 100 m outside (upwind side) of S1, farm holding 3500 fattening pigs. R27 was isolated from a pooled faeces sample collected from S2, farm holding 4100 fattening pigs (500 per unit). The distance between G1 and S2 was ~20 km. The distance between G1/S2 and S1 was ~120 km.

bTested by the CLSI disc diffusion method (CLSI M2-A10 and M100-S21). Carbapenem susceptibility was also tested using Etest strips (bioMérieux, Nürtingen, Germany). Results were interpreted using both CLSI clinical breakpoints and, when available, the EUCAST ECOFF values for public health aspects (www.eucast.org). For fosfomycin and colistin, no CLSI clinical breakpoints or EUCAST ECOFF values were available, and for tigecycline, only the EUCAST ECOFF values were available. All isolates showed fosfomycin and colistin zone diameters greater than or equal to the ones shown by E. coli ATCC 25922 (32 mm and 13 mm, respectively).
severe symptoms (septicaemia and fatal cases) and nosocomial outbreaks. Its main reservoir is poultry and swine. This is the first report on the presence of a carbapenemase-encoding gene in S. enterica isolated from poultry and pig farms. This finding, together with the recent detection of E. coli VIM-1 producers in a pig farm, support the carriage of this gene in different bacterial and animal species. Since the real prevalence of carbapenemase-encoding genes present in zoonotic bacteria or commensals is unknown, livestock carbapenem producers should be included in monitoring programmes. Unfortunately there is still a debate about the methodology for detection (best detection medium, antibiotic and concentration). Although in Germany carbapenems are not licensed for the treatment of livestock animals, the use of any antimicrobial for which the integron- or plasmid-located genes confer resistance (β-lactams, aminoglycosides, sulphonamides, chloramphenical or trimethoprim) could contribute to co-selection. Due to the importance of carbapenems for human treatment, the presence of these genes located on mobile genetic elements in livestock, and the possibility of further transmission via food or direct contact in the community and hospitals, is a public health concern and deserves surveillance.

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Transparency declarations
None to declare.

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

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Analysis of the quinolone-resistance determining region of the gyrA gene and the analogous region of the parC gene in Ureaplasma parvum and Ureaplasma urealyticum detected in first-void urine of men with non-gonococcal urethritis

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