mediated resistance was investigated. As part of the DANMAP surveillance programme (The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme), a total of 137 faecal samples were randomly selected among the healthy pigs at farm level between November 2005 and March 2006. Faecal sample was enriched in MacConkey broth containing 2 mg/L cefotaxime and 3M<sup>TM</sup> Petrifilms<sup>TM</sup> Select E. coli Count Plates (SEC plates) with 2 mg/L cefotaxime were used to identify E. coli. High prevalence of metallo-ß-lactamase and 16S rRNA methylase coproduction among imipenem-resistant Pseudomonas aeruginosa isolates in Brazil. Antimicrob Agents Chemother 2007; 51: 3398–90.

References


Figure 1. Plasmid profile and Southern hybridization analysis. (a) Plasmid profiles of K. pneumoniae R2 and E. coli DH10B (pRS1). (b) Hybridization with the rmtD probe. Lane M, lambda HindIII-digested DNA marker; lane 1, K. pneumoniae R2; lane 2, E. coli DH10B (pRS1).

incorporation of the rmtD gene by multidrug resistance plasmids is a concern.

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Transparency declarations

None to declare.

Detection of a single isolate of CTX-M-1-producing Escherichia coli from healthy pigs in Denmark

Shuyu Wu<sup>1,2</sup>*, Eirini Chouliara<sup>1,2</sup>, Henrik Hasman<sup>1</sup>, Anders Dalsgaard<sup>2</sup>, Antonio Vieira<sup>3,4</sup> and Lars Bogø Jensen<sup>1</sup>

<sup>1</sup>Department of Microbiology and Risk Assessment, National Food Institute, Technical University of Denmark, Bilowsvej 27, DK-1790 Copenhagen V, Denmark; <sup>2</sup>Department of Veterinary Pathobiology, Faculty of Life Sciences, University of Copenhagen, Grønnegårdsvæj 15, DK-1870 Frederiksberg C, Denmark; <sup>3</sup>Department of Microbiology and Risk Assessment, National Food Institute, Technical University of Denmark, Mørkøj Bygade 19, DK-2860 Søborg, Denmark; <sup>4</sup>Department of Large Animal Science, Faculty of Life Sciences, University of Copenhagen, Ridebanevej 12, DK-1870 Frederiksberg C, Denmark

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*Corresponding author. Tel: +45-72346369; Fax: +45-72346001; E-mail: wsx@food.dtu.dk

Sir,

Extended-spectrum ß-lactamase (ESBL)-mediated resistance is an increasing concern in human clinical settings. In Denmark, only a few cases of ESBL-producing Escherichia coli have been reported from food animals,<sup>1–3</sup> however, there was no baseline study on ESBL prevalence among the healthy pig populations in Denmark. In this study, we investigated the prevalence of ESBL-mediated resistance in E. coli isolates obtained from faecal samples of healthy pigs in Denmark. Furthermore, ESBL-related genes and mutations were determined and cephalosporin consumption in pig farms associated with ESBL-mediated resistance was investigated.

As part of the DANMAP surveillance programme (The Danish Integrated Antimicrobial Resistance Monitoring and Research Programme), a total of 137 faecal samples were randomly selected among the healthy pigs at farm level between November 2005 and March 2006.<sup>1</sup> Faecal sample was enriched in MacConkey broth containing 2 mg/L cefotaxime and 3M<sup>TM</sup> Petrifilms<sup>TM</sup> Select E. coli Count Plates (SEC plates) with 2 mg/L cefotaxime were used to identify E. coli with reduced susceptibility to cefotaxime. E. coli appears on SEC plates as dark green to light-blue-green colonies and was subcultured on Mueller–Hinton II agar plates supplemented with 2 mg/L.
### Research letters

Table 1. Phenotypic confirmation test, resistance to non-β-lactams, plasmidic β-lactamase genes and mutations in the promoter of the chromosomal ampC gene in the four E. coli isolates in the study

<table>
<thead>
<tr>
<th>Disc diffusion results</th>
<th>Resistance to non-β-lactams&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Plasmidic bla genes detected</th>
<th>Position of mutations in the promoter of the ampC gene&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td>FOX</td>
<td>CXM</td>
<td>CAZ</td>
</tr>
<tr>
<td>I</td>
<td>R</td>
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<tr>
<td>IV</td>
<td>S</td>
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<td>S</td>
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</tbody>
</table>

FOX, cefoxitin; CXM, cefuroxime; CAZ, ceftazidime; FEP, cefepime; CTX, cefotaxime; STR, streptomycin; TET, tetracycline; SMX, sulfamethoxazole; TMP, trimethoprim; SPE, spectinomycin.

<sup>a</sup>Non-β-lactam antimicrobials tested: apramycin, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, nalidixic acid, neomycin, spectinomycin, streptomycin, sulfamethoxazole, tetracycline and trimethoprim.

<sup>b</sup>Compared with ampC sequence of E. coli K-12.

Cefotaxime. Susceptibility testing for 17 antimicrobials<sup>1</sup> was carried out using a commercially dehydrated panel (Trek Diagnostic Systems, UK) and E. coli strains that showed broad-spectrum cephalosporin (cefotaxim and cefpodoxime) resistance were selected and studied further. Only one colony per sample was further investigated and a total of four broad-spectrum-cephalosporin-resistant E. coli isolates were obtained from the 137 samples. ESBL production was determined by the disc diffusion test using five oxyimino-cephalosporins including cefotaxime and ceftazidime with or without clavulanic acid as described by the CLSI guidelines (Table 1).<sup>3</sup> Based on the obtained phenotype, the presence of TEM, SHV, CTX-M, ACT, FOX and CMY β-lactamase-encoding genes was studied using PCR and obtained amplicons were sequenced. One isolate (E. coli IV) out of the four was highly resistant to cefotaxime and cefotihu but susceptible to ceftazidime and cefotihu. Sequencing of PCR products obtained using primers targeting TEM and CTX-M genes detected the presence of bla<sub>CTX-M-1</sub> and bla<sub>TEM-1b</sub>.<sup>5,6</sup> E. coli IV was able to transfer its cephalosporin resistance to the recipient E. coli K-12 HEHA4 in a conjugation experiment. The remaining three isolates were all susceptible to cefotaxime as well as cefotihu and did not have an ESBL phenotype by the disc diffusion test. PCR and sequence analysis detected mutations in the promoter of the chromosomal ampC gene, determined at positions −42(C→T) and −18(G→A). In addition, two of the isolates carried the bla<sub>TEM</sub> gene (Table 1). PFGE analysis of the four E. coli strains revealed significantly distinguishable PFGE patterns suggesting that they were not clonally related.

Data on cephalosporin consumption from the Danish Veterinary Medicines Statistics Programme (VetStat) indicated that the plotting of cephalosporin consumption of the 137 pig farms did not reveal any clear association between farms with high cephalosporin usage and occurrence of ESBL-producing E. coli.

This study is the first national surveillance of ESBL-producing E. coli from randomly sampled pig farms all over Denmark using selective enrichment. On a positive note, only one positive strain was detected out of 137 pig farms examined over half a year, suggesting a very low prevalence (0.7%) of ESBL-producing E. coli in the Danish pig populations. But it should be noted that the actual occurrence of ESBL-producing E. coli in Danish pig populations could have been underestimated by only testing a single colony from each sample. The first ESBL-producing E. coli from the Danish primary production were isolated in 2005 when two E. coli isolates carrying the bla<sub>CTX-M-1</sub> gene were isolated from diseased pigs as part of the routine diagnostics performed in Denmark.<sup>3</sup> In 2006, this increased to 10 ESBL-producing pathogenic E. coli isolated from diseased Danish pigs and cattle as well as the first ESBL-producing Salmonella Typhimurium isolated from a healthy pig in Denmark. All these isolates carried versions of the bla<sub>CTX-M-1</sub> gene (bla<sub>CTX-M-1</sub>, bla<sub>CTX-M-2</sub> or bla<sub>CTX-M-9</sub>) and were from farms that had used cephalosporins previously (H. Hasman, Technical University of Denmark, unpublished results). However, a very recent Danish study demonstrated 19 CTX-M-1-carrying E. coli isolates from two pig farms with a history of cefotihu usage. No statistical significance between usage of cefotihu and occurrence of ESBL-producing E. coli could be concluded, due to the limited number of farms investigated.<sup>7</sup> Likewise, the number of positive isolates obtained from healthy pigs in our study (where cross-contamination between animals might have taken place) is probably too low to make firm conclusions about a clear association between ESBL production and cephalosporin usage.

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Nosocomial infections caused by multidrug-resistant Pseudomonas putida isolates producing VIM-2 and VIM-4 metallo-β-lactamases

Pierre Bogaerts1*, Te-Din Huang1, Hector Rodriguez-Villalobos2, Caroline Bauraing1, Ariane Deplano2, Marc J. Struelens3 and Youri Glupczynski1

1Laboratoire de Bactériologie, Université catholique de Louvain, Cliniques Universitaires UCL de Mont-Godinne, B-5530 Yvoir, Belgium; 2Service de Microbiologie, Hôpital Erasme-ULB, Université Libre de Bruxelles, B-1070 Brussels, Belgium

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*Corresponding author. E-mail: pierre.bogaerts@uclouvain.be

Sir,

Nosocomial infections caused by multidrug-resistant and carbapenem-resistant Pseudomonas putida isolates have been occasionally reported in severely ill or immunocompromised patients hospitalized in the intensive care unit (ICU). Here, we report briefly the microbiological characteristics of several carbapenem-resistant P. putida isolates producing VIM metallo-β-lactamases (MBLs) at two Belgian university hospitals located in the Brussels area.

Between January 2004 and May 2007, multidrug-resistant P. putida strains originating from 10 inpatients hospitalized at Saint-Luc (hospital 1) and Erasme (hospital 2) university hospitals were characterized for resistance mechanisms to β-lactams. All the isolates were high-level resistant to imipenem and meropenem by disc diffusion testing (no inhibition zone). The 10 patients presented with severe underlying diseases (Table 1) had been hospitalized more than 9 days in ICUs and had all previously received broad-spectrum antimicrobial therapy. All but one of the isolates had been recovered from urine specimens. Bacterial identification to the species level was achieved with Vitek2-GN (bioMérieux) and control growth at 42°C on trypticase soy agar complemented with sheep blood. MICs determined by Etest (AB Biodisk) showed that all isolates were resistant to piperacillin/tazobactam, ceftazidime, aztreonam, imipenem and meropenem and all but one were resistant to cefepime (Table 1). Isolates recovered from hospital 1 were resistant to amikacin, whereas isolates from hospital 2 remained susceptible to this aminoglycoside. Resistance to ciprofloxacin was variable but all isolates remained susceptible to colistin. The MBL screening test was positive both by double-disc method (imipenem versus imipenem-EDTA; Rosco Diagnostica A/S) and by MBL double-sided Etest (imipenem/imipenem-EDTA; AB Biodisk) for all isolates (data not shown). PCR targeting bladIM (FW, 5′-GGC GTT TAT GTT CAT ACT TGG T; RV, 5′-TGG AGA ATT AAC CCA CTC TAT TCC), bladVIM (FW, 5′-TGT CCG TGG TGA TGG TGA TGA GT; RV, 5′-ATT CAG CCA GAT CGG CAT C), various ESBL genes (bladTEM, bladV8, bladPER, bladGES, bladBEL1, bladOKA of Groups 1, 2 and 3, bladOKA-20, bladOKA-18), and penicillinase genes (bladOXA of Groups 1, 2 and 3, CARB1 to 4 and 6: FW, 5′-TGG AAA CGG AAG AAT CCT CGG ACC CAT AAC CA; bladCARB 1 to 4 and 6: FW, 5′-GGA TTA CAA TGG CAA TCA GC; RV, 5′-TGT CGT ATC CCT CAA ATC ACC) was only positive for the bladVIM gene in all 10 isolates and for the bladPER gene in a single isolate (no. 6). Sequencing of the variable region of class I integrons obtained for the different strains revealed two distinct integrons. The first one, isolated from all five isolates from hospital 1, harboured an aacA4 allele coding for the AAC(6′)-Ib aminoglycoside-modifying enzyme explaining the resistance to amikacin, followed by the bladVIM gene. The same integron has already been identified in Pseudomonas aeruginosa isolates reported from Poland and Hungary and presents a specific 170 bp 3′-terminal repeat of the bladVIM gene. The second class I integron, obtained from five strains isolated in hospital 2, revealed a bladVIM-2 gene cassette, following an unidentified open reading frame of 318 nucleotides named orfN. This last sequence is referenced in GenBank under number EU284133. PCR sequencing confirmed that the bladPER gene detected in isolate no. 6 was a bladPER-1 allele. The co-presence of bladPER and bladVIM-2 has been reported in P. aeruginosa and Providencia, but to the best of our knowledge, this is the first description in P. putida. PFGE analysis revealed five PFGE types among the 10 P. putida isolates. Types A and B were recovered from hospital 1, whereas types C, D and E were found in hospital 2. A cluster of four patients showing PFGE type B was found in hospital 1 and another cluster of three patients with PFGE type C was present in hospital 2. Further, the content of the gene cassettes of the P. putida strains also clearly differed between the two centres.