mediated 16S rRNA methyltransferase, NpmA, found in association with the rmtD profiles of K. pneumoniae. This incorporation of the rmtD gene by multidrug resistance plasmids is a concern.

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Transparency declarations
None to declare.

References

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

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Sir,

Extended-spectrum β-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, 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. Faecal sample was enriched in MacConkey broth containing 2 mg/L cefotaxime and 3M Petrifilms 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.
Susceptibility testing for 17 antimicrobials 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 oxymino-cephalosporins including cefotaxim and ceftazidime with or without clavulanic acid as described by the CLSI guidelines (Table 1). 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 cefotaxim and ceftiofur but susceptible to ceftazidim and cefotaxim. Sequencing of PCR products obtained using primers targeting TEM and CTX-M genes detected the presence of bla\textsc{ctx-m-1} and bla\textsc{tem-1b}. 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 cefotaxim as well as ceftiofur but did not have an ESBL phenotype by the disc diffusion test. PCR and sequence analysis detected mutations in the promoter of the chromosomal amp\textsc{c} gene, determined at positions −42(C→T) and −18(G→A). In addition, two of the isolates carried the bla\textsc{tem} 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\textsc{ctx-m-1} gene were isolated from diseased pigs as part of the routine diagnostics performed in Denmark. 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\textsc{ctx-m-1} gene 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 ceftiofur usage. No statistical significance between usage of ceftiofur and occurrence of ESBL-producing E. coli could be concluded, due to the limited number of farms investigated. 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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Transparency declarations

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Research letters

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

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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).1 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 blaIMP (FW, 5'-GGC GGT TAT GTT GTT ACT TCG TF; RV, 5'-TCG AGA ATT AAG CCA CTC TAT TCC), blaVIM (FW, 5'-TGT CCG TGA TGG TGA TGT GA; RV, 5'-ATT CAG CCA GAT CGG CAT C), various ESBL genes (blaTEM, blaPER, blaVEB, blaGES, blaSEL, blaOXA of Groups 1, 2 and 3, blaOXAX, blaOXAX-1B), and penicillinase genes (blaCARB: FW, 5'-TGG AAA CGG GAA AAC GTT GG; RV, 5'-CAC GCG ACC CAT AAC CAC CA; blaCARb of 1 to 4 and 6; FW, 5'-GGA TTA CAA TGG CAA TCA GC; RV, 5'-TGT CTT ATC CCT CAA ATC ACC) was only positive for the blaVIM gene in all 10 isolates and for the blaPER gene in a single isolate (no. 6). Sequencing of the variable region of class 1 integrons obtained for the different strains revealed two distinct integrons. The first one, isolated from all five isolates from hospital 1, harboured an aac6'I allele coding for the AAC(6')-Ia aminoglycoside-modifying enzyme explaining the resistance to amikacin, followed by the blaVIM-4 gene. The same integron has already been sequenced in Pseudomonas aeruginosa isolates reported from Poland and Hungary2,3 and presents a specific 170 bp 3'-terminal repeat of the blaVIM-4 gene. The second class I integron, obtained from the five strains isolated in hospital 2, revealed a blaVIM-2 gene cassette, following an unidentified open reading frame of 318 nucleotides named orfβ. This last sequence is referenced in GenBank under number EU284133. PCR sequencing confirmed that the blaPER gene detected in isolate no. 6 was a blaPER-1 allele. The co-presence of blaPER and blaVIM-2 has been reported in P. aeruginosa4,5 and Providencia,6 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.

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