Transparency declarations
None to declare.

References

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Presence of extended-spectrum β-lactamase-producing Escherichia coli in wild geese

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Sir,

Since 2000, extended-spectrum β-lactamase (ESBL)-producing bacteria have increasingly been detected in humans and animals. Their impact on human health has drawn much attention worldwide. Many reports exist about the diversity of ESBLs among Enterobacteriaceae from food-producing animals.1 Also, for companion animals, several studies have been described.1 Recently some surveys have suggested that European wild birds may act as reservoirs of resistant bacteria2 and might have an epidemiological role in the dissemination of resistance. Therefore, to gain more insight into the role of migratory birds as a reservoir, a large population of wild geese in Belgium was screened for the presence of ceftiofur-resistant Escherichia coli.

For this purpose, cloacal swabs from 396 wild geese (354 Branta canadensis and 42 Anser anser domesticus) originating from six wildlife areas in Belgium were collected and inoculated within 4 h onto MacConkey agar plates (Oxoid Ltd, Basingstoke, UK) supplemented with ceftiofur (8 mg/L). After overnight aerobic incubation at 37°C, suspected E. coli colonies were purified on Columbia agar with 5% sheep blood (blood agar, Oxoid) and phenotypically identified.3 To confirm resistance to the β-lactams, the antimicrobial susceptibility of the E. coli isolates to ampicillin (10 μg), ceftiofur (30 μg) and amoxicillin/clavulanic acid (20/10 μg) (Neo-Sensitabs, Rosco Diagnostica, Taastrup, Denmark) was determined using the disc diffusion test according to the guidelines of the CLSI.3 The β-lactamases of the cultured E. coli were characterized by performing PCR for detection of genes encoding TEM-, SHV-, CTX-M- and CMY-type enzymes, as previously described.5,6 To establish the clonal relationship between the E. coli isolates, multilocus sequence typing (MLST) analysis, using seven conserved housekeeping genes (adk, fumC, gyrB, icd, mdh, purA and recA) (http://mlst.ucc.ie), was performed.7 All PCR products were purified using a Nucleospin Extract II Kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and sequenced using a GeneAmp PCR 9700 Applied Biosystems Sequencer (Foster City, CA, USA). For sequencing, PCR primers were used. The obtained nucleotide sequences were compared with those previously described for bla genes (BLAST database, http://www.ncbi.nlm.nih.gov/BLAST/).

From the 396 faecal samples, two ceftiofur-resistant E. coli isolates were obtained. The isolates originated from geese in the same wildlife area (Donkmeer, Berlare). Characterization and sequencing of the genes encoding the β-lactamases showed that the first E. coli isolate, originating from a Canada goose (B. canadensis), carried a blaTEM gene encoding ESBL SHV-12. The sequence type (ST) of the E. coli isolate after MLST analysis corresponded to ST1079. The second isolate, originating from a wild domestic goose (A. anser domesticus), was found to carry a blaTEM gene encoding ESBL TEM-52. This isolate was assigned to ST1844.

The population of wild domestic and Canada geese in Belgium is estimated at 10 000 birds.8 Since 396 wild geese were swabbed, approximately 4% of the total Belgian population was included in the study. ESBL-producing E. coli were only isolated from two geese (0.5% of the sampled animals). Analysis of the ESBL profile of the two ceftiofur-resistant E. coli isolates in this study resulted in the identification of the genes for TEM-52 and SHV-12. These genes are often present in ceftiofur-resistant E. coli from poultry, cattle, pigs and humans.1 The STs of the two E. coli isolates already existed in the MLST database (http://mlst.ucc.ie). ST1079 was previously isolated from a cow in the UK that died because of extra-intestinal pathogenic E. coli (ExPEC) septicaemia. ST1844 was isolated from a healthy human in France. This demonstrates that the MLST types found in the geese are not restricted to wild birds.

In conclusion, although the role of wild geese as a reservoir of bacteria carrying ESBL-encoding genes seems to be limited at present, the results of this study may indicate that these resistance determinants have disseminated in the natural environment.
Research letters

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References

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Spread of Klebsiella pneumoniae isolates producing OXA-48 β-lactamase in a Tunisian university hospital

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Keywords: K. pneumoniae, OXA-type β-lactamases, mechanisms of resistance

Sir,

The emergence and dissemination of Klebsiella pneumoniae isolates harbouring carbapenemases is a serious problem. Since the initial report of the OXA-48 enzyme in a K. pneumoniae isolate from Turkey in 2001,1 OXA-48 producers have been reported in many countries of the world.2–4 Current reports indicate that OXA-48 producers are widespread, mostly from Mediterranean countries as well as other countries in Europe.2–4 In North Africa, OXA-48 producers have been identified in Morocco and Tunisia.3,4 The outbreaks of OXA-48-producing K. pneumoniae isolates have been described in several cities in Turkey, once in the UK and recently in France.5 In the present report, we describe the spread of OXA-48 associated with CMY-4- and CTX-M-14-producing K. pneumoniae clinical isolates in Sfax University Hospital.

During a 6 month period (October 2009–March 2010), 153 clinical isolates of K. pneumoniae with reduced susceptibility to extended-spectrum cephalosporins and/or imipenem were recovered in Sfax University Hospital. Among these isolates, 21 (13.7%) produced the blaOXA-48 gene. These isolates were recovered from patients in eight different wards.

The antibiogram determined by the disc diffusion method and MICs determined by agar dilution and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) revealed that all isolates were resistant to ticarcillin (MICs >2048 mg/L) (Table 1). All but one isolate were resistant to extended-spectrum cephalosporins, including ceftazidime (MICs 4–1024 mg/L), cefotaxime (MICs 0.5–256 mg/L) and cefepime (MICs 2–64 mg/L). All isolates but one (Kp11) were susceptible to imipenem (MIC90 <2 mg/L; MIC range =0.5–8 mg/L). This isolate, Kp11, was intermediate to imipenem but susceptible to extended-spectrum cephalosporins. However, all isolates were resistant to ertapenem (MIC90 =8 mg/L; MIC range =2–32 mg/L).

The β-lactamase genes detected by PCR as described previously and sequencing in the 21 OXA-48-positive K. pneumoniae isolates were as follows: blaOXA-48 (1 isolate); blaOXA-48 +blaCMY-4 +blaCTX-M-14 (14 isolates); blaOXA-48 +blaCMY-4 +blaCTX-M-14 +blaOXA-1 (3 isolates); blaOXA-48 +blaCMY-4 +blaCTX-M-14 +blaTEM-1 (1 isolate); blaOXA-48 +blaCTX-M-14 +blaOXA-1 (1 isolate); and blaOXA-48 +blaCMY-4 +blaCTX-M-15 (1 isolate).

The transferability of the blaOXA-48 gene to Escherichia coli J53 was observed in 20 OXA-48-producing isolates. However, conjugation and electroporation experiments failed for Kp4, suggesting that in this isolate the blaOXA-48 gene might be chromosomally located. However, the blaOXA-48 gene was shown to be mostly plasmid-borne and associated with insertion sequence IS1999 but not integrons.6 Using a series of PCR primers, two IS1999 insertion sequences were found surrounding the blaOXA-48 gene in all our isolates, as found in the prototype OXA-48-positive K. pneumoniae 11978 isolate from Turkey.1,2

Molecular analysis of E. coli transconjugants showed that the blaOXA-48 and blaCMY-4 genes were detected on the same plasmid, explaining the resistance to β-lactams of the K. pneumoniae isolates and their transconjugants. Plasmid analysis showed