tet(M)-carrying Haemophilus influenzae as a potential reservoir for mobile antibiotic resistance genes

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Sir,

Haemophilus influenzae is an opportunistic pathogen that is found in the nasopharynx of ~75% of healthy adults.1 It is able to cause a variety of different infections and is commonly found in cystic fibrosis patients.1,2,3 Recently, 106 non-typeable H. influenzae samples isolated from cystic fibrosis children in 2007 and 2008 were shown to carry mobile acquired Gram-positive macrolide resistance genes.3,3 These three unrelated isolates (CHH062, CHH082 and CHH126) were resistant to both macrolides (EmR) and tetracycline (TcR). Previously all TcR H. influenzae carried the tet(B) gene generally on Haemophilus plasmids with host range restricted to within the genus.4 With the isolation of the tet(M) gene on a Haemophilus ducreyi plasmid from a strain isolated in 1986, we predicted that the tet(M) gene would move into other species of Haemophilus.4 More recently, Tristram et al.5 predicted that in the future TcR H. influenzae would carry the mobile broad-host range tet(M) gene associated with or without the tet(B) gene.

This study found that all three EmR TcR H. influenzae carried both the tet(M) and tet(B) genes and the two isolates tested could conjugally transfer the tet(M) gene to an unrelated Gram-positive Enterococcus faecalis JH2-2 recipient, while both tet genes could be transferred to a H. influenzae recipient. The data suggest that H. influenzae are now reservoirs for both broad-host range mobile antibiotic resistance genes, which is a shift from that of a recipient of mobile antibiotic resistance genes from unrelated genera in the 1970s, and today these strains are able to act as donors of these genes to unrelated genera.5

The three EmR TcR H. influenzae strains were screened for the presence of the tet(B) and tet(M) genes using previously described PCR assays with appropriate controls,6 and all three isolates were positive for both genes. Plasmid extractions were done and ~60 kb plasmids were identified that carried the tet(B) gene. The plasmids could be conjugally transferred at a frequency ranging from 1.08 × 10^{-1} to 5.0 × 10^{-9}/recipient to the H. influenzae Rd, which is in the range previously reported for transfer of Haemophilus plasmids.4 All the transconjugants examined carried the plasmid and the tet(B) gene, while 10%–15% of the transconjugants carried the tet(M) gene, suggesting that the tet(M) gene was not located on the plasmid. Conjugation was performed with the H. influenzae donors and E. faecalis JH2-2 recipient at 25:1 ratio, as previously described.3 The tet(M) gene transferred at a frequency ranging between 2.39 × 10^{-6} and 3.15 × 10^{-9}/recipient, while the tet(B) gene did not transfer.

This is the first report of the tet(M) gene in H. influenzae. Although the three isolates came from cystic fibrosis patients in North America, it is unlikely that they are unique to TcR H. influenzae strains from North America. However, it is unclear if H. influenzae from people other than cystic fibrosis patients, who normally are treated with antibiotics, also carry strains that harboured the tet(M) gene. Nevertheless, it is of interest that the tet(M) gene, which has the widest host range of the tet genes and has been identified in 37 Gram-negative genera and 35 Gram-positive genera, was recognized in TcR H. influenzae isolated >30 years after TcR H. influenzae carrying the tet(B) genes were described.6

The presence of the mobile tet(M) genes along with the previous identification of mobile erm(A), erm(B), erm(C), ermA and mef(A) macrolide resistance genes in H. influenzae indicates a major shift for H. influenzae from simply being able to acquire antibiotic resistance genes from both Gram-positive and Gram-negative bacteria that could be transferred between the genus Haemophilus to now having mobile elements that are able to conjugally transfer antibiotic resistance genes to unrelated genera within the bacterial community. The presence of the Tn916-like family of conjugative transposons in H. influenzae suggests the potential for antibiotic resistance genes often associated with the Tn916-like elements, aminoglycoside, chloramphenicol and macrolide resistance genes to become more common in the H. influenzae population. Whether surveillance of these mobile genes in the H. influenzae population has value will require further study.

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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. Also, for companion animals, several studies have been described. Recently some surveys have suggested that European wild birds may act as reservoirs of resistant bacteria 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. 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. 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. 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. 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 blaSHV gene encoding ESBL TEM-52. This isolate was assigned to ST1844.

The population of wild domestic and Canada geese in Belgium is estimated at 10000 birds. 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. 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.

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