SHORT COMMUNICATION

Extended spectrum beta-lactamases detected in *Escherichia coli* from gulls in Stockholm, Sweden

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In order to investigate if bacterial antibiotic resistance was present in gull populations in urbanised areas, we conducted a study in which faecal samples from gulls were collected in central Stockholm, Sweden in April and May 2010 and screened for extended spectrum beta-lactamases (ESBL)-type antibiotic resistance. Eighteen of 194 randomly selected *Escherichia coli* isolates harboured ESBL of CTX-M phenotype. Since the bacteria are unlikely to have developed the resistance in gulls, it may indicate leakage of resistant bacteria to the environment. As many gulls find food and shelter in cities around the world and thereby share their habitat with dense human populations, the finding that as many as 9% of gulls carry ESBL-type antibiotic resistance may imply that zoonotic transmission between gulls, humans, and other animals is likely to occur in such places. This study illustrates how ecologically widespread the problem of antibiotic resistance has become and this has implications for future policy making to reduce the spread of bacteria with antibiotic resistance.

Keywords: *antibiotic resistance*; *zoonoses*; *transmission*; *birds*; *ecology*

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Bacterial antibiotic resistance is the cause of excess morbidity and mortality as well as huge economic costs for health care systems worldwide (1). Many bacteria, such as *Escherichia coli* can colonise and/or cause infection in many different animal species, including humans, and zoonotic transmission has been shown to occur. Antibiotic resistance is also a well-recognised clinical challenge in many domestic mammals (2) and in recent years bacteria displaying resistance phenotypes have also been isolated from wild mammals (3) and birds (including birds sampled in the Baltic and Mediterranean regions) (4–9). As wild animal species are unlikely to have received antibiotic treatments, it suggests that transmission has occurred either from contact with waste from infected humans or domestic animals. Identical antibiotic resistance traits have been found in gulls close to human settlements as in local hospital patient samples (6, 7), and ESBL antibiotic resistance may be found in domestic animals and are particularly common in poultry (10).

Large cities are places of extreme human population densities, but also attract wild birds such as gulls and ducks that often congregate wherever there is water. As a consequence, bird droppings contaminate most city landscapes where humans live, eat, and drink providing possible transmission pathways from wild birds to humans. In order to examine the potential of this transmission pathway and to find out the level of wildlife bacterial antibiotic resistance with a focus on ESBL type carriage, we sampled faeces from gulls feeding in the vicinity of the Parliament buildings and the Royal Palace, located in downtown Stockholm, Sweden.

The study

We collected 283 faecal fresh dropping samples from black-headed gulls (*Larus ridibundus*), common gulls

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(*Larus canus*), herring gulls (*Larus argentatus*), and lesser black backed gulls (*Larus fuscus*) during 4 days in 2010; April 14 and April 24, May 26 and May 27. Cotton swab sampling was carried out on the quaysides around the water inlet *Strömmen* that passes through central Stockholm next to the Royal Palace and the Parliament buildings. Faecal material was placed in bacterial freeze medium (Luria Broth, BD, Sparks, MD, USA, in phosphate-buffered saline including 0.45% Na-citrate, 0.1% $MgSO_4$, 1% $(NH_4)_2SO_4$, and 4.4% glycerol). Samples were transported on ice to the laboratory and stored in –70°C for later examination. All samples were subsequently plated on a chromogenic medium (UriSelect 4, Bio-Rad Laboratories, Marnes-La-Coquette, France) and 197 putative *Escherichia coli* (one *E. coli* isolate from each sample, when *E. coli* present in sample) were isolated and species confirmed by biochemical testing. The putative *E. coli* were confirmed by biochemical testing. The antibiotic susceptibility of *E. coli* isolates was tested in accordance with the EUCAST disk diffusion method for antimicrobial susceptibility testing (11) to a panel of 11 antibiotics including tetracycline (TE) 30 μg/disk, ampicillin (AMP) 10 μg/disk, streptomycin (S) 10 μg/disk, chloramphenicol(C) 30 μg/disk, nalidixic acid (NA) 30 μg/disk, cefadroxil (CFR)30 μg/disk, μg/disk, fosfomycin (FOS) 50 μg/disk, tigecycline 15 μg/disk, sulfamethoxazole/trimethoprim 25 μg/disk, nitrofurantoin (F) 100 μg/disk, and mecillinam 10 μg/disk (Oxoid Ltd, Cambridge, UK). These antibiotics were selected to represent antibiotics commonly used against *E. coli* infections in human and veterinary medicine and to provide a general antibiotic susceptibility profile of the faecal samples. *E. coli* strain ATCC 25922 was used as control in all assessments. For detection of ESBL producing isolates specifically, all faecal samples were also enriched in BHI broth (Becton Dickinson, Franklin Lakes, NJ, USA) supplemented with vancomycin (16 mg/L, ICN Biomedicals Inc. Aurora, OH, USA) for 18 hr at 37°C for an initial enrichment of gram negative bacteria in general and, subsequently, inoculated and cultured overnight at 37°C on chromID™ ESBL plates (bioMérieux, Marcy L′Etoile, France) according to manufacturer's instructions. Colonies were isolated and species identity confirmed by biochemical testing. The ESBL production was confirmed by double disc test, one with cefpodoxime and the other with cefpodoxime + clavulanic acid (MAST Diagnostics, Bootle, UK) before genetic characterization. The ESBL producing isolates were analysed by PCR to determinate the presence of $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} enzyme genes using previously described methods (6, 12–14).

Results

Escherichia coli were isolated from 194 of the 283 samples collected (69%) and 35 of those 194 (18%) contained *E. coli* that displayed resistance to either one (n =27, 14%), two (n =4, 2%), or three (n =4, 2%) antibiotics (Table 1). No isolates showed resistance to tigecycline or mecillinam.

Eighteen isolates displayed ESBL harbouring phenotype. Polymerase chain reaction (PCR) analysis of ESBL genotypes showed that these isolates harboured ESBL of CTX-M ($bla_{\text{CTX-M-1}}$, n =16; $bla_{\text{CTX-M-14}}$, n =1) and SHV ($bla_{\text{SHV-12}}$, n =1) types. Seven strains showed a dual presence of TEM-1 ($bla_{\text{TEM-1}}$, not a true ESBL) and CTX-M-1. One strain showed the dual presence of TEM-1 and CTX-M-14, nine strains carried only CTX-M-1, and one strain carried only SHV-12.

Discussion

Escherichia coli was only isolated from 69% of the collected samples in this study. This is not surprising as

Table 1. Antibiotic resistance patterns of the *E. coli* isolates in the study

Antibiotics	Number of resistant isolates	%	
AMP	10	5.15	Resistant to one antibiotic
C	1	0.52	(n =27) 13.92%
F	1	0.52	
FOS	9	4.64	
NA	2	1.03	
TE	4	2.06	
AMP+FOS	2	1.03	Resistant to two antibiotics
AMP+CFR	1	0.52	(n =4) 2.07%
AMP+TE	1	0.52	
AMP+S+SXT	2	1.03	Resistant to three antibiotics
AMP+CFR+F	1	0.52	(n =4) 2.07%
NA+S+TE	1	0.52	

Abbreviations: AMP, ampicillin; C, chloramphenicol; F, nitrofurantoin; FOS, fosfomycin; NA, nalidixic acid; TE, tetracycline; CFR, cefadroxil; SXT, trimethoprim-sulphamethoxazole; S, streptomycin.

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from our experience from analysing more than 10,000 bird faecal samples and from others (15), *E. coli* may not always be isolated from bird faecal samples. While the isolated *E. coli* displayed low general antimicrobial susceptibility to 11 antimicrobial agents, we found a worryingly high number of ESBL isolates ($n = 18$, 9% of strains) predominantly harbouring CTX-M type ESBL genotypes. The ESBL types and variants found (CTX-M-1, -14 and SHV-12) are also common in clinical samples in Sweden, as well as abroad.

Micro-organisms that carry ESBLs are predominantly identified in human isolates relating to hospital outbreaks in Europe as well as worldwide. Our results show that ESBL producing micro-organisms may also be established in gull populations in urbanised areas. Hence, stationary and migratory birds may have an epidemiological role in the dissemination of antibiotic resistance and constitute a potential reservoir of resistance of ESBL type in large cities. There are different possible options for how the birds acquired the resistant *E. coli* strains. However, in our opinion, the most likely explanation is that the gulls had been infected or colonised with ESBL harbouring *E. coli* from the environment. Stockholm has efficient sewage management systems that makes the water in *Strömmen,* extraordinary clean for being in a city centre (fishing and swimming in central Stockholm is encouraged); however, the water may at times be contaminated with untreated wastewater being discharged from the sewer system when flows are large (16). The sampled gull species are omnivorous and often feed both in water and on land in city environments as well as on fields and farmland. They may roam long distances during a day to find food so, although they were in the vicinity of the water inlet Strömmen when samples were collected, they could also have acquired the ESBL-strains elsewhere in Sweden. However, as cases of ESBL infections in humans and domestic animals are rare in Sweden compared to many other countries in Europe (17, 18), perhaps it is more likely that the gulls, which are migratory and spend the winter at lower latitudes in Europe – mainly ice-free parts of Western Europe, Southern Europe (for lesser black backed gulls [*Larus fuscus*] also Africa) – may have acquired the strains in contaminated environments at these locations.

In order to investigate the impact of gulls as a reservoir for human infection by resistant bacteria, the samples in this study were collected in downtown Stockholm right in an area where locals and large amounts of tourists pass daily. The quays and pavements in this area are heavily polluted by bird droppings especially from gulls. Therefore it cannot be ruled out that gulls may directly – via faecal contamination or indirectly via, for example, domestic pets or contamination of water reservoirs – transmit ESBL-strains to humans.

We have showed that bacteria with clinically important resistance genotypes can be isolated from wild animals in the centre of a major European city, and there is no reason why this should be unique to Stockholm. The finding indicates how widespread the problem with antibiotic resistance has become and that, although we may eliminate a resistant strain from the human population, our efforts may be futile as the strains are now also established in other animal species over which we have no control and thus reintroduction may occur.

Conflict of interest and funding

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