Resident wild birds as potential disseminators of antimicrobial resistant bacteria

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Figure 1: An adult female (pen) mute swan with three cygnets, one of which is seen "disembarking" at the feeding trough. Photo: Theo de Waal.

A research team at UCD (see panel) have studied the role of resident wild birds as potential disseminators of antimicrobial resistant bacteria in a large public amenity park in the greater Dublin area

Antimicrobial resistance (AMR) is a significant and rapidly escalating concern globally due to its spread between humans, animals and the environment[1]. The occurrence of multi-drug resistant *Escherichia coli* (*E.coli*) bacteria in wild waterfowl including mute swans and mallard ducks has been documented with these hosts identified as reservoirs of avian pathogenic *E. coli* strains (APEC) and pathogenic human *E. coli* strain serotype H7:O157[2]. One study in Poland highlighted that the mute swan showed the highest number of *E. coli* strain isolates at 59.5 per cent closely followed by the mallard duck at 55.3 per cent[3]. The migratory lifestyle of these wild waterfowl is a contributing factor towards the spread of potentially zoonotic *E. coli* strains and multi-drug resistant (MDR) strains between various ecosystems across Europe and other countries.

Thus, wild water birds may pose a potential risk to human and animal health by transmitting MDR *E. coli* strains to water bodies via contaminated faeces[4].

Research on wild water birds acting as hosts for MDR *E.coli* has been widely documented in various countries worldwide. However, there is little data available on wild water birds acting as hosts for MDR *E.coli* in local public amenities in Ireland. This research project identified one public water amenity in the greater Dublin area where the only water source for the water bodies was from surface water run-off in the immediate area. These water bodies were inhabited by wild waterfowl which were made up of mute swans (Figure 1), mallard ducks, seagulls and other seabirds. The water bodies were located along popular walking trails where dogs were allowed direct access. Therefore, the aim of this project was to determine if *E.coli* was present in these water bodies and whether the *E. coli* identified were

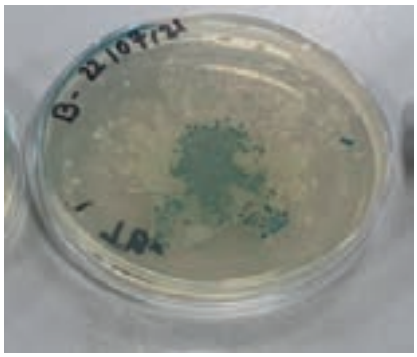

Figure 2: TBX agar plate showing blue *Escherichia coli* colony growth following culturing and incubation. Photo: Chloe Maloney.

resistant to certain veterinary and human antibiotics.

MATERIALS AND METHODS

Collection, Filtration and Culturing

Water sampling was carried out weekly for four consecutive weeks from four water bodies in a Dublin public amenity area. Five 100 ml water samples were collected and pooled at each site. Samples of 100ml from each site were vacuum filtered using a sterile 0.45μm nitrocellulose filter membrane. Buffer Peptone Water (BPW) was added to rinse each membrane by vortexing. Samples (100μl) were cultured on i) TBX (Tryptone Bile X-glucuronide (Figure 2); ii) TBX with 1mg/ml cefotaxime; and iii) TBX with 1mg/ml ciprofloxacin. These plates were incubated for 18-24 hours at 37°C. Potential *E. coli* isolates were sub-cultured onto MacConkey agar (Figure 3) and then checked by antibiotic disc diffusion on Mueller Hinton agar[5].

Figure 3: *Escherichia coli* colonies were sub-cultured onto MacConkey agar to ensure pure colonies were obtained for subsequent carrying out of an antibiotic disc diffusion test. Photo: Chloe Maloney.

Antibiotic Disc Diffusion Test

Antibiotic resistance trends were determined for seven antibiotics commonly used in veterinary and human medicine (Figure 4)[5].

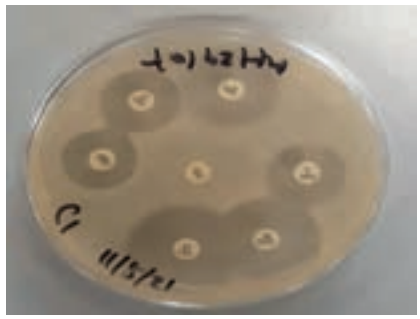

Figure 4: Antibiotic susceptibility patterns of *Escherichia coli* isolated from the public amenity water bodies. Photo: Chloe Maloney.

The European Committee of Antimicrobial Susceptibility Testing (EUCAST) Zone of Diffusion chart was used to classify each isolate as resistant (R), intermediate (INT), or sensitive (S) dependent on the zone of inhibition (Table 1 overleaf).

MALDI-TOF Mass Spectrometer

The MALDI-TOF Mass Spectrometer (bioMerieux Vitek MS) facility in the UCD School of Agriculture and Food Science was used to identify *E. coli* and other coliform isolates (Figure 5)[6].

Figure 5: MALDI-TOF Mass Spectrometer used for bacterial identification. Photo: Chloe Maloney.

RESULTS AND DISCUSSION

Microbiology culture results displayed in Table 2 showed *E. coli* growth on 11/20 (55%) culture plates across the four-week study period. Of these 11 plates, 46 *E. coli* colonies were isolated from the four water bodies analysed. Tap water culture samples, used as controls, showed no growth of *E. coli*. *Escherichia coli* are almost exclusively faecal in origin, and the presence of *E. coli* is suggestive of water faecal contamination from resident wildlife including mallard ducks and swans. From the literature, the incidence rate of *E. coli* strain isolates in mute swans is 59.5 per cent, closely followed by the mallard duck at 55.3 per cent[3]. *Escherichia coli* growth was only seen on "TBX only" culture plates, with no *E. coli* growth seen on "TBX + Ciprofloxacin" and "TBX + Cefotaxime" culture plates. All three TBX plates, across the four weeks, displayed some coliform growth in the form of white or cream colonies of various sizes.

In terms of antimicrobial resistance, the *E. coli* detected had higher levels of resistance to certain antibiotics as shown in Table 3. Ninety-six per cent of *E. coli* isolates were resistant to cephalexin. Cephalexin is a first generation cephalosporin commonly used in canines. It is known to be clinically resistant to *E. coli*[6]. *Escherichia coli* resistance to cefpodoxime, a third generation cephalosporin, was lower at 35 per cent.

Tetracycline is widely known in veterinary medicine for its broad-spectrum activity against bacterial infections[7]. In the present study, 85 per cent of *E. coli* isolates were resistant to tetracycline. In Poland, a study on wild birds which included mute swans and mallard ducks, found that 50 per cent of *E. coli* isolates were resistant to tetracycline[2].

This study found that only 15 per cent of *E. coli* isolates were resistant to enrofloxacin and sulfamethoxazole/trimethoprim. Fluoroquinolones are categorised as "highest priority critically important antimicrobials" (HPCIA) in both human and veterinary medicine[8] with enrofloxacin (Baytril) being widely used in both companion and food producing animals to treat gram-negative and gram-

Antibiotic discs used	Disc content (µg)	Zone diameter breakpoints (mm)		
		R ≤	INT	S ≥
Amoxicillin-clavulanic acid	30	13	14-17	18
Cephalexin	30	14	15-17	18
Cefpodoxime	10	17	18-20	22
Enrofloxacin	5	17	18-21	22
Gentamicin	10	12	13-14	15
Tetracycline	30	14	15-18	19
Sulfamethoxazole/ Trimethoprim	25	10	11-15	16

Table 1. EUCAST Zone of Diffusion chart, displaying the disc contents in µg and diameter breakpoints measured in mm, was used to determine the resistance and susceptiblity levels of the *Escherichia coli* isolates towards the antimicrobials[5].

Week	Number of "TBX only" culture plates submitted	Number of "TBX only" culture plates positive for *Escherichia coli*	Total number of *Escherichia coli* colonies isolated from "TBX only" culture plates
1	5	3	15
2	5	3	13
3	5	3	15
4	5	2	3
Total	20	11	46

Table 2. Culture demographics from the four-week culture period.

positive infections[9]. Enrofloxacin is partially metabolised to ciprofloxacin in animals, and high levels of ciprofloxacin resistance in multi-drug resistant *E. coli* isolates have been found in the mallard duck in Poland at 46.8 per cent[2]. Therefore, the low levels of resistance in the Irish water bodies studied towards enrofloxacin and no resistance to ciprofloxacin is an interesting finding.

Sulfamethoxazole/Trimethoprim is a human preparation based on a combination of the antimicrobial sulfamethoxazole and the antibiotic trimethoprim, whereas trimethoprim and sulfadiazine is the veterinary preparation. Both combinations can be used in veterinary medicine[10].

The low resistance levels of the *E. coli* isolates towards the human preparation of sulfamethoxazole/ trimethoprim is promising as it is used to treat a range of gram-negative and gram-positive bacterial infections in companion and domestic farm animals[10].

The *E. coli* isolates displayed relatively high resistance levels at 30 per cent towards amoxicillin/ clavulanic acid and gentamicin. Amoxicillin-clavulanic acid (Co-Amoxiclav, Synulox) is a commonly used veterinary and human antibiotic that has a broad spectrum of activity against gram-negative and gram-positive species. However, it is not indicated for gram negative bacteria including *E. coli*, as it is known to be clinically resistant[11].

It has been documented that there is growing resistance towards this beta-lactamase inhibitor combination. It has also been suggested that its use should be reserved alongside the critically important antimicrobials including the third and fourth generation cephalosporins and fluoroquinolones to slow the growing resistance levels[12, 13].

The findings of this study highlight the need for monitoring of water quality of all water bodies to which the public have direct access. Currently, there is a European Council Directive (76/160/EEC) which only applies to sites where bathing is not prohibited, and the sites are frequented by a large number of bathers[14]. Under the Directive, local authorities are responsible for sampling of waters at the bathing sites in their areas. Analysis of microbiological parameters such as total coliforms and faecal coliforms are carried out. Ideally, this Directive should be amended to include shallow water bodies with public access. Dogs can still drink/bathe in these waters and the public can still access them to paddle in, if they wished.

The MADLI-TOF Mass Spectrometer identified a number of other coliforms

Antibiotic	Antibiotic Class	per cent Resistant *E. coli* Isolates (n=46)	Animal/Human Use
Enrofloxacin	Fluoroquinolone	15 per cent (n=7)	Animal
Tetracycline	Tetracycline	85 per cent (n=39)	Animal + Human
Sulfamethoxazole/Trimethoprim	Sulphonamide	15 per cent (n=7)	Animal + Human
Cephalexin	1st Generation Cephalosporin	96 per cent (n=44)	Animal + Human
Gentamicin	Aminoglycoside	30 per cent (n=14)	Animal + Human
Cefpodoxime	3rd Generation Cephalosporin	35 per cent (n=16)	Human
Amoxicillin/Clavulanic acid	Penicillin + beta-lactam	30 per cent (n=14)	Animal + Human
Ciprofloxacin	Fluoroquinolone	0 per cent (n=0)	Human
Cefotaxime	3rd Generation Cephalosporin	0 per cent (n=0)	Human

Table 3. Antibiotic resistance levels of *Escherichia coli* isolates.

in the water bodies that were obtained from the culture plates (Table 4). While the MALDI-TOF database is very extensive, the *E. coli* strains and serotypes could not be identified due to their protein profile not being in the database. However, the *E. coli* bacteria were confirmed as members of the *E. coli* family which further confirmed that *E. coli* is present in the water bodies. Similarly, 31.25 per cent of isolates could not be identified as there were no matching profiles in the MALDI-TOF database.

MALDI-TOF Mass Spectrometer identification results (n=16)	
Pseudomonas fluorenscens	**6.25% (n=1)**
Pectobacterium cartovarum	**6.25% (n=1)**
Pseudomonas cuatrocienegasensis	**6.25% (n=1)**
Citrobacter freundii	**6.25% (n=1)**
Pseudomonas putida	**6.25% (n=1)**
Enterbacter cloaca	**6.25% (n=1)**
Klebsiella oxytoca	**6.25% (n=1)**
Escherichia coli	**25% (n=4)**
No identification	**31.25% (n=5)**

Table 4. Coliforms identified using the MADLI-TOF Mass Spectrometer.

CONCLUSION

1. Microbiological analysis of the water samples concluded that *E. coli* is present in these four public amenity water bodies. The areas surrounding these water bodies are popular trails where dogs are regularly taken for walks. If water is ingested, there is the potential for the health of both the owners and their pets to be affected. This interplay between humans, animals and the environment highlights the relevance of regular microbiological monitoring of our public water amenities.
2. The MALDI-TOF Mass Spectrometer did not identify the specific strain of *E. coli*. However, it is still potentially a zoonotic pathogen and diligence must be taken if dogs are given unrestricted access by their owners to these water bodies.
3. This study focused on *E. coli*, but it is likely that there are various other pathogens in these waters that may have zoonotic potential and owners should take precaution around open stagnant water bodies to protect their own health as well as their pets.
4. Water body sites with public access should not be assumed to be safe. The public should be alerted to this fact by the placing of local authority public health warning notices in each vicinity.
5. A strong trend of resistance towards tetracycline and cephalexin was observed. The high levels of *E. coli* resistance to some of the antibiotics tested in this study further supports the escalating trends of resistance towards commonly used antibiotics in human and veterinary medicine. This study highlights that the resistance levels to certain antibiotics are low. However, it is probable that these resistance levels will continue to increase if antibiotics are not used wisely. This in turn will have serious implications for the future of One Health.

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