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Wild birds as biological indicators of environmental pollution: antimicrobial resistance patterns of *Escherichia coli* and enterococci isolated from common buzzards (*Buteo buteo*)

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A total of 36 *Escherichia coli* and 31 enterococci isolates were recovered from 42 common buzzard faecal samples. The *E. coli* isolates showed high levels of resistance to streptomycin and tetracycline. The following resistance genes were detected: bla_{TEM} (20 of 22 ampicillin-resistant isolates), *tet*(A) and/or *tet*(B) (16 of 27 tetracycline-resistant isolates), *aadA1* (eight of 27 streptomycin-resistant isolates), *cmlA* (three of 15 chloramphenicol-resistant isolates), *aac(3)-II* with/without *aac(3)-IV* (all seven gentamicin-resistant isolates) and *sul1* and/or *sul2* and/or *sul3* [all eight sulfamethoxazole/trimethoprim-resistant (SXT) isolates]. *intI1* and *intI2* genes were detected in four SXT-resistant isolates. The virulence-associated genes *fimA* (type 1 fimbriae), *papC* (P fimbriae) and *aer* (aerobactin) were detected in 61.1, 13.8 and 11.1 % of the isolates, respectively. The isolates belonged to phylogroups A (47.2 %), B1 (8.3 %), B2 (13.9 %) and D (30.5 %). For the enterococci isolates, *Enterococcus faecium* was the most prevalent species (48.4 %). High levels of tetracycline and erythromycin resistance were found among our isolates (87 and 81 %, respectively). Most of the tetracycline-resistant strains carried the *tet*(M) and/or *tet*(L) genes. The *erm*(B) gene was detected in 80 % of erythromycin-resistant isolates. The *vat*(D) and/or *vat*(E) genes were found in nine of the 17 quinupristin–dalfopristin-resistant isolates. The enterococcal isolates showing high-level resistance for kanamycin, gentamicin and streptomycin contained the *aph(3′)-IIIa, aac(6′)-aph(2″)* and *ant(6)-Ia* genes, respectively. This report reveals that common buzzards seem to represent an important reservoir, or at least a source, of multi-resistant *E. coli* and enterococci isolates, and consequently may represent a considerable hazard to human and animal health by transmission of these isolates to waterways and other environmental sources via their faecal deposits.

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INTRODUCTION

Microbial resistance to antibiotics is a worldwide problem in human and veterinary medicine. Commonly, it is usual that the principal risk factor for an increase in this situation is the extensive use of antibiotics leading to the dissemination of resistant bacteria and resistance genes in animals and humans (van den Bogaard & Stobberingh, 2000). The appearance of multi-resistant bacteria of human and veterinary origin is probably accompanied by co-contamination of the environment often leading to serious health concerns (Grobbel *et al.*, 2007).

Bacteria may present resistance to antibiotics under selective pressure, but they may also acquire antibiotic resistance determinants without direct exposure to an antibiotic

Abbreviations: ESBLs, extended-spectrum β-lactamases; SXT, sulfamethoxazole/trimethoprim.

through horizontally mobile elements including conjugative plasmids, integrons and transposons (Middleton & Ambrose, 2005). These mobile elements can simply transfer antibiotic resistance genes from one bacterium to another (Coque *et al.*, 2008).

The bacteria of the normal flora of the gut, such as *Escherichia coli* and enterococci, can easily acquire and transfer resistance genes. These commensal bacteria, which constitute a reservoir of resistance genes for pathogenic bacteria, can thus be used as indicators of changes in antimicrobial resistance (Caprioli *et al.*, 2000).

Antibiotic resistance in faecal indicator bacteria could have a number of consequences. For example, *E. coli* and enterococci have become more efficient human nosocomial pathogens (Jett *et al.*, 1994) as they have developed increased antibiotic resistance.

The common buzzard is a medium to large bird of prey, with a geographical distribution that covers most of Europe and also extends into Asia. As a great opportunist, it is well adapted to a varied diet of pheasants, rabbits, other small mammals, snakes and lizards, and can often be seen walking over recently ploughed fields looking for worms and insects (IUCN, 2010).

In addition to the currently common detection of multi-resistant bacteria in areas with high human density (Cole *et al.*, 2005), the emergence of such bacteria in more remote areas such as high mountain regions is even more alarming (Dolejska *et al.*, 2007). Although wild birds have only rare contact with antimicrobial agents, in disagreement with the existence of direct selective pressure, they can be contaminated or colonized by resistant bacteria. Water contact and acquisition via food seem to be the major routes of transmission of resistant bacteria of human or veterinary origin to wild animals (Cole *et al.*, 2005). Wild birds in general may therefore represent reservoirs of resistant bacteria and genetic determinants of antimicrobial resistance (Dolejska *et al.*, 2007).

Monitoring the prevalence of resistance in indicator bacteria such as faecal *E. coli* and enterococci in different populations such as animals, patients and healthy humans makes it feasible to compare the prevalence of resistance and to detect the transfer of resistant bacteria or resistance genes from animals to humans and vice versa (Martel *et al.*, 2001). However, few reports of the level of antimicrobial resistance in *E. coli* and enterococci of wild animals have been published (Nulsen *et al.*, 2008; Poeta *et al.*, 2005b, 2007b; Radhouani *et al.*, 2009; Silva *et al.*, 2010).

The aim of the present study was to analyse the prevalence of antimicrobial resistance and the mechanisms implicated in faecal *E. coli* isolates and *Enterococcus* species of common buzzards in Portugal.

METHODS

Samples and bacteria. Forty-two faecal samples from common buzzards (*Buteo buteo*) of Portugal were recovered from September 2007 to February 2008. All the faecal samples were collected individually from each animal, and were obtained in collaboration with the Center of Collecting, Welcome and Handling of Wild Animals (CRATAS), located in the Trás-os-Montes and Alto Douro University (Portugal), which receives injured animals found in its natural environment. None of the animals had been fed previously by humans or had received antibiotics. The common buzzard is fairly well distributed throughout the Portuguese territory, being the only species of bird of prey found in all regions of the country. The majority of the birds came from the centre and north of the country. For *E. coli* isolation, samples were seeded in Levine agar plates and incubated for 24 h at 37 °C. Colonies with a typical *E. coli* morphology were selected and identified by classical biochemical methods (Gram staining, and catalase, oxidase, indol, methyl-red Voges–Proskauer, citrate and urease tests) and using the API 20E system (bioMérieux).

For enterococcal isolation, faecal samples were diluted and spread on Slanetz–Bartley agar plates and incubated for 48 h at 37 °C. Colonies with a typical enterococcal morphology (one per sample) were identified by cultural characteristics, Gram staining, catalase test and bile-aesculin reaction and by biochemical tests using the API ID20 Strep system (bioMérieux). Enterococci were further identified to the species level by PCR using primers and conditions for the different enterococcal species, as described previously (Torres *et al.*, 2003).

Antimicrobial susceptibility test. Antibiotic susceptibility was tested by the agar disc diffusion method as recommended by the CLSI (2010). The susceptibility of the *E. coli* isolates was tested for 16 antibiotics: ampicillin (10 μg), amoxicillin/clavulanic acid (20 μg + 10 μg), cefoxitin (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), aztreonam (30 μg), imipenem (10 μg), gentamicin (10 μg), amikacin (30 μg), tobramycin (10 μg), streptomycin (10 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), sulfamethoxazole/trimethoprim (SXT) (1.25 μg + 23.75 μg), tetracycline (30 μg) and chloramphenicol (30 μg). *E. coli* ATCC 25922 was used as a quality-control strain. Additionally, a screening test for detection of extended-spectrum β-lactamases (ESBLs) was carried out by a double disc diffusion test (Bradford, 2001; CLSI, 2010).

The susceptibility of the enterococcal isolates was tested for 11 antibiotics: vancomycin (30 μg), teicoplanin (30 μg), ampicillin (10 μg), streptomycin (300 μg), gentamicin (120 μg), kanamycin (120 μg), chloramphenicol (30 μg), tetracycline (30 μg), erythromycin (15 μg), quinupristin–dalfopristin (15 μg) and ciprofloxacin (5 μg), by the disc diffusion method (CLSI, 2010). Only the category of high-level resistance was considered for streptomycin, gentamicin and kanamycin. *Enterococcus faecalis* strain ATCC 29212 and *Staphylococcus aureus* strain ATCC 25923 were used as quality controls.

Antibiotic resistance genes. The presence of genes encoding TEM and SHV β-lactamases was studied by PCR in all ampicillin-resistant isolates, using primers and conditions reported previously (Briñas *et al.*, 2002). The following genes were studied by PCR: *tet*(A), *tet*(B), *tet*(C), *tet*(D) and *tet*(E) (in tetracycline-resistant isolates), *aadA1* (in streptomycin-resistant isolates), *aac(3)-II* and *aac(3)-IV* (in gentamicin-resistant isolates), *aac(6′)-Ib* (in amikacin-resistant isolates), *cmlA* (in chloramphenicol-resistant isolates) and *sul1*, *sul2* and *sul3* (in SXT-resistant isolates). The presence of the *intI1* and *intI2* genes, encoding class 1 and 2 integrases, respectively (Radhouani *et al.*, 2009), and genes encoding different virulence factors (*fimA*, *papGIII*, *stx*, *cnf1*, *papC* and *aer*) was also verified by PCR using primers and conditions described previously (Ruiz *et al.*, 2002).

Resistant enterococci isolates were tested by PCR for detection of the following resistance genes: *erm*(B) (in erythromycin-resistant isolates), *tet*(M) and *tet*(L) (in tetracycline-resistant isolates),

aph(3′)-IIIa (in kanamycin-resistant isolates), *aac(6′)-aph(2″)* (in gentamicin-resistant isolates), *ant(6)-Ia* (in streptomycin-resistant isolates) and *vat*(D) and *vat*(E) (in quinupristin–dalfopristin-resistant isolates), using primers and conditions reported previously (Aarestrup *et al.*, 2000; Leener *et al.*, 2005; Torres *et al.*, 2003). Specific PCR assays for detection of the resolvase gene *tdnX* and *int* genes were also used in *tet*(M)-positive isolates, to demonstrate the presence of the Tn*5397*-like and Tn*916*/Tn*1545*-like transposons, respectively (Agersø *et al.*, 2006).

Positive and negative controls were used in all PCRs, from the strain collection of the University of Trás-os-Montes and Alto Douro (Portugal). DNA sequencing was used to verify the identity of the gene products of at least one isolate randomly selected for each gene.

Detection of phylogenetic groups. The *E. coli* isolates were assigned to one of the four main phylogenetic groups, A, B1, B2 and D, following a PCR strategy published previously based on the presence or absence of the *chuA* and *yjaA* gene or the DNA fragment TSPE4.C2 (Clermont *et al.*, 2000).

RESULTS

Bacteria isolation

Thirty-six *E. coli* isolates were recovered from the 42 common buzzard faecal samples (85.7 %), whilst enterococci were detected in 31 (73.8 %) of the faecal samples studied. *Enterococcus faecium* was the most prevalent species detected in common buzzards (48.4 %), followed by *Enterococcus faecalis* (16.1 %), *Enterococcus hirae* and *Enterococcus durans* (each 12.9 %). Three enterococci isolates could not be identified to the species level and are referred to below as *Enterococcus* species.

Antimicrobial resistance among *E. coli* isolates

Table 1 shows the percentages of the 36 *E. coli* isolates that were resistant to each of the antimicrobials tested. The double disc synergy test for detection of ESBLs was negative for all our *E. coli* isolates.

A high percentage of resistance to streptomycin and tetracycline was observed in our *E. coli* isolates (75 %). More than 60 % of the isolates were resistant to ampicillin. The percentages of *E. coli* isolates that were resistant to ciprofloxacin, amikacin, cefoxitin, tobramycin or chloramphenicol ranged from 50 to 41.7 %. Amoxicillin/clavulanic acid, nalidixic acid, SXT and gentamicin resistances were also present in *E. coli* isolates (38.9, 33.3, 22.2 and 19.4 %, respectively). Lower percentages of resistance were observed to imipenem and aztreonam (5.5 and 2.8 %, respectively). All the isolates were susceptible to ceftazidime and cefotaxime. It is interesting to underline the high diversity of the resistance phenotypes; in fact, 34 different phenotypic profiles were observed in our *E. coli* isolates.

The presence of antibiotic resistance genes was studied by PCR in all resistant *E. coli* isolates. The presence of β-lactamase genes was investigated in all 22 ampicillin-resistant isolates, with the bla_{TEM} gene detected in 20 of them and the bla_{SHV} gene not being detected. The *aac(3)-II* or *aac(3)-IV* gene, encoding an aminoglycoside acetyltransferase that modifies gentamicin, was found in the seven gentamicin-resistant isolates. In addition, the *aadA1* gene, encoding an aminoglycoside adenyltransferase that modifies streptomycin, was detected in eight of the 27 streptomycin-resistant isolates of this study. The *tet*(A) and/or *tet*(B) genes, associated with an active efflux system, were identified in 16 of the 27 tetracycline-resistant isolates. The *cmlA* gene was found in three of the 15 chloramphenicol-resistant isolates. A total of eight *E. coli* isolates presented the SXT-resistant phenotype and the *sul1* and/or *sul2* and/or *sul3* genes were detected in all of them.

The *intI1* gene encoding class 1 integrase and the *intI2* gene encoding class 2 integrase were found in four and one of the eight SXT-resistant isolates, respectively.

Phylogenetic groups and virulence factor genes among *E. coli* isolates

Table 2 presents the phylogenetic groups of the 36 *E. coli* isolates in relation to the virulence factor genes. Most of the isolates belonged to phylogenetic group A (17 isolates) or D (11 isolates), and only five isolates corresponded to B2 group and three isolates to B1 group. All the *E. coli* isolates from groups B1 and B2 carried the *fimA* gene. This virulence factor gene was also detected in almost 53 and 46 % of our *E. coli* isolates, respectively. It is interesting to point out that almost all the isolates that carried the *aer* and *papC* genes belonged to the B2 and D phylogenetic groups.

Antimicrobial resistance among enterococci isolates

Table 1 shows the percentage of antimicrobial resistance according to enterococcal species. All 31 enterococci isolates were resistant to one or more than one antibiotic agent. A higher level of resistance was observed for tetracycline (87.1 %) and erythromycin (80.6 %), with a moderate percentage of resistance to quinupristin–dalfopristin (65.4 %) and streptomycin (35.5 %). Almost 30 % of our isolates showed resistance to ciprofloxacin, kanamycin and chloramphenicol. Low percentages were observed for ampicillin (20 %) and gentamicin (12.9 %). Three ampicillin-resistant *Enterococcus faecium* isolates were detected in our report. No teicoplanin- or vancomycin-resistant enterococci were identified in this study.

From the 27 tetracycline-resistant enterococcal isolates, the *tet*(M) (eight isolates), *tet*(L) (five isolates) and *tet*(M) + *tet*(L) (seven isolates) genes were detected.

Genes specific for the Tn*916*/Tn*154* transposons were detected in eight (two *Enterococcus faecium*, two *Enterococcus faecalis*, three *Enterococcus durans* and one *Enterococcus hirae*) of the 15 *tet*(M)-positive isolates, and specific sequences of Tn*5397* were also identified in one *Enterococcus durans* isolate. The presence of the *erm*(B) gene was investigated in the 25

Table 1. Distribution of antibiotic resistance in *E. coli* and *Enterococcus* species isolated from faecal samples of common buzzards

NT, Not tested.

Antimicrobial agent	No. (%) of *E. coli* isolates (*n*=36)	No. (%) of *Enterococcus* isolates by species				
		E. faecium (*n*=15)	*E. faecalis* (*n*=5)	*E. durans* (*n*=4)	*E. hirae* (*n*=4)	*Enterococcus* spp. (*n*=3)
Ampicillin	22 (61.1)	3 (20)	0	0	0	0
Amoxicillin/clavulanic acid	14 (38.9)	NT	NT	NT	NT	NT
Cefoxitin	16 (44.4)	NT	NT	NT	NT	NT
Cefotaxime	0	NT	NT	NT	NT	NT
Ceftazidime	0	NT	NT	NT	NT	NT
Aztreonam	1 (2.8)	NT	NT	NT	NT	NT
Imipenem	2 (5.5)	NT	NT	NT	NT	NT
Gentamicin	7 (19.4)	3 (20)	0	0	1	0
Amikacin	17 (47.2)	NT	NT	NT	NT	NT
Tobramycin	15 (41.7)	NT	NT	NT	NT	NT
Streptomycin	27 (75)	4 (26.7)	1	3	2	1
Nalidixic acid	12 (33.3)	NT	NT	NT	NT	NT
Ciprofloxacin	18 (50)	5 (33.3)	2	1	0	1
Sulfamethoxazole/trimethoprim	8 (22.2)	NT	NT	NT	NT	NT
Tetracycline	27 (75)	14 (93.3)	4	4	3	2
Chloramphenicol	15 (41.7)	3 (20)	1	2	2	1
Vancomycin	NT	0	0	0	0	0
Teicoplanin	NT	0	0	0	0	0
Kanamycin	NT	4 (26.7)	2	2	1	0
Erythromycin	NT	13 (86.7)	3	4	3	2
Quinupristin–dalfopristin*	NT	8 (53.3)	–	3	4	2
Susceptible to all antibiotics	1 (2.8)	0	0	0	0	0

*Susceptibility for this drug combination was not tested in *Enterococcus faecalis* isolates.

Table 2. Virulence factor genes and phylogenetic groups detected among 36 *E. coli* isolates recovered from common buzzards

Virulence factor gene detected	No. (%) of *E. coli* isolates by phylogenetic group			
	A (*n*=17)	B1 (*n*=3)	B2 (*n*=5)	D (*n*=11)
fimA	9 (52.9)	3	5	5 (45.5)
aer	0	0	2	2 (18.2)
papC	0	1	1	3 (27.3)

erythromycin-resistant isolates and was found in 80 % of these isolates (ten *Enterococcus faecium*, two *Enterococcus faecalis*, three *Enterococcus durans*, three *Enterococcus hirae* and two *Enterococcus* species isolates). The streptogramin A resistance genes *vat*(D) and/or *vat*(E) were found in nine of the 17 quinupristin–dalfopristin-resistant isolates (five *Enterococcus faecium*, two *Enterococcus durans* and two *Enterococcus hirae*). The *catA* gene was present in one of the chloramphenicol-resistant isolates (*Enterococcus durans*). In addition, the enterococcal isolates showing high-level resistance to gentamicin and streptomycin contained the *aac(6′)-aph(2″)* and *ant(6)-Ia* genes, respectively. Furthermore, the *aph(3′)-IIIa* gene was found in all the isolates with high-level resistance to kanamycin (four *Enterococcus faecium*, two *Enterococcus faecalis*, two *Enterococcus durans* and one *Enterococcus hirae*).

DISCUSSION

Few data exist about the susceptibility to antimicrobial agents of *E. coli* and enterococcal isolates of healthy wild animals (Guenther *et al.*, 2010; Silva *et al.*, 2010), and even fewer in common buzzards. Usually, the reports are restricted to analysis of ESBL-containing *E. coli* and vancomycin-resistant enterococci (Literak *et al.*, 2010; Pinto *et al.*, 2010; Poeta *et al.*, 2009; Radhouani *et al.*, 2009, 2010a).

In our study, it is important to point out that, of the 36 *E. coli* isolates, 35 of them showed resistance to one or more than one antibiotic. The most prevalent resistances were to streptomycin, tetracycline and ampicillin. High rates of resistance to the same antibiotics were detected in *E. coli* isolated from migratory Canadian geese (Middleton & Ambrose, 2005), comparable to our results, whilst antimicrobial susceptibility data for wild birds have been restricted to black-headed gulls from the Czech Republic (Dolejska *et al.*, 2007), yellow-legged gulls from Portugal (Radhouani *et al.*, 2009) and European wild birds of different species (Guenther *et al.*, 2010). Interestingly, the data indicate that both the synanthropic species, such as pigeons or gulls, which have frequent contact to humans, and bird species living in more rural areas and birds of prey seem to play a role as carriers of multi-resistant isolates.

Almost all ampicillin-resistant *E. coli* isolates from buzzards harboured the bla_{TEM} gene. The TEM β-lactamase is the most common mechanism of ampicillin resistance in *E. coli* from different origins (Briñas *et al.*, 2002). In our report, all the gentamicin-resistant isolates carried the *aac(3)-II* and/or *aac(3)-IV* genes. These genes, which present cross-resistance to other aminoglycosides, can be mobilized on multi-resistance elements, so that the spread of gentamicin-resistant determinants is likely to be selected by antimicrobial agents other than gentamicin (Jakobsen *et al.*, 2007). The detection of *tet*(A) and/or *tet*(B) genes in almost 60 % of our tetracycline-resistant isolates shows that the main mechanism of tetracycline resistance in *E. coli* isolates from common buzzards is by active efflux.

All our SXT-resistant *E. coli* isolates carried *sul* genes. This high occurrence is similar to that reported in previous studies (Soufi *et al.*, 2009; Vinué *et al.*, 2010). Four of the eight SXT-resistant isolates possessed the *sul3* gene, highlighting the high ability of this gene to disseminate in different populations, possibly due to the efficient genetic structure in which this gene is included.

In our study, three of the eight SXT-resistant isolates carried class 1 integrons, and one of them contained both class 1 and class 2 integrons. The high prevalence of integrons is a cause of concern, mainly due to the significant association of integrons with ampicillin resistance and even with multi-resistance phenotypes. The presence of integrons among commensal *E. coli* from common buzzards is a cause for alarm because this genetic structure is very efficient for the acquisition of antimicrobial resistance genes, which could be transmitted to other bacteria by mobile elements such as plasmids and transposons. Integrons appear to occur not only among clinical isolates of *E. coli* but also among commensal strains, even in wild animals.

It is well recognized that *E. coli* consists of a number of distinct phylogroups and that isolates of the different phylogroups differ in their ecological niches, life-history characteristics and propensity to be the origin of diseases. Consequently, much can be learnt by assigning a strain of *E. coli* to one of the recognized phylogroups (Gordon *et al.*, 2008). In our study, phylogenetic typing revealed an affiliation of a high proportion of multi-resistant strains to groups A and D. The same results have been obtained in poultry meat (Soufi *et al.*, 2009) and wild animals (Poeta *et al.*, 2007a; Radhouani *et al.*, 2009, 2010a). At least one virulence-associated gene (*fimA*, *papC* or *aer*) was detected in 22 of the 36 isolates studied (61 %). The same was observed in *E. coli* isolates from poultry meat (Soufi *et al.*,

2009). The emergence of potentially highly virulent isolates in combination with a multi-resistance phenotype is alarming, as a possible consequence would be a severe clinical outcome concomitant with serious limitations in antimicrobial treatment.

Among our enterococcal isolates, *Enterococcus faecium* and *Enterococcus faecalis* were the most predominant enterococcal species in the faecal samples of common buzzards. This observation is in agreement with those of other studies performed in animals (Aarestrup *et al.*, 2000; Kojima *et al.*, 2010) and particularly in wild animals in Portugal (Poeta *et al.*, 2007a; Silva *et al.*, 2010).

It is important to highlight the presence of the three ampicillin-resistant *Enterococcus faecium* isolates. Recently, it has been relatively common to find *Enterococcus faecium* resistant to this antibiotic, as a result of modifications in its penicillin-binding proteins (PBP5), although this resistance phenotype has been identified more often in isolates of human origin (Billström *et al.*, 2008). Our results are consistent with the results of other studies in humans, poultry and pets (Poeta *et al.*, 2005a) and in wild animals (Poeta *et al.*, 2007a; Silva *et al.*, 2010).

In our report, the *erm*(B), *tet*(M) and/or *tet*(L) genes identified were frequently associated together in the same strain. The *erm*(B) gene is frequently linked with the *tet*(M) gene on the highly mobile conjugative transposon Tn*1545*, which predominates in clinically important Gram-positive bacteria (De Leener *et al.*, 2004). It has been suggested that there may be a correlation between the level of antimicrobial resistance in faecal bacteria from animals and the level of contact of these animals with people (Radhouani *et al.*, 2010b).

The common buzzards included in this study were localized in different natural areas in the north and the centre of Portugal, where they make their nests. They are large predatory birds at the top of the food chain. This fact could be one answer to the high rates of antimicrobial resistance found in *E. coli* and enterococci isolates from these birds. The data presented here suggest that wild birds are common carriers of multi-resistant faecal bacteria, and are thus probably involved in the transmission of antimicrobial resistance into the environment. In particular, common buzzards seem to represent an important reservoir, or at least a source, of multi-resistant *E. coli* and enterococci isolates, and consequently may represent a considerable hazard to human and animal health by transmission of these isolates to waterways and other environmental sources via their faecal deposits. Most obviously, *E. coli* and enterococci of common buzzards seem to reveal the same resistance patterns as isolates isolated from other animals, thus highlighting the need for thorough future epidemiological studies to gain a more detailed understanding of the transmission mode of resistant bacteria to wild birds and back into the environment.

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