



Antimicrobial resistance profiles of from common European wild bird species

Sebastian Guenther, Mirjam Grobbel, Antina Lübke-Becker, Andreas Goedecke, Nicole D. Friedrich, Lothar H. Wieler, Christa Ewers

► To cite this version:

Sebastian Guenther, Mirjam Grobbel, Antina Lübke-Becker, Andreas Goedecke, Nicole D. Friedrich, et al.. Antimicrobial resistance profiles of from common European wild bird species. *Veterinary Microbiology*, 2010, 144 (1-2), pp.219. 10.1016/j.vetmic.2009.12.016 . hal-00597833

HAL Id: hal-00597833

<https://hal.science/hal-00597833>

Submitted on 2 Jun 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Antimicrobial resistance profiles of *E. coli* from common European wild bird species

Authors: Sebastian Guenther, Mirjam Grobbel, Antina Lübke-Becker, Andreas Goedecke, Nicole D. Friedrich, Lothar H. Wieler, Christa Ewers



PII: S0378-1135(09)00607-5
DOI: doi:10.1016/j.vetmic.2009.12.016
Reference: VETMIC 4713

To appear in: *VETMIC*

Received date: 12-8-2009
Revised date: 7-12-2009
Accepted date: 9-12-2009

Please cite this article as: Guenther, S., Grobbel, M., Lübke-Becker, A., Goedecke, A., Friedrich, N.D., Wieler, L.H., Ewers, C., Antimicrobial resistance profiles of *E. coli* from common European wild bird species, *Veterinary Microbiology* (2008), doi:10.1016/j.vetmic.2009.12.016

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

SHORT COMMUNICATION**TITLE**

Antimicrobial resistance profiles of *E. coli* from common European wild bird species

AUTHORS

Sebastian Guenther¹, Mirjam Grobbel¹, Antina Lübke-Becker¹, Andreas Goedecke², Nicole D. Friedrich³, Lothar H. Wieler¹ and Christa Ewers¹

ADRESSES

¹ Institut für Mikrobiologie und Tierseuchen, Fachbereich für Veterinärmedizin, Freie Universität Berlin, Philippstrasse 13, 10115 Berlin Germany

² ProRing e.V. Germany

³ Fachbereich Veterinärmedizin, Justus von Liebig Universität Gießen, Frankfurter Str. 94 35392 Gießen Germany

***CORRESPONDING AUTHOR**

Dr. Sebastian Guenther

Mailing adress: Institut für Mikrobiologie und Tierseuchen
Philippstrasse 13, Berlin, D-10115, Germany

Phone: 0049-30-2093 6028

Fax: 0049-30-2093 6067

E-mail: guenther.sebastian@vetmed.fu-berlin.de

KEY WORDS

Antimicrobial resistance, MIC, wild birds

ABSTRACT

The emergence and spread of multiresistant bacteria in natural environments constitutes a serious impact on animal and human health. To gain more insight into the role of wild birds as carriers and reservoir of multiresistant *E. coli* we tested a broad spectrum of common European bird species for the occurrence of *E. coli* strains and their antimicrobial resistance by minimal inhibitory concentration testing and PCR analysis of several resistance genes. Nine of the 187 *E. coli* isolates (4.8%) exhibited multiresistant phenotypes including resistances against beta-lactams, aminoglycosides, fluoroquinolones, tetracyclines and sulfonamides. By comparing avian *E. coli* resistance frequencies with frequencies known for *E. coli* isolated from livestock and companion animals analogous profiles were identified. Multiresistant *E. coli* strains were isolated from synanthropic avian species as well as from birds of prey, waterfowl and passerines. By that, all these avian hosts are suggested to represent a considerable reservoir of resistant *E. coli* strains. Consequently wild birds might constitute a potential hazard to human and animal health by transmitting multiresistant strains to waterways and other environmental sources via their faecal deposits.

1. Introduction

The emergence of multiresistant bacteria of human and veterinary origin (Grobbel et al., 2007) is inevitably accompanied by co-contamination of the environment presumably leading to a great health concern (Martinez, 2009). In addition to the currently frequent detection of multiresistant bacteria in areas with high human density (Cole et al., 2005), their emergence in more remote areas like high mountain regions or the arctic is even more alarming (Caprioli et al., 1991; Sjölund et al., 2008). Although wild birds have only rare contact with antimicrobial agents, arguing against the existence of direct selective pressure on birds can nevertheless be infected or colonized by resistant bacteria. Water contact and acquisition via food seem to be major aspects of transmission of resistant bacteria of human or veterinary origin to wild animals (Cole et al., 2005; Kozak et al., 2009). Wild birds or wild animals in general could therefore serve as reservoirs of resistant bacteria and genetic determinants of antimicrobial resistance (Dolejska et al., 2007).

As knowledge on the distribution of resistant *E. coli* in wild birds is limited to certain species only (Cole et al., 2005; Dolejska et al., 2007) we performed a pilot study to verify the occurrence of antimicrobial resistant *E. coli* in a broad spectrum of European wild bird species with a focus on the detection of multiresistant strains. The study included samples from passerines, waterfowl and birds of prey (Tab.1) from two areas of Germany, one rural (Eichsfeld region, Northern Thuringia, approx. 100 inhabitants/km², predominantly villages) and one medium-sized urban agglomeration area (conurbation of Gießen, Middle Hesse, approx. 300 inhabitants/km²). Results were compared with those published for livestock and companion animals in order to verify whether wild birds reflect the resistance patterns observed for domesticated animals. The major aim of our study was to get more detailed knowledge on possible avian reservoirs of multiresistant *E. coli* strains in the environment which could constitute a potential hazard to human and animal health by transmission of multiresistant strains to waterways and environmental sources via faecal deposits.

2. Methods

Avian species were tested for faecal *E. coli* via cloacal swabs taken while ringing the birds in the rural Eichsfeld region from January to August 2008. Furthermore cloacal swabs and in case of dissection of animals organ samples were taken during entrance evaluation in a rescue station for injured wild birds in a veterinary hospital in the conurbation of Gießen from August to September 2006.

Overall 275 samples from 226 birds were taken resulting in a total of 201 putative *E. coli* isolates cultured from the faeces (n=160), heart (n=6), liver (n=9), lung (n=13), spleen (n=6) and kidney (n=7). On the avian host species level we isolated *E. coli* from 40 of 55 avian species tested. From each faecal swab only one colony was included in further processing. Multiple organ samples were taken if birds were dissected (n=22) and also one colony per sample was further investigated. After exclusion of multiple isolates from the same clone via RAPD-PCR (P1: 5'- GCGATCCCCA-3', P2: 5'- GTGGATGCGA-3'), especially in case of processing multiple organ samples from one bird, 188 unique *E. coli* isolates were further processed. Several bird species were represented by larger numbers of animals tested, other species were only sampled once due to the frequency of appearance while ringing/entrance evaluation (Tab.1). Bacterial species determination was performed using CHROMagar orientation (Chromagar, Paris, France). Colonies with red or white colour were further characterised by classical biochemical methods (Winkle, 1979). Table 1 summarizes the distribution of *E. coli* isolates among the different avian host species.

Preselection of multiresistant *E. coli* isolates was performed on freshly prepared Mueller-Hinton-agarose plates containing estimated breakpoint concentrations of six different antimicrobial substances (ampicillin (≥ 32 µg/ml), streptomycin (≥ 64 µg/ml), spectinomycin (≥ 128 µg/ml), chloramphenicol (≥ 32 µg/ml), gentamicin (≥ 16 µg/ml) and tetracycline (≥ 16 µg/ml) to determine their phenotypic resistance pattern. Overall 17.1 % (n=32) of the 187

isolates showed a phenotypic resistance to at least one of the above mentioned antimicrobial substances. Multiresistant isolates were further selected by processing only those which showed phenotypical resistance against three or more antimicrobial substances as determined (n=15).

Six isolates (3.3%) with positive confirmatory test for the production of extended spectrum beta lactamases were included in an additional study (CLSI, 2008). Antimicrobial susceptibility testing of the preselected isolates against 30 antimicrobials was performed via broth microdilution method (Micronaut breakpoint plates, Genzyme Diagnostics, Rüsselsheim Germany) according to the standards given by the Clinical and Laboratory Standards Institute (CLSI, 2008). The presence of genes encoding resistances against tetracycline (*tet(A)*, *tet(B)*, *tet(C)*), sulfonamides (*sul1*, *sul2*, *sul3*), streptomycin-spectinomycin (*aadA*, *strA*, *strB*), and apramycin-gentamicin (*aac(3)-IV*) was detected by PCR as previously published by Kozak et al. (2009). Phylogenetic grouping of *E. coli* strains was performed by a triplex PCR (Clermont et al., 2000).

3. Results and Discussion

MIC testing revealed that nine of the preselected fifteen *E. coli* isolates showed multiresistant phenotypic profiles (Tab. 2). We detected high rates of resistance to ampicillin (9/15), amoxicillin (9/15), ticarcillin (9/15), cephalothin (7/15), streptomycin (7/15), sulfadimethoxine (12/15) and tetracycline (7/15). Resistance for cefazolin (3/15), ceftiofur (2/15), gentamicin (2/15), neomycin (5/15), chloramphenicol (4/15) and fluoroquinolones (2/15) was less often observed. For six of the preselected isolates (IMT12313, IMT16287, IMT17789, IMT17794, IMT16295, and IMT16304) with phenotypic resistance in the agar with antibiotics prescreening results were not confirmed in MIC testing. In most of the cases the inhibitory concentration of the respective compounds was near but not above the breakpoint. This discrepancy is explicable by the well known lack of accuracy and sensitivity

of the method making use of agar containing antibiotics which predominantly serves as a qualitative, not a quantitative screening test. As such, it is well accepted and an often applied and suitable pre-screening method. The most abundant pattern observed was combined resistance to ampicillin, tetracycline, sulfamethoxazole and streptomycin. Strains with highest numbers of resistances were isolated from the following avian hosts: Pigeons (IMT16378, IMT16296, both from Hesse), Mute Swan (IMT12317, Hesse), White-throated Dipper (IMT17784, Eichsfeld region), Buzzard (IMT16369, IMT16305, and IMT12313, all from Hesse), Grey Heron (IMT16376, Hesse) and Garden Warbler (IMT17799, Eichsfeld region). There was no significant difference in the isolation frequency of multiresistant strains between rural or urban areas (4.4 % rural Eichsfeld region, 5.0 %, urban area of Gießen). PCR based analysis of genetic determinants of resistance confirmed the phenotypic results as shown in Tab. 2.

While Cole et al. (2005), detected high rates of resistance to ampicillin, streptomycin, cephalothin and sulfathiazole in *E. coli* isolated from migratory Canadian geese, which is comparable to our results, antimicrobial susceptibility data for wild birds from Central Europe are limited to Black-headed Gulls from the Czech Republic. Dolejska et al. (2007) found 29 % of the *E. coli* isolates investigated to show a resistance against at least one antimicrobial agent with comparable resistance patterns to those of the present study. Interestingly our data indicates that not only synanthropic species, such as pigeons, which have frequent contact to humans, also bird species living in more rural areas like waterfowl and birds of prey, seem to play a role as carriers of multiresistant strains, as isolates from both groups were present among the multiresistant samples. Taking a closer look at the 40 species examined, there were six birds of prey (15 %), nine waterfowl and avian species with frequent water contact (22.5 %) and 25 passerines (62.5 %). Among the nine multiresistant strains from avian hosts the first two groups mentioned were highly prevalent and especially birds of prey constitute one of the major host groups of resistant strains (n=3, all buzzards). It is well known that avian

predators can acquire resistant microorganisms via their prey (Marrow et al., 2009). In this context the recently shown spread of resistant bacteria from livestock to small wild animals in close proximity to farms (Kozak et al., 2009) has to be kept in mind as possible explanation for the occurrence of resistant bacteria in birds of prey.

Besides waterfowl (n=2, Mute Swan and Grey Heron) and synanthropic species (n=2, both pigeons) also passerines from rural areas (Garden Warbler, White-throated Dipper) were present among the hosts of resistant bacteria. The involvement of waterfowl species is not surprising as species of this group have already been described as carriers of resistant bacteria which might most likely be due to the uptake of such microorganisms via contaminated water (Cole et al., 2005; Dolejska et al., 2007).

In case of the Garden Warbler one could speculate on an acquisition via the insectivorous lifestyle habit of the bird. Indeed, an appearance of resistant bacteria on insects has already been described for cockroaches (Pai et al., 2005). Transmission to the White-throated Dipper may be due to the aqueous lifestyle of this species, similar to the situation in classical waterfowl. Taken together, for all bird species examined, dissemination via ingestion could be supposed, requiring further analysis in the future.

A comparative view on the resistance patterns of the *E. coli* isolates described in our study with those of *E. coli* isolates originating from livestock animals in Germany and Europe reveals an obvious correlation. Guerra et al. (2003) found that the most common antimicrobial resistances observed among *E. coli* from swine, poultry and cattle were against tetracycline, ampicillin, aminoglycosides and sulfamethoxazole (Guerra et al., 2003). In line with these results Grobbel et al. (2007) observed high rates of antimicrobial resistance among *E. coli* isolates from swine, horses, dogs and cats against tetracycline, ampicillin and sulfamethoxazole, while the resistance rates against aminoglycosides were lower. In case of the Netherlands and Sweden Van den Bogaard et al. found high rates of resistance to tetracyclines, aminopenicillins and aminoglycosides in *E. coli* from pig farms (Van den

Bogaard et al., 2000). Furthermore antimicrobial resistance data obtained for *E. coli* isolates of canine and feline companion animals from Portugal showed correlations with our data as high rates of resistance were found for tetracycline, ampicillin, sulfamethoxazole and the aminoglycoside gentamicin in these animals, substantiating that resistances against these agents are the most abundant worldwide (Costa et al., 2008).

Phylogenetic typing revealed an affiliation of a high proportion of multiresistant strains to group B2 (7/15) while the remaining strains were distributed among phylogenetic groups A (n=4), B1 (n=1), and D (n=3) (Tab. 2). Taking into account all isolates analyzed in this study (antimicrobial resistant or susceptible) B2 strains were detected less often than all other phylogenetic groups (B2: 15.3 %; A: 31.9 %; B1: 25.2 %; D:27.6 %). It has been previously assumed that the B2 group to a large extent consists of highly virulent extraintestinal pathogenic strains, be it commensal or clinical isolates (Le Gall et al., 2007). Our findings stand in contrast to previous studies, where B2 strains have been described as “low resistance strains” in contrast to group A, B1, and D strains, which are more often found to bear resistances (Bukh et al., 2009). The emergence of potentially highly virulent strains in combination with a multiresistant phenotype is alarming as a possible consequence would be a severe clinical outcome concomitant with serious limitations in antimicrobial therapy.

The data presented here suggest that birds are frequent carriers of multiresistant faecal bacteria, thus likely being involved in the transmission of antimicrobial resistance into the environment. In particular birds of prey, waterfowl and passerines seem to represent a notable reservoir or at least source of multiresistant *E. coli* strains, and therefore may constitute a considerable hazard to human and animal health by transmission of these strains to waterways and other environmental sources via their faecal deposits. Most noticeably, *E. coli* of wild birds seem to reveal the same resistance patterns as strains isolated from domestic animals, thus highlighting the need for thorough future epidemiological studies to gain a more detailed

202 understanding of the transmission mode of resistant bacteria to wild birds and back into the
203 environment.

204

205 Acknowledgements

206 We would like to thank I. Diel and B. Lauzat for their excellent technical assistance.

207 Funding

208 This work was supported by grants from the Federal Ministry of Education and Research

209 Network Zoonosis (FBI-Zoo, Grant no. 01KI07120).

210

- 211 Bukh, A.S., Schonheyder, H.C., Emmersen, J.M., Sogaard, M., Bastholm, S., Roslev, P., 2009, *Escherichia coli*
 212 phylogenetic groups are associated with site of infection and level of antibiotic resistance in
 213 community-acquired bacteraemia: a 10 year population-based study in Denmark. J. Antimicrob.
 214 Chemother. 64, 163-168.
- 215 Caprioli, A., Donelli, G., Falbo, V., Passi, C., Pagano, A., Mantovani, A., 1991, Antimicrobial resistance and
 216 production of toxins in *Escherichia coli* strains from wild ruminants and the alpine marmot. J. Wildl.
 217 Dis. 27, 324-327.
- 218 Clermont, O., Bonacorsi, S., Bingen, E., 2000, Rapid and simple determination of the *Escherichia coli*
 219 phylogenetic group. Appl. Environ. Microbiol. 66, 4555-4558.
- 220 CLSI 2008. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated
 221 from animals; approved standard - Third Edition. In CLSI document M31-A3 (Wayne, PA, U.S.A.,
 222 CLSI).
- 223 Cole, D., Drum, D.J., Stalknecht, D.E., White, D.G., Lee, M.D., Ayers, S., Sobsey, M., Maurer, J.J., 2005, Free-
 224 living Canada geese and antimicrobial resistance. Emerg. Infect. Dis. 11, 935-938.
- 225 Costa, D., Poeta, P., Saenz, Y., Coelho, A.C., Matos, M., Vinue, L., Rodrigues, J., Torres, C., 2008, Prevalence
 226 of antimicrobial resistance and resistance genes in faecal *Escherichia coli* isolates recovered from
 227 healthy pets. Vet. Microbiol. 127, 97-105.
- 228 Dolejska, M., Cizek, A., Literak, I., 2007, High prevalence of antimicrobial-resistant genes and integrons in
 229 *Escherichia coli* isolates from Black-headed Gulls in the Czech Republic. J. Appl. Microbiol. 103, 11-
 230 19.
- 231 Grobbel, M., Lübke-Becker, A., Alesik, E., Schwarz, S., Wallmann, J., Werckenthin, C., Wieler, L.H., 2007,
 232 Antimicrobial susceptibility of *Escherichia coli* from swine, horses, dogs and cats as determined in the
 233 BfT-GermVet monitoring program 2004-2006. Berl. Munch. Tierarztl. Wochenschr. 120, 391-401.
- 234 Guerra, B., Junker, E., Schroeter, A., Malorny, B., Lehmann, S., Helmuth, R., 2003, Phenotypic and genotypic
 235 characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and
 236 poultry. J. Antimicrob. Chemother. 52, 489-492.
- 237 Kozak, G.K., Boerlin, P., Janecko, N., Reid-Smith, R.J., Jardine, C., 2009, Antimicrobial resistance in
 238 *Escherichia coli* isolates from swine and wild small mammals in the proximity of swine farms and in
 239 natural environments in Ontario, Canada. Appl. Environ. Microbiol. 75, 559-566.
- 240 Le Gall, T., Clermont, O., Gouriou, S., Picard, B., Nassif, X., Denamur, E., Tenaillon, O., 2007, Extraintestinal
 241 virulence is a coincidental by-product of commensalism in B2 phylogenetic group *Escherichia coli*
 242 strains. Mol. Biol. Evol. 24, 2373-2384.
- 243 Marrow, J., Whittington, J.K., Mitchell, M., Hoyer, L.L., Maddox, C., 2009, Prevalence and antibiotic-resistance
 244 characteristics of *Enterococcus* spp. Isolated from free-living and captive raptors in central Illinois. J.
 245 Wildl. Dis. 45, 302-313.
- 246 Martinez, J.L., 2009, The role of natural environments in the evolution of resistance traits in pathogenic bacteria.
 247 Proc. Biol. Sci. 276, 2521-2530.
- 248 Pai, H.H., Chen, W.C., Peng, C.F., 2005, Isolation of bacteria with antibiotic resistance from household
 249 cockroaches (*Periplaneta americana* and *Blattella germanica*). Acta. Trop. 93, 259-265.
- 250 Sjölund, M., Bonnedahl, J., Hernandez, J., Bengtsson, S., Cederbrant, G., Pinhassi, J., Kahlmeter, G., Olsen, B.,
 251 2008, Dissemination of multidrug-resistant bacteria into the Arctic. Emerg. Infect. Dis. 14, 70-72.
- 252 Van den Bogaard, A.E., London, N., Stobberingh, E.E., 2000, Antimicrobial resistance in pig faecal samples
 253 from The Netherlands (five abattoirs) and Sweden. J. Antimicrob. Chemother. 45, 663-671.
- 254 Winkle, S., 1979, Mikrobiologische und serologische Diagnostik. Jena: VEB Gustav Fischer Verlag.

255
256

Table 1

Occurrence and distribution of *E. coli* among avian host species and results of pre-screening for antimicrobial resistances with agar containing antibiotics (TH Thuringia, H Hesse, AMP ampicillin, C chloramphenicol, GN gentamicin, SP spectinomycin, S streptomycin, TE tetracycline),

Avian host species	No. and origin (TH/H) of birds sampled	No. of <i>E. coli</i> isolated	No. of <i>E. coli</i> after exclusion of double isolates	No. of phenotypic resistant isolates, (resistance profiles), type of sample
Barn Owl (<i>Tyto alba</i>)	6/3	9	9	1 (AMP,SP,GN), faecal
Blackcap (<i>Sylvia atricapilla</i>)	14/0	1	1	-
Black-headed Gull (<i>Chroicocephalus ridibundus</i>)	2/0	1	1	-
Blue Tit (<i>Cyanistes caeruleus</i>)	4/0	1	1	-
Brambling (<i>Fringilla montifringilla</i>)	2/0	1	1	-
Chaffinch (<i>Fringilla coelebs</i>)	5/0	-	-	-
Common Buzzard (<i>Buteo buteo</i>)	0/58	58	55	1 (AMP, S, TE), faecal 1 (AMP, GN, SP), faecal 1 (AMP, GN, S, TE), faecal 1 (SP, S), faecal 3 (S), 2 faecal, 1 lung 1 (SP), faecal 2 (S, TE), faecal 2 (TE), faecal
Chiffchaff (<i>Phylloscopus collybita</i>)	1/0	-	-	-
Common Kestrel (<i>Falco tinnunculus</i>)	7/5	6	6	1 (AMP, SP, TE), faecal
Common Kingfisher (<i>Alcedo atthis</i>)	1/0	-	-	-
Common Redstart (<i>Phoenicurus phoenicurus</i>)	1/0	-	-	-
Common Swift (<i>Apus apus</i>)	1/0	-	-	-
Common Treecreeper (<i>Certhia familiaris</i>)	1/0	-	-	-
Dunnock (<i>Prunella modularis</i>)	8/0	3	3	-
Egyptian Goose (<i>Alopochen aegyptiacus</i>)	0/1	-	1	-
Eurasian Blackbird (<i>Turdus merula</i>)	8/20	28	20	1 (AMP, SP, S), faecal 1 (AMP, SP), faecal
Eurasian Collared Dove (<i>Streptopelia decaocto</i>)	0/2	2	2	-
Eurasian Coot (<i>Fulica atra</i>)	0/2	-	2	-
Eurasian Jay (<i>Garrulus glandarius</i>)	4/1	4	3	-
Eurasian Nuthatch (<i>Sitta europaea</i>)	1/0	1	1	-
Eurasian Siskin (<i>Carduelis spinus</i>)	6/0	-	-	-

Avian host species	No. and origin (TH/H) of birds sampled	No. of <i>E. coli</i> isolated	No. of <i>E. coli</i> after exclusion of double isolates	No. of phenotypic resistant isolates, (resistance profiles), type of sample
Eurasian Sparrowhawk (<i>Accipiter nisus</i>)	0/3	3	3	-
European Robin (<i>Erithacus rubecula</i>)	5/0	4	4	-
European Starling (<i>Sturnus vulgaris</i>)	0/5	5	5	3 (TE), faecal
Firecrest (<i>Regulus ignicapillus</i>)	3/0	-	-	-
Rock Pigeon (<i>Columba livia</i>)	0/18	18	18	1 (AMP, SP, S), faecal 2 (AMP, C, GN, SP, S), faecal 1 (TE), faecal 1 (AMP, S), faecal
Garden Warbler (<i>Sylvia borin</i>)	3/0	2	2	1 (AMP, SP, S, TE), faecal
Goshawk (<i>Accipiter gentilis</i>)	0/4	4	4	1 (SP, S, TE), lung
Grasshopper Warbler (<i>Locustella naevia</i>)	1/0	-	-	-
Great Cormorant (<i>Phalacrocorax carbo</i>)	0/2	2	2	-
Great Spotted Woodpecker (<i>Dendrocopos major</i>)	1/2	3	3	-
Great Tit (<i>Parus major</i>)	1/0	1	1	-
Greater White-fronted Goose (<i>Anser albifrons</i>)	0/2	2	1	-
Green Woodpecker (<i>Picus viridis</i>)	0/2	2	2	-
Greenfinch (<i>Carduelis chloris</i>)	1/0	-	-	-
Grey Heron (<i>Ardea cinerea</i>)	0/4	4	2	1 (AMP, SP, S, TE), faecal
Grey Wagtail (<i>Motacilla cinerea</i>)	1/0	-	-	-
Jackdaw (<i>Corvus monedula</i>)	3/0	1	1	-
Long-eared Owl (<i>Asio otus</i>)	0/1	1	1	-
Long-tailed Tit (<i>Aegithalos caudatus</i>)	1/0	-	-	-
Mallard duck (<i>Anas platyrhynchos</i>)	0/3	3	3	1 (SP), faecal
Marsh Warbler (<i>Acrocephalus palustris</i>)	5/0	2	2	-
Mute Swan (<i>Cygnus olor</i>)	0/1	1	1	1 (AMP, C, S, TE), faecal
Nightingale (<i>Luscinia megarhynchos</i>)	1/0	1	1	-
Reed Bunting (<i>Emberiza schoeniclus</i>)	1/0	1	1	-
Reed Warbler (<i>Acrocephalus scirpaceus</i>)	3/0	1	1	-
Rook (<i>C. frugilegus frugilegus</i>)	0/14	14	14	1 (SP), faecal
Serlin (<i>Serinus serinus</i>)	3/0	-	-	-
Song Thrush (<i>Turdus philomelos</i>)	3/0	1	1	-

Avian host species	No. and origin (TH/H) of birds sampled	No. of <i>E. coli</i> isolated	No. of <i>E. coli</i> after exclusion of double isolates	No. of phenotypic resistant isolates, (resistance profiles), type of sample
Tree Sparrow (<i>Passer montanus</i>)	1/7	6	6	-
Whitethroat (<i>Sylvia communis</i>)	3/0	1	1	-
White-throated Dipper (<i>Cinclus cinclus</i>)	2/0	2	2	1 (AMP, SP, S, TE), faecal 1 (SP, S), faecal
Willow Tit (<i>Poecile montanus</i>)	1/0	-	-	-
Winter Wren (<i>Troglodytes troglodytes</i>)	1/0	-	-	-
Yellowhammer (<i>Emberiza citrinella</i>)	1/0	1	1	-
Total number	277	201	188	32

262

263

264

265

266

Table 2: Results of minimal inhibitory testing of fifteen, via disk diffusion method preselected *E. coli* isolates (Micronaut breakpoint plates, Genzyme Diagnostics, Rüsselsheim, Germany). Grey shaded boxes indicate multiresistant isolates.

			Beta-Lactams										Aminoglycosides					
Strain number	Avian host	Phylogenetic group	Amoxicillin	Ampicillin	Ticarcillin	Amoxicillin/clavulanic acid	Ticarcillin/clavulanic acid	Ceftiofur	Cefazolin	Cefoxitin	Cefpodoxime	Cephalothin	Imipenem	Amikacin	Gentamicin	Spectinomycin	Streptomycin	Neomycin
IMT12313	Buzzard	D	>16	>16	>64	≤4/2	16/2	0,5	≤8	≤2	≤2	8	≤1	≤4	1	16	256	≤2
IMT12317	Mute Swan	B2	>16	>16	>64	8/4	16/2	0,5	≤8	4	≤2	>16	≤1	≤4	1	16	512	>32
IMT16287	Blackbird	B2	2	2	≤8	≤4/2	≤8/2	0,5	≤8	4	≤2	16	≤1	≤4	1	16	≤8	≤2
IMT16295	Barn Owl	A	4	4	≤8	≤4/2	≤8/2	0,5	≤8	8	≤2	>16	≤1	≤4	≤1	16	≤8	≤2
IMT16296	Pigeon	D	>16	>16	>64	32/16	16/2	1	>16	>16	≤2	>16	≤1	≤4	>8	>64	512	>32
IMT16304	Goshawk	B2	4	4	≤8	≤4/2	≤8/2	0,5	≤8	8	≤2	16	≤1	≤4	≤1	16	≤8	≤2
IMT16305	Buzzard	B2	>16	>16	>64	8/4	16/2	0,5	≤8	≤2	≤2	16	≤1	≤4	2	16	≤8	>32
IMT16369	Buzzard	B2	>16	>16	>64	8/4	16/2	0,5	≤8	4	≤2	8	≤1	≤4	1	16	512	>32
IMT16376	Grey Heron	B1	>16	>16	>64	8/4	16/2	1	≤8	4	≤2	>16	≤1	≤4	1	>64	64	≤2
IMT16378	Pigeon	A	>16	>16	>64	32/16	16/2	2	>16	>16	≤2	>16	≤1	≤4	>8	>64	1024	>32
IMT16400	Pigeon	D	8	4	≤8	≤4/2	≤8/2	0,5	≤8	4	≤2	16	≤1	≤4	≤0,5	16	≤8	≤2
IMT17784	White-throated Dipper	B2	>16	>16	>64	8/4	16/2	0,5	≤8	4	≤2	>16	≤1	≤4	1	16	1024	≤2
IMT17789	Chaffinch	A	4	2	≤8	≤4/2	≤8/2	0,5	≤8	4	≤2	8	≤1	≤4	1	64	≤8	≤2
IMT17794	Common Kestrel	B2	4	4	≤8	≤4/2	16/2	0,5	>16	4	≤2	16	≤1	8	2	16	≤8	≤2
IMT17799	Garden Warbler	A	>16	>16	>64	8/4	16/2	0,5	≤8	4	≤2	>16	≤1	≤4	≤1	16	1024	≤2

269
270
271

272 Table 2 continued

Strain number	Avian host	Fluoroquinolones			Sulphonamides			Phenicoles		Tetracyclines		Macrolides		Others		Resistance genes
		Enrofloxacin	Marbofloxacin	Orbifloxacin	Sulphadimethoxine	Sulphathiazole	Thrimethoprim/ Sulfamethoxazole	Florfenicol	Chloramphenicol	Tetracyclin	Oxytetracycline	Erythromycin	Tylosin tartrate	Novobiocin	Rifampin	
IMT12313	Buzzard	0,5	≤0,25	2	>256	>256	>2/38	4	8	>8	>8	>4	>20	>4	>2	<i>tetA/tetB/strA/sul2</i>
IMT12317	Mute Swan	≤0,12	≤0,25	≤1	>256	>256	≤0,5/9,5	4	>16	>8	>8	>4	>20	>4	>2	<i>tetA/tetB/strA/sul2</i>
IMT16287	Blackbird	≤0,12	≤0,25	≤1	>256	64	≤0,5/9,5	4	8	1	1	>4	>20	>4	>2	<i>tetA/sul2/strA/strB</i>
IMT16295	Barn Owl	≤0,5	≤0,25	≤1	128	≤32	≤0,5/9,5	8	8	≤2	1	>4	>20	>4	>2	<i>tetA/tetB/strA</i>
IMT16296	Pigeon	>4	>2	>4	>256	>256	1/19	>8	>16	4	1	>4	>20	>4	>2	<i>tetA/tetB</i>
IMT16304	Goshawk	≤0,5	0,5	2	>256	128	≤0,5/9,5	4	≤4	>8	>8	>4	>20	>4	>2	<i>tetA/tetB/sul2/strA</i>
IMT16305	Buzzard	≤0,12	≤0,25	≤1	128	64	≤0,5/9,5	2	8	1	1	>4	>20	>4	>2	<i>tetA/tetB/strA</i>
IMT16369	Buzzard	≤0,12	≤0,25	≤1	>256	>256	>2/38	4	≤4	>8	>8	>4	>20	>4	>2	<i>tetA/tetB/strA/sul2</i>
IMT16376	Grey Heron	≤0,12	≤0,25	≤1	>256	64	1/19	8	8	>8	>8	>4	>20	>4	>2	<i>tetA/tetB/sul2/strA/aadA2</i>
IMT16378	Pigeon	>4	>2	>4	>256	>256	1/19	>8	>16	2	2	>4	>20	>4	>2	<i>tetA/tetB/strA/sul2</i>
IMT16400	Pigeon	≤0,12	≤0,25	≤1	128	64	≤0,5/9,5	2	≤4	1	0,5	>4	>20	>4	>2	<i>tetA/tetB/sul2/strA</i>
IMT17784	White-throated Dipper	0,5	≤0,25	≤1	>256	>256	≤0,5/9,5	4	>16	>8	>8	>4	>20	>4	>2	<i>tetA/tetB/sul2/strA</i>
IMT17789	Chaffinch	≤0,12	≤0,25	≤1	>256	≤32	≤0,5/9,5	8	8	1	1	>4	>20	>4	>2	<i>tetA/tetB/sul2/strA/strB/aadA2</i>
IMT17794	Common Kestrel	≤0,12	≤0,25	≤1	>256	64	≤0,5/9,5	4	8	1	1	>4	>20	>4	>2	<i>tetA/tetB/sul2</i>
IMT17799	Garden Warbler	≤0,5	≤0,25	≤1	>256	>256	>2/38	8	8	>8	>8	>4	>20	>4	>2	<i>tetA/tetB/sul2/strA/strB</i>

273
274

275 IMT = Institut für Mikrobiologie und Tierseuchen (Institute of Microbiology and Epizootics)