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1 **SHORT COMMUNICATION**2 **TITLE**3 **Antimicrobial resistance profiles of *E. coli* from common European wild bird species**

4

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23 **KEY WORDS**

24 Antimicrobial resistance, MIC, wild birds

25

26 ABSTRACT

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

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46 **1. Introduction**

47 The emergence of multiresistant bacteria of human and veterinary origin (Grobbel et al.,
48 2007) is inevitably accompanied by co-contamination of the environment presumably leading
49 to a great health concern (Martinez, 2009). In addition to the currently frequent detection of
50 multiresistant bacteria in areas with high human density (Cole et al., 2005), their emergence in
51 more remote areas like high mountain regions or the arctic is even more alarming (Caprioli et
52 al., 1991; Sjölund et al., 2008). Although wild birds have only rare contact with antimicrobial
53 agents, arguing against the existence of direct selective pressure on birds can nevertheless be
54 infected or colonized by resistant bacteria. Water contact and acquisition via food seem to be
55 major aspects of transmission of resistant bacteria of human or veterinary origin to wild
56 animals (Cole et al., 2005; Kozak et al., 2009). Wild birds or wild animals in general could
57 therefore serve as reservoirs of resistant bacteria and genetic determinants of antimicrobial
58 resistance (Dolejska et al., 2007).

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

72

73 **2. Methods**

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

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

93 Preselection of multiresistant *E. coli* isolates was performed on freshly prepared Mueller-
94 Hinton-agarose plates containing estimated breakpoint concentrations of six different
95 antimicrobial substances (ampicillin (≥ 32 $\mu\text{g/ml}$), streptomycin (≥ 64 $\mu\text{g/ml}$), spectinomycin
96 (≥ 128 $\mu\text{g/ml}$), chloramphenicol (≥ 32 $\mu\text{g/ml}$), gentamicin (≥ 16 $\mu\text{g/ml}$) and tetracycline (≥ 16
97 $\mu\text{g/ml}$) to determine their phenotypic resistance pattern. Overall 17.1 % (n=32) of the 187

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

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

112

113 3. Results and Discussion

114 MIC testing revealed that nine of the preselected fifteen *E. coli* isolates showed multiresistant
115 phenotypic profiles (Tab. 2). We detected high rates of resistance to ampicillin (9/15),
116 amoxicillin (9/15), ticarcillin (9/15), cephalothin (7/15), streptomycin (7/15),
117 sulfadimethoxine (12/15) and tetracycline (7/15). Resistance for cefazolin (3/15), cefoxitin
118 (2/15), gentamicin (2/15), neomycin (5/15), chloramphenicol (4/15) and fluoroquinolones
119 (2/15) was less often observed. For six of the preselected isolates (IMT12313, IMT16287,
120 IMT17789, IMT17794, IMT16295, and IMT16304) with phenotypic resistance in the agar
121 with antibiotics prescreening results were not confirmed in MIC testing. In most of the cases
122 the inhibitory concentration of the respective compounds was near but not above the
123 breakpoint. This discrepancy is explicable by the well known lack of accuracy and sensitivity

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

136 While Cole et al. (2005), detected high rates of resistance to ampicillin, streptomycin,
137 cephalothin and sulfathiazole in *E. coli* isolated from migratory Canadian geese, which is
138 comparable to our results, antimicrobial susceptibility data for wild birds from Central Europe
139 are limited to Black-headed Gulls from the Czech Republic. Dolejska et al. (2007) found 29
140 % of the *E. coli* isolates investigated to show a resistance against at least one antimicrobial
141 agent with comparable resistance patterns to those of the present study. Interestingly our data
142 indicates that not only synanthropic species, such as pigeons, which have frequent contact to
143 humans, also bird species living in more rural areas like waterfowl and birds of prey, seem to
144 play a role as carriers of multiresistant strains, as isolates from both groups were present
145 among the multiresistant samples. Taking a closer look at the 40 species examined, there were
146 six birds of prey (15 %), nine waterfowl and avian species with frequent water contact (22.5
147 %) and 25 passerines (62.5 %). Among the nine multiresistant strains from avian hosts the
148 first two groups mentioned were highly prevalent and especially birds of prey constitute one
149 of the major host groups of resistant strains (n=3, all buzzards). It is well known that avian

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

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

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

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

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

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

193
194 The data presented here suggest that birds are frequent carriers of multiresistant fecal
195 bacteria, thus likely being involved in the transmission of antimicrobial resistance into the
196 environment. In particular birds of prey, waterfowl and passerines seem to represent a notable
197 reservoir or at least source of multiresistant *E. coli* strains, and therefore may constitute a
198 considerable hazard to human and animal health by transmission of these strains to waterways
199 and other environmental sources via their faecal deposits. Most noticeably, *E. coli* of wild
200 birds seem to reveal the same resistance patterns as strains isolated from domestic animals,
201 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

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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

257 Table 1

258 Occurrence and distribution of *E. coli* among avian host species and results of pre-screening
 259 for antimicrobial resistances with agar containing antibiotics (TH Thuringia, H Hesse, AMP
 260 ampicillin, C chloramphenicol, GN gentamicin, SP spectinomycin, S streptomycin, TE
 261 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
Serin (<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

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

Strain number	Avian host	Phylogenetic group	Beta-Lactams										Aminoglycosides					
			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

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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>

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