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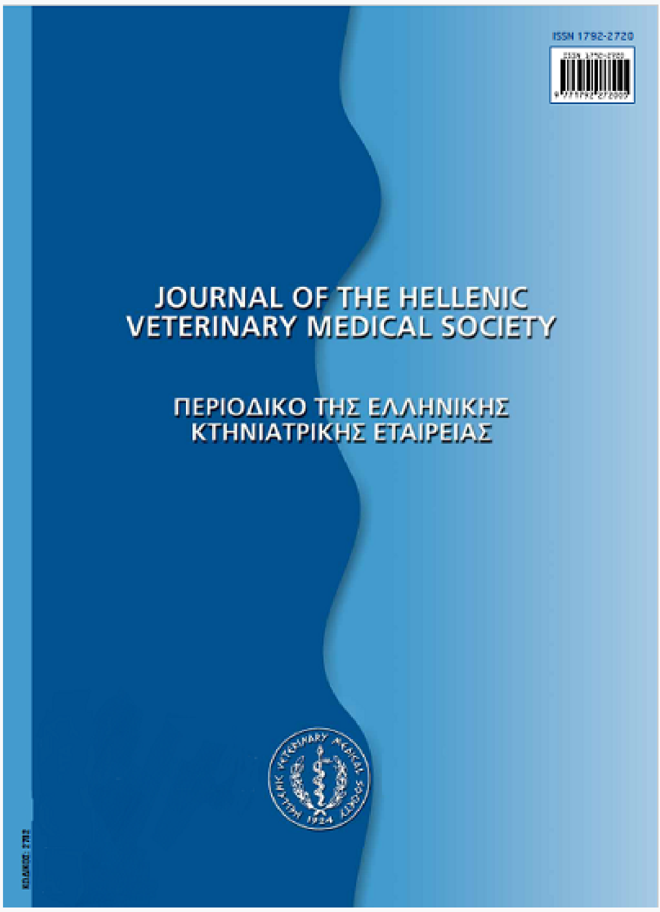

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S. SEIFI, M. R. SHIRZAD

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Antimicrobial susceptibility of *Escherichia coli* isolated from free range poultry or wild birds at the southern Caspian Sea coast of Iran

Seifi S.[1], Shirzad M.R.[2]

[1] *Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran*

[2] *Douk Avian Diseases Research Center, Veterinary Directorate General of Mazandaran Province, Iran*

ABSTRACT. Objective of this study was the investigation of antimicrobial resistance of 95 *Escherichia coli* strains from faecal samples of wild birds and free range poultry in Mazandaran province (southern Caspian Sea coast of Iran), which were isolated during the period May to December 2012. Isolation rate was 59% or 30% from samples of free range poultry or wild birds, respectively. All isolates from free range poultry were susceptible to amikacin, danofloxacin, enrofloxacin, gentamicin, neomycin, whereas all were resistant to carbenicillin and erythromycin. In strains from wild birds, most common resistance was detected to be to carbenicillin and tetracycline. Multi-drug resistance was evident in all isolates from free range poultry and in 26% of those from wild birds. It is postulated that that wild birds may be infected by antibiotic resistant strains of *E. coli* and may serve as reservoirs of such strains.

Keywords: antibiotic resistance, *Escherichia coli*, free range, southern Caspian Sea, wild bird.

Correspondence: S. Seifi,
Faculty of Veterinary Medicine,
Amol University of Special Modern Technologies, Amol, Iran.
E-mail: saeedseifi@umz.ac.ir

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INTRODUCTION

Although the largest area of the territory of Iran is dry, there are several some wetland systems of importance for a wide variety of waterfowl species. The south Caspian Sea, with 700 km of sandy shoreline, and the fresh-water lakes, marshes and brackish lagoons in Mazandaran, Gilan and Gorgan Bay provide a complex of breeding and wintering areas for waterfowl, almost unequaled in the Western palearctic (Scott, 1989). Regular censuses in recent years have estimated the mid-winter population of ducks, geese, swans and coots at over 1.000.000 individuals, with, perhaps, as many birds passing through that area in spring and autumn. During the spring and autumn migration seasons, large numbers of shorebirds pass through the south Caspian on their way between breeding grounds in the Arctic and wintering grounds in the Persian Gulf and East and South Africa (Martins and Hirschfeld, 1998). Wild bird populations can be vectors of various microorganisms, which can be excreted in faeces, thus contributing to increased level of contamination of drinking water reservoirs. Moreover, wild birds can transmit those pathogens to humans directly by contaminating agricultural fields or surface waters used for drinking, recreation or crop irrigation (Simpson, 2002; Cole et al., 2005). The free range poultry sector has a significant economical importance in rural and peri-urban areas of northern of Iran. Farmers depend on this sector as one of the main sources for their income, thus it is important to maintain this sector and help it to grow. Free-range chickens reared under extensive management systems scavenge for food and receive little or no veterinary care (Ojo et al., 2012). Consequently, they are highly exposed to numerous pathogens that limit their production capacity.

Objective of this study was to study *Escherichia coli* isolates from wild birds and free range poultry, in order to determine their susceptibilities to various antimicrobial agents, which are used as common therapeutic antibacterial agents.

MATERIALS AND METHODS

Sample collection

A total of 192 fresh faecal samples were collected by cloacal swabs from clinically healthy wild birds and free-range poultry, specifically from 24 swans (*Cygnus olor* or *C. cygnus*), 21 Northern shovelers (*Anas clypeata*), 18 Eurasian coots (*Fulica atra*), 48 ducks, 32 chickens, 28 turkeys and 21 geese, on the southern Caspian Sea coast of Iran, during the period May to December 2012. Samples were sent to the laboratory within 3 to 4 hours of collection, appropriately packaged.

Characterization of the isolates

Samples were inoculated onto MacConkey's agar (Merck, Darmstadt, Germany) plates and incubated at 37 °C for 24 hours. Suspected *E. coli* colonies were subsequently inoculated on Eosin Methylene Blue (EMB) agar (HiMedia, Mumbai, India) and incubated as above. Isolates were tested for Gram-staining, sulfide-indole-motility, methyl red, Voges-Proskauer, citrate, catalase and triple sugar iron reaction and, then, stored on nutrient agar until used for antimicrobial susceptibility tests (Holt et al., 1996).

Antibiotics susceptibility test

One colony from each plate was tested for susceptibility to various antimicrobial agents as detailed below, by using the disc diffusion method (Clinical and Laboratory Standards Institute, 2008). Bacterial cultures were prepared by inoculating the colonies into sterile distilled water, to reach an inoculum turbidity equivalent to a 0.5 McFarland turbidity standard. Disks of the following antibacterial agents were applied on Mueller-Hinton (Merck, Darmstadt, Germany) media: ampicillin (10 μg), carbencillin (100 μg), tetracycline (30 μg), oxytetracycline (30 μg), erythromycin (15 μg), flumequine (FM/30 μg), florfenicol (30 μg), enrofloxacin (5 μg), ciprofloxacin (5 μg), danofloxacin (10 μg), neomycin (30 μg), streptomycin (10 μg), amikacin (30 μg), gentamicin (10 μg), lincomycin + spectinomycin (15 μg + 200 μg). All antibiotic discs obtained from Padtan Teb (Tehran, Iran). *E. coli* strains ATCC 25922 was used as reference strain.

RESULTS

E. coli isolation

E. coli was isolated from 95 (76 free range and 19 wild) samples, with an isolation rate of 59% and 30% from free range poultry and wild birds, respectively.

Antimicrobial susceptibility in free range poultry

All *E. coli* isolates from free range poultry were susceptible to amikacin, danofloxacin, enrofloxacin, gentamicin and neomycin, whereas all were resistant to carbenicillin and erythromycin (Table 1). High rates of resistance also were detected against colistin, tetracycline and ampicillin. Multi-drug resistance was evident in all *E. coli* isolates; the pattern varied from two to 12 drugs (Table 2). In total, 32 isolates generated 18 different patterns of antimicrobial resistance (Table 3).

Antimicrobial susceptibility in wild birds

Most commonly, isolates were resistant to carbenicillin and tetracycline (Table 1). Multi-drug resistance was found in 26% of the isolates.

DISCUSSION

Wild birds can be reservoirs of various pathogenic bacteria, including *E. coli* (Simpson, 2002; Pennycott et al., 2006), as well as of antibiotic-resistant strains or antibiotic resistance genes (Cole et al., 2005; Dolejska et al., 2007). Antimicrobial resistance of *E. coli* strains in livestock and human populations is well documented (Faldynova et al., 2003; Grape et al., 2003; Guerra et al., 2003). Interaction with human and agricultural waste materials may spread pathogenic bacteria, including antibiotic-resistant isolates to wildlife, potentially creating an additional environmental reservoir of these organisms. Migratory birds in close contact with humans or domestic animals, e.g., free range poultry, may play a role in the dissemination of these pathogens over long distances and large areas.

Very low resistance to fluoroquinolones (ciprofloxacin, danofloxacin, enrofloxacin, norfloxacin) was evident in this study, whilst isolates had increased resistance to erythromycin, carbenicillin, tetracycline and ampicillin. The study corroborates the findings of Kwak et al. (2005), who reported high resistance to tetracycline and carbenicillin in free range chicken. Also, Dolejska et al. (2007) and Guenther et al. (2010) have also reported increased

Table 1. Antimicrobial drug resistance in Escherichia coli isolates from wild or free range birds in Northern Iran

Antimicrobial drug	Proportion (%) of isolates from free range poultry resistant to each drug	Proportion (%) of isolates from wild birds resistant to each drug
Amikacin	0	0
Ampicillin	44	10
Carbencillin	100	25
Ciprofloxacin	7	8
Colistin	83	17
Danofloxacin	0	0
Enrofloxacin	0	0
Erythromycin	100	18
Florfenicol	3	0
Flumequine	5	0
Gentamycin	0	0
Lincospectin	21	9
Neomycin	0	0
Oxitetracycline	32	7
Tetracycline	53	22

Table 2. Frequency of multi-drug resistance in Escherichia coli isolates from wild or free range birds in Northern Iran.

No of antimicrobial drugs, in which resistance was recorded	No (%) of isolates from free range poultry	No (%) of isolates from wild birds
≥2	76 (100)	5 (26)
≥3	76 (100)	4 (21)
≥4	67 (88)	3 (16)
≥5	61 (80)	2 (11)
≥6	53 (70)	1 (5)
≥7	44 (58)	0 (0)
≥8	23 (30)	0 (0)
≥9	18 (24)	0 (0)
≥10	13 (17)	0 (0)
≥11	8 (11)	0 (0)
≥12	3 (4)	0 (0)
≥13	2 (3)	0 (0)
≥14	0 (0)	0 (0)

resistance to ampicillin, streptomycin and tetracycline in black-headed gulls and common European wild bird species, respectively.

Ojo et al. (2012) have reported increased resistance (average 47%) to fluoroquinolones in free-range chickens in Nigeria. Increased use of fluoroquinolones instead of older-generation antimicrobial agents leads to gradual emergence of bacterial strains resistant to these antibacterials (Kurutepe et al., 2005). Our results are close to results of Obeng et al. (2012), who have not found increased resistance to fluoroquinolones in *E. coli* isolates from healthy commercial and free-range chickens in Australia.

A frequent occurrence of *E. coli* isolates resistant to antibiotics in wild and free range birds may reflect antibiotic resistance in livestock (poultry, swine, cattle) and human population as wild birds currently pass through or near animal farms seeking for food. In addition, wild fowl are in continued contact with water sources or reservoirs, where wastewater from farms and cities is discharged.

The high multi-antibiotic resistance observed in *E. coli* from free range and wild birds can be explained by the extensive use of antibiotics in poultry and cross-resistance with free range or wild birds isolates. Although free range chickens hardly receive any modern veterinary attention, they maintain close contact through various ways with microorganisms from other hosts in their environment.

Increased multi-drug resistance has been reported in *E. coli* isolates in many countries, including Iran (Cabassi et al., 2004; Khoshkhoo and Peighambari, 2005; Ozawa et al., 2008; Carneiro et al., 2010; Li et al., 2010). This situation in *E. coli* isolates of avian origin has emphasized the importance of antimicrobial susceptibility tests to select efficient antimicrobial agent against this pathogen. In our study, all *E. coli* isolates demonstrated that pattern of multi-drug resistance in number of antibacterial agents varied between 2 to 12, which is comparable with relevant previous work (Khoshkhoo and Peighambari, 2005). Wild birds are able to act as carriers of such *E. coli* isolates and spread them around. Guenther et al. (2010) studied 187 *E. coli* isolates from faeces and internal organs of wild birds and showed that 8% of isolates were multi-drug resistant. In Iran, free range poultry keep in open system, so the contact with wild birds, in addition to the increased risk of diseases transmission, could act

Table 3. Drug resistance patterns in Escherichia coli isolates from wild or free range birds in Northern Iran.

Drug-resistance pattern	No of isolates in the pattern
CB, E, CL, AM	2
CB, E, CL	2
CB, E, AM	2
CB, E, CL, TE	2
CB, E, CL, TE, OTC	2
CB, E, CL, AM, S	2
CB, E, CL, AM, TE, OTC	2
CB, E, CL, AM, S, TE, OTC	2
CB, E, CL, S, TE	2
CB, E, CL, AM, TE	2
CB, E, CL, AM, S, TE	1
CB, E, CL, TE	1
CB, E, CL, AM, S, LP	1
CB, E, CL, AM, S, LP, TE	1
CB, E, CL, S, LP, TE, OTC	1
CB, E, CL, S, LP	1
CB, E, CL, S, LP, C, FF, TE, OTC	1
CB, E, CL, AM, FM, LP, C, TE, OTC	1

CB: carbenicillin, E: erythromycin, CL: colistin, TE: tetracycline, OTC: oxytetracycline, AM: ampicillin, S: streptomycin, LP: lincomycin+spectinomycin, FF= florfenicol, C= ciprofloxacin.

as potential sources of antimicrobial resistant isolates.

Drug-resistant bacterial isolates of animal origin may spread into human population by direct contacts and through animal-origin foods (Soulsby, 2008). These bacteria may colonize humans and the genes coding for antibiotic resistance can be transferred to the bacteria of natural micro-flora of people or to pathogenic bacteria. Resistant bacteria present in the environment may infect other animals and then, through the food chain, may return back to humans (Hawkey, 2008).

CONCLUDING REMARKS

The present study showed relatively increased antibiotic resistance among *E. coli* isolates from free range poultry in northern Iran and indicates that wild birds can be a host reservoir of resistant *E. coli* strains, probably reflecting the presence of such strains in their sources of food and/or water.

CONFLICT OF INTEREST STATEMENT

The authors report no conflict of interest.

REFERENCES

Cabassi CS, Taddei S, Predari G, Galvani G, Ghidini F, Schiano E, Cavirani S (2004) Bacteriologic findings in ostrich (*Struthio camelus*) eggs from farms with reproductive failures. Avian Dis 48:716-722.

Carneiro C, Araújo C, GonÇalves A, Vinué L, Somalo S, Ruiz E, Uliyakina I, Rodrigues J, Igrejas G, Poeta P, Torres C (2010) Detection of CTX-M-14 and TEM-52 extended-spectrum beta-lactamases in fecal *Escherichia coli* isolates of captive ostrich in Portugal. Foodborne Pathog Dis 7:991-994.

Clinical and Laboratory Standards Institute (2008) Performance Standards for Antimicrobial Susceptibility Testing, 8th informational supplement. CLSI, Wayne.

Cole D, Drum DJV, Stallknecht DE, White DG, Lee MD, Ayers S, Sobsey M, Maurer JJ (2005) Free-living Canada geese and antimicrobial resistance. Emerg Infect Dis 11:935-938.

Dolejska M, Cizek A, Literak I (2007) High prevalence of antimicrobial resistant genes and integrons in *Escherichia coli* isolates from Black-headed Gulls in the Czech Republic. J Appl Microbiol 103:11-19.

Faldynova M, Pravcova M, Sisak F, Havlickova H, Kolackova I, Cizek A, Karpiskova R, Rychlik I (2003) Evolution of antibiotic resistance in *Salmonella enteric serovar Typhimurium* strains isolated in Czech Republic between 1984 and 2002. Antimicrob Agents Chemother 47:2002-2005.

Grape M, Sundstrom L, Kronvall G (2003) Sulphonamide resistance gene sul3 found in *Escherichia coli* isolates from human sources. J Antimicrob Chemother 52:1022-1024.

Guenther S, Grobbel M, Lübke-Becker A, Goedecke A, Friedrich ND, Wieler LH, Ewers C (2010) Antimicrobial resistance profiles of *Escherichia coli* from common European wild bird species. Vet Microbiol 144:219-225.

Guerra B, Junker E, Schroeter A, Malorny B, Lehmann S, Helmuth R (2003) Phenotypic and genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine and poultry. J Antimicrob Chemother 52:489-492.

Hawkey PM (2008) Molecular epidemiology of clinically significant antibiotic resistance genes. Br J Pharmacol 153:406-413.

Holt JH, Krieg NR, Sneath PHA, Staley JT, Williams ST (1996) Determinative Bacteriology, 9th edn. Williams & Wilkins, Baltimore.

Khoshkhoo PH, Peighambari SM (2005) Drug resistance patterns and plasmid profiles of *Escherichia coli* isolated from cases of avian colibacillosis. J Fac Vet Med Univ Tehran 60:97-105.

Kurutepe S, Surucuoglu S, Sezgin C, Gazi H, Gulay M, Ozbakkaloglu B (2005) Increasing antimicrobial resistance in *Escherichia coli* isolates from community acquired urinary tract infection during 1998-2003 in Manisa, Turkey. Jpn J Infect Dis 58:159-161.

Kwak HJ, Lee WW, Kim JH, Chung KT, Woo BG, Lee GR, Lee DS (2005) Antimicrobial Susceptibility and Plasmid Profile of *E. coli* Isolated from Wild Bird. Annual Rep Busan Metropolitan City Inst Heal Environ 15:86-92.

Li L, Jiang ZG, Xia LN, Shen JZ, Dai L, Wang Y, Huang SY, Wu CM (2010) Characterization of antimicrobial resistance and molecular determinants of beta-lactamase in *Escherichia coli* isolated from chickens in China during 1970-2007. Vet Microbiol 144:505-510.

Martins RP, Hirschfeld E (1998) Comments on the limits of the Western Palearctic in Iran and the Arabian Peninsula. Sandgrouse 20:108-134.

Obeng AS, Rickard H, Ndi O, Sexton M, Barton M (2012) Antibiotic resistance, phylogenetic grouping and virulence potential of *Escherichia coli* isolates from the faeces of intensively farmed and free-range poultry. Vet Microbiol 154:305-315.

Ojo OE, Ogunyinka OG, Agbaje M, Okuboye JO, Kehinde OO, Oyekunle MA (2012) Antibiogram of *Enterobacteriaceae* isolated from free-range chickens in Abeokuta, Nigeria. Vet Arhiv 82:577-589.

Ozawa M, Harada K, Kojima A, Asai T, Sameshima T (2008) Antimicrobial susceptibilities, serogroups, and molecular characterization of avian pathogenic *Escherichia coli* isolates in Japan. Avian Dis 52:392-397.

Pennycott TW, Park A, Mather HA (2006) Isolation of different serovars of *Salmonella enterica* from wild birds in Great Britain between 1995 and 2003. Vet Rec 158:817-820.

Scott DA (1989) Birds in Iran. Encyclopedia Iranica 4:265-272.

Simpson VR (2002) Wild animals as reservoirs of infectious diseases in the UK. Vet J 163:128-146

Soulsby L (2008) The 2008 Garrod lecture: antimicrobial resistance – animals and the environment. J Antimicrob Chemother 62:229-233.