

Antimicrobial resistance in *Enterococcus* spp. isolated from laying hens of backyard poultry flocks

Fabrizio Bertelloni¹, Claudia Salvadori¹, Annalisa Moni², Domenico Cerri¹, Paolo Mani¹,
Valentina Virginia Ebani¹

¹ Department of Veterinary Science, University of Pisa, Italy

² Dipartimento di Prevenzione, UOC Sanità Animale, Azienda USL Massa Carrara, Italy

Bertelloni F, Salvadori C, Moni A, Cerri D, Mani P, Ebani VV. Antimicrobial resistance in *Enterococcus* spp. isolated from laying hens of backyard poultry flocks. *Ann Agric Environ Med.* 2015; 22(4): 665–669. doi: 10.5604/12321966.1185771

Abstract

Introduction and objective. Enterococci belonging to human and animal gastrointestinal flora are widely-distributed in the environment. They are opportunistic bacteria that can cause severe infections, with the ability to acquire, express and transfer antimicrobial resistance. The aim of the present study was to investigate antimicrobial resistance profiles of *Enterococcus* spp. strains isolated from cloacal swabs of laying hens of small backyard flocks.

Materials and methods. Susceptibility to 21 antimicrobial agents was tested by the disc diffusion method in 115 *Enterococcus* spp. strains. Vancomycin and ampicillin minimum inhibitory concentrations and high-level aminoglycoside resistance tests were also performed.

Results. Isolates showed resistance mainly to aminoglycosides, erythromycin, fluoroquinolones, tetracycline and nitrofurantoin. 19 (16.5%) isolates showed a high level of resistance to streptomycin, but no high level resistance to gentamycin. No significant resistance was detected for vancomycin. Several strains (45; 39.1%) showed combined resistance to macrolides, lincosamides and streptogramin B. 61 (53%) isolates were classified as multidrug-resistant (MDR) and 6 (5.2%) strains as possibly extensively drug-resistant (XDR). *E. faecium* was the most prevalent antimicrobial resistant species, followed by *E. faecalis* and *E. durans*.

Conclusions. The results show that the risk of dissemination of antimicrobial resistant enterococci is related not only to the birds of large commercial flocks, but also to the birds of small backyard flocks. Thus, laying hens of hobby flocks, which share the outside environment with people, could represent a hazard for public health by providing a conduit for the entrance of resistance genes into the community.

Key words

Enterococcus spp., laying hens, antimicrobial resistance, cloacal swabs, hobby poultry

INTRODUCTION

Enterococci (genus *Enterococcus*) belong to the gastrointestinal flora of humans and animals and are widely distributed in the environment, such as terrestrial and water habitats [1]. As residents of the human and animal gastrointestinal tract, enterococci bring numerous benefits, such as probiotic activity and bacteriocins production. However, enterococci have raised research interests due to their pathogen role as opportunistic bacteria that can cause severe human infections, mainly as nosocomial infections, and for their strong ability to acquire, express and transfer antimicrobial resistance [1, 2]. In the gastrointestinal habitat, enterococci are in a suitable position to acquire resistance genes from other commensals, which may further transfer to other more pathogenic bacteria [3]. The clinically most important species in human beings are *E. faecalis* and *E. faecium* that can be involved in urinary tract infections, endocarditis, wound infections, sepsis and neonatal infections [4, 5]. *E. faecalis* is the most pathogenic species and *E. faecium* is the most involved in the acquisition and transfer of antimicrobial resistance [5].

The mechanism for antibiotic resistance can be intrinsic to enterococci or acquired through mutations of intrinsic genes, or horizontal exchange of genetic elements encoding resistance determinants [6]. The latter mechanism is the most important in the antimicrobial resistance of enterococci, and involves mobile genetic elements such as plasmids and transposons [3].

Enterococci are involved also as etiological agents of infections in veterinary medicine, such as mastitis in cattle, diarrhea in swine and cattle, as well as endocarditis, septicemia, spondylitis, and amyloid arthropathy in poultry [7, 8].

Humans can be colonized or infected with resistant enterococci through close contact with animals or through consumption of animal products [9, 10].

The presence of multidrug resistant enterococci has been detected worldwide on poultry farms in Australia [11], Canada [12, 13] and Malaysia [14], as well as in Europe: e.g., Sweden [15], Denmark [16], France [17], the Czech Republic [18] and Lithuania [8]. Continual and careful monitoring programmes are necessary to obtain data on the occurrence and trends in antimicrobial resistance, and consequently, for establishing intervention strategies [19].

The health status of backyard poultry flocks is generally poorly investigated. In particular, studies on the dissemination of multidrug resistant enterococci among commercial poultry have been carried out, but to the best

Address for correspondence: Valentina Virginia Ebani, Department of Veterinary Science, University of Pisa, Viale delle Piagge, 2; 56124 Pisa, Italy
E-mail: valentina.virginia.ebani@unipi.it

Received: 10 March 2015; accepted: 14 April 2015

of the authors' knowledge no data are available about this concern in hobby poultry.

The aim of the presented study was to investigate the antimicrobial resistance profiles of *Enterococcus* spp. strains isolated from cloacal swabs of laying hens of small backyard poultry flocks.

MATERIALS AND METHOD

Sampling. 157 cloacal swabs were collected from healthy laying hens of 24 different hobby poultry flocks in the Massa Carrara province of central Italy. The birds were geographically separated with no transfer between farms. Backyard flocks were characterized by a small number of raised animals (10–50) that live in open spaces or small fenced areas. Swabs were collected by the random capture of the animals, and the swabs were kept at 4 °C until bacteriological examinations.

Bacterial isolation. Within 24 hours of collecting, the swabs were sewn directly on Kanamycin Aesculin Azide Agar (KAAA, Oxoid Ltd., Basingstoke, UK) and incubated at 42±1 °C for 18–24 hours. From plates with growth of colonies typical for enterococci, at least one colony was subcultured on KAAA. Isolates were stored at -80 °C in Brain Heart Infusion Broth (BHI, Oxoid) for further investigations.

Antimicrobial susceptibility testing – Disc diffusion method. Isolates were tested by the standard disc diffusion method of Kirby-Bauer [20] on Mueller Hinton Agar (Oxoid) incubated at 35 ±1 °C for 18–24 hours. The following antimicrobial molecules (Oxoid) were tested: amoxicillin-clavulanic acid (30 µg), ampicillin (10 µg), cephalothin (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), enrofloxacin (5 µg), erythromycin (10 µg), gentamycin (10 µg), linezolid (30 µg), neomycin (10 µg), nitrofurantoin (300 µg), oxacillin (1 µg), quinupristin-dalfopristin (15 µg), rifampicin (30 µg), streptomycin (10 µg), teicoplanin (30 µg), tetracycline (30 µg), trimethoprim (5 µg), vancomycin (30 µg). Results were interpreted following EUCAST breakpoint Tables and, where not possible, according to NCCLS indications [21, 22]. Reference strains *E. faecalis* ATCC 29212 and *E. faecium* ATCC 19434 were used as controls.

Minimum inhibitory concentration (MIC). MIC for vancomycin and ampicillin were performed on microplates [23]. Concentrations from 0.5 – 256 µg/mL were used to test vancomycin MIC, and concentrations 8 – 256 µg/mL were used for MIC of ampicillin. Microplates were incubated at 37±1 °C in a humid chamber.

High level aminoglycoside resistance (HLAR). As indicated by CLSI Performance Standards for antimicrobial susceptibility tests, isolates that showed resistance to gentamicin and/or streptomycin by the disc diffusion method, were tested for resistance to high concentration of gentamicin (500 µg/mL) and streptomycin (1,000 µg/mL) [23].

Classification of acquired resistance. To classify isolated strains for expression of acquired resistance, the standardized international terminology proposed by Magiorakos *et al.*,

2012 [24] has been used in this study. For enterococci, aminoglycosides, carbapenems, fluoroquinolones, glycopeptides, glycolcyclines, lipopeptides, oxazolidinones, penicillins, streptogramins and tetracycline categories should be tested. Criteria for defining acquired resistance are: multidrug-resistant (MDR) strain when it is non-susceptible to ≥1 agent in ≥3 antimicrobial categories; extensively drug-resistant (XDR) strain when it is non-susceptible to ≥1 agent in all but ≤ 2 categories; and pandrug-resistant (PDR) strain when it is non-susceptible to all antimicrobial agents listed.

Since not all proposed molecules were tested in this study, only MDR or possibly XDR strains could be detected.

Species identification. *Enterococcus* spp. isolates classified as MDR or possibly XDR was examined for species identification with API 20 STREP (Bio Mérieux Italia, Bagno a Ripoli, Fi, Italy). Apiweb V 1.1.0 software was used as interpretative criteria.

RESULTS

Bacterial isolation. 115 *Enterococcus* isolates were obtained from 157 cloacal swabs.

Antimicrobial susceptibility testing – Disc diffusion method. All 115 isolates were tested for antimicrobial susceptibility with the disc diffusion method; results shown on Table 1. All isolates were not susceptible to oxacillin and most of them were not susceptible to cephalothin (86.9%) and trimethoprim (95.7%). Moreover, isolates were more frequently non-susceptible to the aminoglycosides category (100% of isolates were non-susceptible to neomycin and 93% were non-susceptible to streptomycin), macrolides (87.8% of isolates were non-susceptible to erythromycin), fluoroquinolones (86.1% of isolates were non-susceptible to enrofloxacin) and lincosamides (81.7% of isolates were non-susceptible to clindamycin). Over half of the isolates were non-susceptible to ciprofloxacin (70.4%), tetracycline (65.2%), gentamicin (60%) and quinupristin-dalfopristin (53.9%). A moderate resistance was evident for nitrofurantoin (48.7%), ampicillin (29.6%), tigecycline (26.1%), rifampicin (22.6%) and chloramphenicol (19.1%). Only a limited number of isolates were not susceptible to glycopeptides (vancomycin 10% and teicoplanin 11%) and to association of amoxicillin-clavulanic acid (13%).

A total of 101 resistance patterns were identified and all *Enterococcus* spp. isolates were resistant to at least 2 different categories of antibiotics, with 106 (92.17%) isolates being resistant to 5 or more antibiotics. Two isolates were susceptible only to vancomycin, one isolate was susceptible only to ampicillin and to amoxicillin-clavulanic acid, 6 isolates were susceptible only to 3 tested molecules, and 9 isolates were susceptible to 4 tested antibiotic molecules.

Minimum inhibitory concentration (MIC). All isolates characterized by a non-susceptibility to vancomycin and/or ampicillin with Kirby-Bauer test were tested for determination of MIC of these molecules. For vancomycin, 7 isolates showed MIC=1 mg⁻¹; one isolate showed MIC=2 mg⁻¹, and 2 isolates showed MIC=4 mg⁻¹. For ampicillin, 22 isolates showed MIC ≤8 mg⁻¹, 7 isolates with MIC=16 mg⁻¹, 4 isolates with MIC=32 mg⁻¹ and one isolate with MIC=64 mg⁻¹.

Table 1. Antimicrobial resistance expression of *Enterococcus* spp. isolates as result by disc diffusion method. Number and percentage of isolates resistant to 21 antibiotics are shown

Antibiotic	Susceptible (No. isolates)	%	Intermediate (No. isolates)	%	Resistant (No. isolates)	%	No-susceptible: intermediate + resistant (No. isolates)	%
Amoxicillin-clavulanic acid (AMC)	100	87.0	13	11.3	2	1.7	15	13.0
Ampicillin (AMP)	81	70.4	1	0.9	33	28.7	34	29.6
Cephalotin (KF)	15	13.0	20	17.4	80	69.6	100	86.9
Chloramphenicol (C)	93	80.9	13	11.3	9	7.8	22	19.1
Ciprofloxacin (CIP)	34	29.6	61	53.0	20	17.4	81	70.4
Clindamycin (DA)	21	18.3	9	7.8	85	73.9	94	81.7
Enrofloxacin (ENR)	16	13.9	32	27.8	67	58.3	99	86.1
Eritromycin (E)	14	12.2	59	51.3	42	36.5	101	87.8
Gentamycin (CN)	46	40.0	37	32.2	32	27.8	69	60.0
Linezolid (LZD)	74	64.3	15	13.0	26	22.6	41	35.6
Neomycin (N)	0	0.0	12	10.4	103	89.6	115	100
Nitrofurantoin (F)	59	51.3	14	12.2	42	36.5	56	48.7
Oxacillin (OX)	0	0.0	0	0.0	115	100	115	100
Quinupristin-dalfopristin (QD)	53	46.1	23	20.0	39	33.9	62	53.9
Rifampicin (RD)	89	77.4	7	6.1	19	16.5	26	22.6
Streptomycin (S)	8	7.0	10	8.7	97	84.3	107	93.0
Teicoplanin (TEC)	104	90.4	9	7.8	2	1.7	11	9.6
Tetracycline (TE)	40	34.8	9	7.8	66	57.4	75	65.2
Tigecycline (TGC)	85	73.9	14	12.2	16	13.9	30	26.1
Trimethoprim (W)	5	4.3	83	72.2	27	23.5	110	95.7
Vancomycin (VA)	105	91.3	7	6.1	3	2.6	10	8.7

Table 2. Resistance patterns of strains identified as *E. faecalis* and classified as MDR

Resistance pattern	Strain
CIP; KF; DA; ENR; E; CN; N; QD; RD; S; TE	37; 38
KF; DA; ENR; E; CN; LZD; N; F; QD; RD; S; TE; TGC	39
KF; CIP; DA; ENR; E; CN; LZD; N; QD; RD; TE; TGC	46
KF; C; CIP; DA; ENR; E; CN; LZD N; F; QD; RD; S (HLRA); TE; TGC	43
CIP; DA; ENR; E; CN; N; QD; S (HLRA); TE	229
CIP; DA; ENR; E; CN; N; QD; TE; TGC	40
AMC; AMP; KF; CIP; DA; ENR; CN; N; QD; TGC	65
AMP; KF; C; CIP; DA; ENR; E; CN; LZD; N; QD; TE; TGC	92
CIP; DA; ENR; E; CN; LZD; N; QD; TE	219
CIP; DA; ENR; E; CN; LZD; N; QD; TE; TGC	36
KF; C; DA; ENR; E; CN; LZD; N; QD; RD; TE; TGC	33
KF; C; CIP; DA; ENR; E; CN; LZD; N; QD; RD; S (HLRA); TE; TGC	41; 45

HLAR – High Level Aminoglycoside Resistance

High level aminoglycoside resistance (HLAR). 106 isolates that were non-susceptible to gentamicin and/or streptomycin with Kirby-Bauer test, were tested for high level aminoglycoside resistance. None of the tested isolates showed a high level resistance to gentamicin, whereas 19 (16.5%) isolates showed high level resistance to streptomycin.

Classification of acquired resistance. Following MDR, XDR and PDR classification, 61 (53%) strains were classified as MDR and 6 (5.2%) strains as possibly XDR bacteria (Tab. 2, 3, 4 and 5).

Species identification. 67 isolates, characterized as MDR and possibly XDR, were identified by API 20 STREP: 48 strains were identified as *E. faecium*, 14 as *E. faecalis* and 5 as *E. durans* (Tab. 2, 3, 4 and 5).

DISCUSSION

In this study, expression of antimicrobial resistance was evaluated on strains of *Enterococcus* spp. isolated from cloacal swabs collected from laying hens raised in small backyard flocks.

As expected, almost all isolates showed resistance to oxacillin, cephalothin, and trimethoprim because of intrinsic resistance to these molecules [6] that were not considered for the evaluation of resistance patterns. Indeed, 101 resistance patterns were identified. This high variability could be related to the high number of tested molecules; however, high variability has been frequently found in other studies performed on poultry enterococci [12, 13]. Most of the isolates showed a resistance to aminoglycoside molecules, particularly neomycin (100% of isolates) and streptomycin (93% of isolates). However, enterococci are intrinsically resistant to clinically achievable concentrations of aminoglycosides due to inability to enter the cell (*E. faecalis*), and for enzyme-mediated resistance or sterically-hindered ribosome target site (*E. faecium*). Intrinsic high level resistance to neither gentamicin nor streptomycin has been described in enterococci [6].

HLAR has been tested on all isolates that showed a no-susceptible phenotype in the Kirby-Bauer test for

Table 3. Resistance patterns of strains identified as *E. faecium* and classified MDR

Resistance pattern	Strain
DA; ENR; E; N; QD; S; TE	49
KF; DA; E; N; QD; S; TE	84; 164
AMP; KF; DA; ENR; N; S; TE	113
AMP; KF; DA; ENR; E; N; S; TE	122
KF; CIP; DA; ENR; E; CN; LZD; N; QD; RD; S (HLRA); TE	101
AMP; KF; CIP; DA; ENR; E; N; QD; RD; S (HLRA); TE	58
AMC; KF; CIP; DA; ENR; E; CN; N; F; TE	80
AMP; KF; DA; ENR; E; CN; N; F; S (HLRA); TE	110
AMP; KF; CIP; DA; ENR; E; CN; N; QD; S; TE	108; 120
CIP; DA; ENR; E; CN; N; QD; S; TE	239
CIP; DA; ENR; E; CN; N; QD; S; TE; TGC	40
KF; CIP; DA; ENR; E; CN; N; QD; S; TE	31; 109; 168
AMC; AMP; KF; CIP; DA; ENR; CN; N; QD; S; TGC	65
C; KF; DA; ENR; E; N; F; QD; S; TE	57
AMC; KF; C; CIP; DA; ENR; E; CN; LZD; N; F; S	82
KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; S; TE; TGC	177
AMC; AMP; KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; S; TE; TGC	107
AMC; AMP; KF; CIP; ENR; E; CN; N; F; S; TE	76
AMC; KF; CIP; DA; ENR; E; CN; N; F; S (HLRA); TE	79
AMC; AMP; KF; CIP; DA; ENR; E; CN; N; F; S; QD	86
KF; CIP; DA; ENR; E; N; F; QD; S; TE	54
KF; CIP; DA; ENR; E; N; F; S; TEC; TE	226
KF; CIP; DA; ENR; E; N; F; QD; S; TEC; TE	162
KF; CIP; DA; ENR; E; CN; LZD; N; S (HLRA)	190
KF; CIP; DA; ENR; E; LZD; N; QD; S; TE	118
AMP; KF; CIP; ENR; E; CN; LZD; N; F; S; TE	104
KF; CIP; ENR; E; CN; LZD; N; F; S; TGC	91
KF; CIP; DA; ENR; E; CN; LZD; N; F; QD; S	75
AMC; AMP; KF; CIP; ENR; E; CN; LZD; N; F; S	78
KF; CIP; DA; ENR; E; CN; LZD; N; F; QD; S (HLRA); TE; TGC	103; 223
AMP; KF; CIP; DA; ENR; E; CN; LZD; N; F; QD; S; TE; TGC	114
AMC; AMP; KF; CIP; DA; ENR; E; CN; LZD; N; F; QD; S; TE	167
KF; DA; ENR; E; CN; LZD; N; F; QD; S; TE; TGC	175
KF; CIP; DA; ENR; E; LZD; N; F; QD; S; TE; TGC	158
AMP; KF; CIP; DA; ENR; E; LZD; N; F; QD; S; TE; TGC	173
AMP; KF; CIP; DA; ENR; E; N; F; RD; S; TE	94
AMP; KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; RD; S; TE	93
AMP; KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; RD; S; TE	133
KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; RD; S (HLRA); TEC; TE	189
KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; RD; S; TEC; TE; TGC	188
KF; CIP; DA; ENR; E; CN; N; QD; RD; S (HLRA); TEC; TE	105
KF; CIP; DA; ENR; E; CN; LZD; N; F; QD; RD; S; TEC; TE; TGC	179
AMC; AMP; KF; CIP; DA; ENR; E; CN; LZD; N; F; QD; RD; S; TE; TGC	127

HLAR – High Level Aminoglycoside Resistance

streptomycin and/or gentamycin. 19 isolates showed an HLAR to streptomycin, but high level resistance to gentamycin was not observed. In the absence of HLAR, enterococci with lower resistance to cell wall active agents, such as penicillin or ampicillin, may be susceptible to synergistic killing of aminoglycoside-penicillin combination therapy [6, 23].

Table 4. Resistance patterns of strains identified as *E. durans* and classified as MDR

Resistance pattern	Strain
KF; CIP; DA; ENR; E; CN; N; QD; S (HLRA)	81; 119
AMC; AMP; KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; TE; TGC	157
AMP; CIP; DA; ENR; E; CN; N; F; TE	160
AMC; AMP; KF; CIP; N; DA; ENR; E; CN; F; QD; RD; TE; TGC	64

HLAR - High Level Aminoglycoside Resistance

Table 5. Resistance patterns of strains classified as possibly XDR

Resistance pattern	Strain	Species
AMP (HLR); KF; C; DA; ENR; E; LZD; N; QD; S (HLRA); TE; TGC	87	<i>E. faecium</i>
KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; S (HLRA); TEC; TE; TGC	42	<i>E. durans</i>
AMP; KF; C; CIP; DA; ENR; E; CN; LZD; N; QD; RD; S (HLRA); TEC; TE	98	<i>E. faecium</i>
AMP; KF; C; CIP; DA; ENR; E; CN; LZD; N; QD; RD; S (HLRA); TEC; TE; TGC	90	<i>E. faecium</i>
AMC; AMP; KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; RD; TEC; TE; TGC	128	<i>E. faecium</i>
AMC; AMP; KF; C; CIP; DA; ENR; E; CN; LZD; N; F; QD; RD; TEC; TE; TGC	222	<i>E. durans</i>

HLAR - High Level Aminoglycoside Resistance

Regarding erythromycin, 87.8% of isolates was no-susceptible, similar to other studies on enterococci isolated from turkeys [18] and broiler chickens [8, 11, 12, 13, 16, 17]. Also in the report of European Food Safety Authorities regarding years 2005–2011, macrolide resistance was the most frequently observed in poultry enterococci [19]. Cross-resistance among macrolide-lincosamide-streptogramin (MLS_B resistance) is well characterized in enterococci and 3 mechanisms of acquired resistance have been described: methylation of 23S rRNA, active efflux or inactivating enzymes [6, 18]. In the current study, 45 isolates showed a concomitant resistance to erythromycin, clindamycin and quinupristin-dalfopristin, suggesting an acquired MLS_B resistance.

Diffuse resistance was detected to fluoroquinolones: 86.1% of isolates were non-susceptible to enrofloxacin and 70.4% were non-susceptible to ciprofloxacin. Fluoroquinolones have been introduced on a large scale in veterinary therapy in recent years, leading to a high prevalence of resistant enterococcal isolates in certain animal groups [8].

Resistance to tetracycline has been detected in 65.2% of isolated strains. Tetracycline resistance has an importance due to its association with other antimicrobial molecules resistances [4].

In agreement with other authors [16], moderate resistance was observed to rifampicin and chloramphenicol, but these molecules are poorly used in the treatment of *Enterococcus* infections [6].

Moderate resistance was observed for linezolid and tigecycline. The mechanisms of tigecycline resistance are unknown, whereas linezolid resistance can be mediated by mutation or an acquired gene that can be transferred from staphylococci to enterococci [6].

Glycopeptide resistance, mainly to vancomycin, has raised much research interest during last 25 years, because vancomycin is considered a drug of 'last resort' in human medicine for the treatment of infections by multi-resistant

enterococci and methicillin resistant *Staphylococcus aureus* [5]. In the presented study, a low percentage of isolates showed resistance to teicoplanin and vancomycin with the Kirby-Bauer test. MIC did not confirm the resistance to vancomycin, suggesting that the results of the Kirby-Bauer method for this antibiotic must be confirmed by other tests [25].

61 of the isolates in the current were classified as MDR, and 6 as possibly XDR bacteria. The strains have been identified as *E. faecium*, *E. faecalis* and *E. durans*, all potential pathogens for humans [1]. *E. faecium* strains are usually host-specific, but this species could act as a very efficient donor of antimicrobial resistance genes to other enterococci, or more pathogenic bacteria, as well as between different hosts. The same clonal multidrug resistant *E. faecalis* strains have been detected in animals and humans affected by sepsis and endocarditis, suggesting a direct risk of infection from animal to humans [26].

CONCLUSIONS

The presence of multidrug resistant enterococci in hobby poultry flocks could represent a hazard for public health, considering the close contact between humans and animals. Contamination of the environment and human furniture with the faeces of poultry harbouring resistant enterococci is a risk for all members of the farm family, including children and elders, providing a conduit for the entrance of resistance genes into the community where further transmission is possible.

Backyard poultry flocks have more risk factors compared to commercial ones. Hens and broilers, kept for personal consumption of eggs and meat, usually share the same outside environment with their owners. Moreover, eggs from hobby poultry are not submitted to dipping, thus faeces contamination of shells may facilitate enterococci infection of consumers.

Backyard poultry could be a source of infection also for other domestic animals, such as dogs, cat or pigeons, that can further infect humans or amplify the distribution of resistant strains in the environment.

In conclusion, the presented study, although performed in a restricted geographical area and on a limited number of birds, contribute to the verification of the presence of multi-drug resistant enterococci in the environment, and determination of their antimicrobial resistance profiles. The obtained results underline that not only commercially-kept poultry, but also backyard birds could have an epidemiological role in the amplification and transmission of antimicrobial resistant bacteria.

REFERENCES

- Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR, Harwood VJ. Enterococci in the environment. *Microbiol Mol Biol Rev.* 2012; 4: 685–706.
- Domig KJ, Mayer HK, Kneifel W. Methods used for the isolation, enumeration, characterization and identification of *Enterococcus* spp. 1. Media for isolation and enumeration. *Int J Food Microbiol.* 2003; 88: 147–164.
- Hegstad K, Mikalsen T, Coque TM, Werner G, Sundsfjord A. Mobile genetics elements and their contribution to the emergence of antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium*. *Clin Microbiol Infect.* 2010; 16: 541–554.
- Hammerum AM. Enterococci of animal origin and their significance for public health. *Clin Microbiol Infect.* 2012; 18: 619–625.
- Nilsson O. Vancomycin resistant enterococci in farm animals—occurrence and importance. *Infect Ecol Epidemiol.* 2012; 2: doi10.3402/iee.v2i0.16959.
- Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in enterococcus. *Virulence* 2012; 5: 421–433.
- Robbins KM, Suyemoto MM, Lyman RL, Martin MP, Barnes HJ, Borst LB. An outbreak and source investigation of enterococcal spondylitis in broiler caused by *Enterococcus cecorum*. *Avian Dis.* 2012; 56: 768–773.
- Šeputienė V, Bogdaitė A, Ružauskas M, Sužiedėlienė E. Antibiotic resistance genes and virulence factors in *Enterococcus faecium* and *Enterococcus faecalis* from diseased farm animals: pigs, cattle and poultry. *Pol J Vet Sci.* 2012; 3: 431–438.
- Sørensen TL, Blom M, Monnet DL, Frimodt-Møller N, Poulsen RL, Espersen F. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. *N Engl J Med.* 2001; 16: 1161–1166.
- Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev.* 2011; 4: 718–733.
- Obeng AS, Rickard H, Ndi O, Sexton M, Barton M. Comparison of antimicrobial resistance patterns in enterococci from intensive and free range chickens in Australia. *Avian Pathol.* 2013; 1: 45–54.
- Diarra MS, Rempel H, Champagne J, Masson L, Pritchard J, Topp E. Distribution of antimicrobial resistance and virulence genes in *Enterococcus* spp. and characterization of isolates from broiler chickens. *Appl Environ Microbiol.* 2010; 24: 8033–8043.
- Furtula V, Jackson CR, Farrell EG, Barrett JB, Hiott LM, Chambers PA. Antimicrobial resistance in *Enterococcus* spp. isolated from environmental samples in an area of intensive poultry production. *Int J Environ Public Health.* 2013; 10: 1020–1036.
- Getachew Y, Hassan L, Zakaria Z, Abdul Aziz S. Genetic variability of vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* isolates from humans, chickens, and pigs in Malaysia. *Appl Environ Microbiol.* 2013; 79: 4528–4533.
- Nilsson O, Greko C, Bengtsson B. Environmental contamination by vancomycin resistant enterococci (VRE) in Swedish broiler production. *Acta Vet Scand.* 2009; 51: 49.
- Aarestrup FM, Agerso Y, Gerner-Smidt P, Madsen M, Jensen LB. Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. *Diagn Microbiol Infect Dis.* 2000; 37: 127–137.
- Petsaris O, Miszczak F, Gicquel-Bruneau M, Perrin-Guyomard A, Humbert F, Sanders P, Leclercq R. Combined antimicrobial resistance in *Enterococcus faecium* isolated from chickens. *Appl Environ Microbiol.* 2005; 5: 2796–2799.
- Jaglic Z, Vlkova H, Bardon J, Michu E, Cervinkova D, Babak V. Distribution, characterization and genetic bases of erythromycin resistance in staphylococci and enterococci originating from livestock. *Zoonoses Pub Health.* 2012; 59: 202–211.
- Garcia-Migura L, Hendriksen RS, Fraile L, Aarestrup FM. Antimicrobial resistance of zoonotic and commensal bacteria in Europe: the missing link between consumption and resistance in veterinary medicine. *Vet Microbiol.* 2014; 170: 1–9.
- Bauer A, Kirby WMN, Sherris JC, Turk M. Antibiotic susceptibility testing by a standardized single disc method. *Am J Pathol.* 1966; 45: 493–496.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Seventeenth Informational Supplement. Clinical and Laboratory Standards Institute document M100-S17 [ISBN 1–56238–625–5]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087–1898 USA, 2007.
- EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 3.1, 2013. <http://www.eucast.org>
- Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition. Clinical and Laboratory Standards Institute document M7-A7 [ISBN 1-56238-587-9]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087–1898 USA, 2006.
- Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2012; 18: 268–281.
- Andrews JM. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother.* 2001; 48 Suppl 1: 5–16.
- Hammerum AM, Lester CH, Heuer OE. Antimicrobial-resistant enterococci in animals and meat: a human health hazard? *Foodborne Pathog Dis.* 2010; 10: 1137–1146.