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ANTIMICROBIAL RESISTANCE OF *ENTEROCOCCUS* SPP. ISOLATED FROM ANIMAL-DERIVED FOOD

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Abstract: The current work aims to test the antimicrobial resistance of 92 *Enterococcus* spp. isolates from dairy products, eggs and meat in the Autonomous Province of Vojvodina, Serbia. As confirmed by PCR or MALDI TOF, *E. faecalis* was the most frequently encountered species (51.08%), followed by *E. faecium* (39.13%), *E. hirae* (6.52%), *E. thailandicus* (2.17%) and *E. durans* (1.08%). Generally, the most frequent resistance phenotype in all isolates was to tetracycline (34.78%), erythromycin (27.17%), doxycycline (21.73%) and streptomycin (13.04%). The phenotypic resistance to antimicrobials was less prevalent in enterococci isolates from dairy products than in meat isolates. Out of the 92 enterococci isolates, 16 (17.39%) were multidrug-resistant (MDR), primarily those from poultry (38.09%) and pork meat (21.05%). Resistance to fluoroquinolones was confirmed only in MDR enterococci isolates from poultry meat (28.57%). Resistance to vancomycin, ampicillin, linezolid, teicoplanin and tigecycline was not detected.

Key words: *Enterococcus, antimicrobial resistance, animal-derived food*

INTRODUCTION

Enterococcus species are members of the *Enterococcaceae* family, order *Lactobacillales*, class *Bacilli*, and phylum *Firmicutes* (Schleifer & Kilpper-Bälz, 1984; Ružičkova, Vitezova & Kushkevych, 2020). The genus has constantly been revised and is now comprised of over 60 well-described and identified species of known habitats, tropisms, and metabolic and phenotypic characteristics (DSMZ, 2020). Their natural habitats are the guts of various animal species, from insects to vertebrates. *E. faecium* and *E. faecalis* are the most prominent species and generally contribute to less than 1% of the culturable gut microbiota isolated from healthy people (Gilmore, Clewell, Ike & Shankar, 2014; Bortolaia, Espinosa-Gongora & Guardabassi, 2016). Enterococci are also found in soil and sand, ambient waters, and aquatic and terrestrial vegetation.

Due to their evolutionary extreme resilience to unfavourable environmental conditions, they can thrive at both 10 ºC and 45 ºC, even in 6.5% sodium chloride solution, and pH 9.6. In addition, they may survive at 60 ºC for 30 minutes (Franz, Holzapfel & Stiles, 1999).

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Since the 1970s, enterococci have more often been identified as the causative agents of healthcare-associated infections (Boccella et al., 2021; Guan et al., 2024), for example, of the urinary tract and postoperative wounds, endocarditis, neonatal infections, abdominal, pelvis and central nervous system infections. On these occasions, *E. faecalis* comprised 80-90%, and *E. faecium* 5-15% of the clinical isolates (Jett, Huycke & Huycke, 1994; Edmond et al., 1995; Cetinkaya, Falk & Mayhall, 2000; Gilmore et al., 2014; Ružičkova et al., 2020). In the period from 2002 to 2008, these two species taken together were the third cause of bacteriemia in Europe and America, causing roughly 11–13% of all cases (Schaberg, Culver & Gaynes, 1991; Jett et al., 1994; De Kraker et al., 2013).

The importance of enterococci in healthcare-associated infections results from their intrinsic resistance/tolerance to frequently used antibiotics (cephalosporins, β-lactams, sulphonamides, clindamycin and aminoglycosides) and the acquisition of antimicrobial resistance either by chromosomes, conjugative or non-conjugative plasmids or transposons, which occurs efficiently (Willems, et al., 2005; Palmer, Kos & Gilmore et al., 2010; Prabaker & Weinstein, 2011; Hammerum, 2012). The wide use of antibiotics in human and veterinary medicine led to the selection of novel combinations of traits in some species, mainly *E. faecalis* and *E. faecium* (Gilmore et al., 2014), and the prevalence of drug-resistant *E. faecalis* strains are on the increase over time (Guan et al., 2024). In food-producing animals, avoparcin, gentamicin, and virginiamycin applied as growth promotors or for therapeutic reasons resulted in the emergence of vancomycin- and gentamicin-resistant enterococci and quinupristin/dalfopristin-resistant *E. faecium* (Hammerum, Lester & Heuer, 2010). Vancomycin-resistant enterococci (VRE) have been detected in poultry and pork in Europe (Bates, Jordens & Griffiths, 1994; McDonald, Kuehnert, Tenover & Jarvis, 1997; Shepard and Gilmore, 2002).

The pathogenicity of enterococci, connected with traits such as transmissibility, invasiveness and toxicity, is disputable and has not been established (Flint, 2002; Anderson et al., 2016; Ružičkova et al., 2020). The pathogennicity and capability of causing foodborne ailments of *E. faecalis* and *E. faecium* have been suspected, but remain to be verified (Franz et al., 1999; Flint, 2002). The colonization of the human GIT by resistant enterococci strains originating from food poses no risk to the outbreak of clinical infections (Gordts, Van Landuyt, Ievene, Vandamme & Goosens, 1995; Aestrup, 1995; Adams, 1999; Cetinkaya et al., 2000; Chajecka-Wierzchowska, Zadernowska & Laniewska-Trokenheim, 2017). The controversy of these bacteria pertaining to food safety derives from the fact that the food chain may be the pathway for spreading strains containing virulence factors and resistance to antimicrobials to humans. What is more, bacterial transfer with food may endanger significantly more people via both contaminated food consumption and handling (Klare et al., 1995; Franz et al., 1999; De Kraker et al., 2013; Bortolaia et al., 2016). The risk from enterococci present in food originates from the possible horizontal transfer of resistance determinants of clinical interest to the human gut microbiota, both within their genus and to other bacteria and transmission of multidrug-resistant enterococci (Johnston & Jaykus, 2004; Palmer et al., 2010; Bortolaia et al., 2016).

Routine control of the production and distribution of animal-derived foods does not comprise testing for the presence of *Enterococcus* species. Their number/concentration is not limited, unlike of those of coliform bacteria and *E. coli*. The current work is aimed to test the antimicrobial resistance of enterococci isolated from animal-derived foods from retail facilities in Bačka and Srem (Autonomous Province of Vojvodina, Serbia).

MATERIALS AND METHODS

Isolation and identification of *Enterococcus* spp.

Samples: The research was conducted in January and February 2023, on the following samples of animal-derived foods: dairy products (n=22), meat (n=65), eggs and egg powder (n=5), randomly obtained from retail facilities in Bačka and Srem (Autonomous Province of Vojvodina, Serbia).

Isolation of enterococci. Food samples were enriched in the peptone water (BK084HA, BioKar Diagnostics) at 37 °C for 24 h. The next day, 1 mL of peptone water was transferred to Slanetz and Bartley agar plates

(CM0377, Oxoid). After 24 h or 48 h of incubation at 37 °C, single colonies were transferred to Bile Aesculin Agar (CM0888, Oxoid) to obtain pure cultures. Colonies characteristic of *Enterococcus* spp. were then transferred to Tryptone soya broth (Merck), and after 24 h of incubation at 37 ºC, the cultures were frozen with 20% glycerol for further work.

Species identification. Isolates of enterococci were identified to the genus level by the polymerase chain reaction (PCR) using the protocol by the Danish Technical University (Parte, 2018). Briefly, DNA was isolated after boiling bacteria in distilled water for 10 minutes. The reaction mixture was from the commercial company BioLine, and the master mix contained hot-start polymerase. Cycling conditions were as follows: initial step to activate polymerase at 95 °C for 15 minutes, 30 cycles at 94 °C 90 s, at 50 °C 90 s, at 72 °C 60 s and final extension at 72 °C 10 min, hold at 4 °C. The primers used for the PCR were for the determination of two *Enterococcus* species: *E. faecalis* E1-5'ATCAAGTACAGTTAGTCTT-3' and E2-5'-ACGATTCAAAGCTAACTG-3', *E. faecium* F1-5'-GCAAGGCTTCTTAGAGA-3' and F2-5'-CATCGTGTAAGCTAACTTC-3'. The PCR reaction yielded a product of 941 bp for *E. faecium* and 550 bp for *E. faecalis*. For species identification, 9 isolates that were not identified by PCR were processed by MALDI-TOF mass spectrometry. MALDI-TOF mass spectra were obtained using a Microflex LT/SH Biotyper spectrometer (Bruker Daltonics, Germany) equipped with a nitrogen laser (337 nm) under the control of flexControl software ver. 3.4 (Bruker Daltonics).

Antibiotic susceptibility testing. Antibiotic sensitivity test was performed using the disk diffusion method. The results were interpreted using the protocols of the Clinical and Laboratory Standards Institute CLSI M100, 2022 and the EUCAST document 2022. The following disks (BioRad, Mames-la-Coquette, France) were used: Ampicillin (AMP) 10 µg, Ciprofloxacin (CIP) 5 µg, Erythromycin (ERY)15 µg, Chloramphenicol (CHL) 30 µg, Tetracycline (TET) 30 µg, Gentamicin (GMN) 10 µg, Nitrofurantoin (FTN) 300 µg, Fosfomycin (FOS) 200 µg, Quinupristin-dalfopristin (QDF) 15 µg, Linezolid (LZD) 30 µg, Vancomycin (VAN) 30 µg, Teicoplanin (TEC) 30 µg, Tigecycline (TGC) 15 µg, Doxycycline (DOX) 30 µg, Moxifloxacin (MXF) 5 µg, Norfloxacin (NXN) 10 µg, Levofloxacin (LVX) 5 µg, Streptomycin (HLS300) 300 µg. For the quality control, *E. coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used.

RESULTS AND DISCUSSION

Enterococcus spp. were isolated from all of the tested samples (n=92) of animal-derived foods: 22 isolates were obtained from dairy products, 65 from meat and meat products, and 5 isolates from eggs and egg powder samples. As confirmed by PCR or MALDI TOF, *E. faecalis* was the most frequently encountered species (n=47, 51.08%), followed by *E. faecium* (n=36, 39.13%), *E. hirae* (n=6, 6.52%), *E. thailandicus* (n=2, 2.17%) and *E. durans* (n=1, 1.08%). *E. faecalis* and *E. faecium* were isolated from all types of food samples; six strains of *E. hirae* were isolated from beef meat (n=5) and pork meat (n=1). Two isolates, from beef and pork meat, were identified as *E. thailandicus*, and a single isolate *E. durans* was isolated from poultry meat (Table 1).

Table 1.
Sources, serotypes and resistotypes of *Enterococcus* spp. isolates

Serial No.	Source of isolates (food item)	Species (PCR/MALDI TOF)	Resistotypes	Resistance to Antibiotic Classes
Dairy products (n=22)				
1	Cheese	*E. faecalis*	-	-
2	Edamer	*E. faecalis*	-	-
3	Gauda	*E. faecalis*	-	-
4	Cheese	*E. faecalis*	-	-
5	Cheese	*E. faecalis*	-	-
6	Milk powder	*E. faecium*	-	-
7	Milk powder	*E. faecium*	-	-
8	Casein	*E. faecium*	-	-
9	Whey powder	*E. faecium*	-	-
10	Condensed milk	*E. faecium*	-	-
11	Sheep yoghurt	*E. faecium*	-	-

Table 1. Continued

12	Butter	*E. faecium*	-	-
13	Cake	*E. faecium*	-	-
14	Cheese	*E. faecium*	ERY	1
15	Blue cheese	*E. faecium*	FOS	1
16	White cheese	*E. faecalis*	STR300	1
17	Milk powder	*E. faecium*	FOS	1
18	Milk powder	*E. faecium*	ERY	
19	Whey powder	*E. faecium*	ERY	1
20	Condensed milk	*E. faecium*	ERY	1
21	Butter	*E. faecium*	ODF	1
22	Ice cream	*E. faecium*	ERY, FTN, QDF, STR300	4
Eggs and egg powder (n= 5)				
23	Egg powder	*E. faecium*	-	-
24	Table eggs	*E. faecalis*	-	-
25	Table eggs	*E. faecalis*	-	-
26	Quail eggs	*E. faecalis*	ERY	1
27	Table eggs	*E. faecalis*	ERY, TET, STR300	3
Pork meat (n=19)				
28	Pork meat	*E. faecalis*	-	-
29	Pork meat	*E. faecium*	-	-
30	Pork meat	*E. faecalis*	-	-
31	Smoked ribs	*E. faecium*	-	-
32	Pork meat	*E. hirae*	-	-
33	Pork meat	*E. faecalis*	-	-
34	Pork meat	*E. faecalis*	-	-
35	Pork meat	*E. faecalis*	-	-
36	Pork meat	*E. faecalis*	-	-
37	Gyros	*E. faecium*	-	-
38	Pork meat	*E. faecalis*	TET	1
39	Pork meat	*E. faecalis*	TET	1
40	Pork meat	*E. faecalis*	TET, DOX	1
41	Pork meat	*E. faecalis*	TET, DOX	1
42	Smoked pork meat	*E. thailandicus*	TET, FTN	2
43	Pork meat	*E. faecium*	ERY, TET, DOX, STR300	3
44	Pork meat	*E. faecalis*	ERY, TET, DOX, STR300	3
45	Pork	*E. faecium*	ERY, GMN, QDF	3
46	Pork meat	*E. faecium*	ERY, TET, DOX, STR300	3
Beef meat (n=25)				
47	Barbeque meat	*E. hirae*	-	-
48	Kebab	*E. hirae*	-	-
49	Rijet	*E. faecalis*	-	-
50	Kebab	*E. faecium*	-	-
51	Ground meat	*E. hirae*	-	-
52	Kebab	*E. hirae*	-	-
53	Kebab	*E. faecium*	-	-
54	Kebab	*E. faecalis*	-	-
55	Barbeque sausage	*E. faecium*	-	-
56	Meat with spices	*E. hirae*	-	-
57	Sausage	*E. faecalis*	-	-
58	Sausage smoked	*E. faecalis*	-	-
59	Burger	*E. faecalis*	GMN	1
60	Cattle's heart	*E. faecalis*	TET	1
61	Kebab	*E. faecium*	ERY	1
62	Kebab	*E. faecium*	ERY	1
63	Kebab	*E. faecalis*	FTN	1
64	Barbeque sausage	*E. faecalis*	TET	1
65	Barbeque sausage	*E.faecalis*	TET, DOX	1
66	Sausage	*E. faecium*	ERY	1
67	Barbeque sausage	*E. faecalis*	TET, DOX	1
68	Kebab	*E. thailandicus*	TET, FTN, DOX	2
69	Veal meat	*E. faecalis*	TET, STR300	2
70	Kebab	*E. faecalis*	ERY, TET, STR300	3
71	Intestine	*E. faecalis*	ERY, CHL, TET, DOX	3
Poultry meat (n=21)				
72	Chicken meat	*E. faecium*	-	-
73	Chicken meat	*E. faecium*	-	-

Table 1. Continued

74	MSM	*E. faecium*	-	-
75	MSM	*E. faecium*	-	-
76	Chicken barbeque meat	*E. faecalis*	-	-
77	Chicken breast meat	*E. faecalis*	TET	1
78	Chicken file	*E. faecalis*	TET, DOX	1
79	Chicken drumstick	*E. faecalis*	TET, DOX	1
80	Chicken meat	*E. faecium*	TET, DOX	1
81	MSM	*E. faecalis*	TET	1
82	MSM	*E. faecalis*	TET	1
83	Chicken kebab	*E. faecalis*	ERY, TET, DOX	2
84	MSM	*E. faecalis*	ERY, TET, DOX	2
85	MSM	*E. faecalis*	ERY, TET, STR300	3
86	MSM	*E. faecalis*	ERY, TET, DOX, CIP, MFX	3
87	MSM	*E. faecium*	TET, DOX, QDF, STR300	3
88	Chicken skin	*E. faecalis*	ERY, STR300, GMN, CIP, MXF, NXN, LVX,	4
89	MSM	*E. faecalis*	ERY, STR300; TET, DOX, MXF, NXN, LVX,CIP,	4
90	MSM	*E. faecalis*	ERY, TET, DOX, CHL, CIP, NXN, LVX	4
91	Chicken meat	*E. faecium*	ERY, TET, DOX, CHL, CIP, MXF, NXN, LVX	5
92	Chicken skin	*E. durans*	ERY, TET, DOX, FTN, CIP, MXF, NXN, LVX	5

Erythromycin (ERY), Fosfomycin (FOS), Chloramphenicol (CHL), Streptomycin (HLS300), Gentamicin (GMN), Tetracycline (TET), Doxycycline (DOX), Ciprofloxacin (CIP), Moxifloxacin (MXF), Quinupristin-dalfopristin (QDF), Levofloxacin (LVX), Norfloxacin (NXN), Nitrofurantoin (FTN)

For easier comprehension, in Table 1, the order of the isolates (ordinal numbers) was created in sequence depending on the determined resistotype (from those susceptible to all antibiotics to multidrug-resistant). The total number of isolates susceptible to all antibiotics was 43 (46.73%): 13 isolates from dairy products (59.09%), 12 from beef meat (48%), 10 from pork meat (52.63%), 5 from poultry meat (23.80%), and 3 from eggs (60%) (Table 1). Resistance to a single antibiotic/antibiotic class was determined in 33 isolates (35.86%), most frequently to tetracycline/doxycycline (15.2%) and erythromycin (8.60%) (Table 1). Three isolates, one from pork and beef meat each and one from chicken skin, were resistant to high doses of gentamycin. Resistance to ciprofloxacin was detected in 6 (6.52%) enterococci isolates from poultry meat, among which five were also resistant to moxifloxacin and levofloxacin, and four to norfloxacin and levofloxacin. In the current research, the other types of food were free from enterococci resistant to fluoroquinolones (Table 2).

Resistance to three or more distinct classes of antibiotics (multidrug-resistant, MDR) was detected in 16 isolates (17.39%): *E. faecalis* (n=9; 9.78%), *E. faecium* (n=6; 6.50%), and *E. durans* (n=1; 1.0%). In total, 14 (21.50%) isolates from meat were MDR and also one obtained from table eggs and one from ice cream. (Table 3). Resistance to vancomycin (VAN), ampicillin (AMP), linezolid (LZD), teicoplanin (TEC) and tigecycline (TGC) was not detected in *Enterococcus* species isolates in this research. Antimicrobial resistance is a global problem, which is why the corresponding body of knowledge has been growing continuously. In this research, resistance to various antibiotic classes was detected in *Enterococcus* spp. isolates from dairy products, eggs and meat.

Generally, the most frequent resistance phenotype in all isolates was to tetracycline, found in 32 isolates (34.78%), followed by erythromycin resistance, detected in 25 isolates (27.17%), doxycycline in 20 isolates (21.73%) and streptomycin in 12 isolates (13.04%) (Table 2). Unsurprisingly, tetracycline resistance has been confirmed to be among the most common acquired resistance in food isolates of *Enterococcus* species (Peters, Mac,Wichmann-Schauer & Eller-broek, 2003; Johnston & Jaykus, 2004; due to its widespread use in animal production (Hammerum, 2012). In the current work, 71.42% of enterococci isolates from poultry meat were resistant to tetracycline. Owing to the extensive use of this antimicrobial in poultry production, tetracycline-resistant enterococcus isolates are commonly detected in a high percentage of poultry products, evenas high as 91% (Kročko et al., 2011) or 87.5% (Rożanska, Lewtak-Piłat

& Osek, 2015). *E. faecalis* meat isolates were most often resistant to tetracycline: 29.2% (Golob et al., 2019). Moreover, high levels of clinical resistance were confirmed in *E. faecalis* and *E. faecium*: tetracycline resistance (45-100%) was detected in poultry and pig samples (Makarov et al., 2022), and most of the *E. faecalis* and *E. faecium* poultry isolates in Zambia were resistant to tetracycline (89.2%) and ampicillin and erythromycin (68.9%) (Mwikuma et al., 2023). The *Enterococcus* isolates from pig farms in China showed high prevalence of resistance to medically important antibiotics, such as ampicillin (50.9% for *E. faecium* and 19.6% for *E. faecalis*), erythromycin (83.0% for *E. faecium* and 91.1% for *E. faecalis*), and tetracycline (79.2% for *E. faecium* and 100% for *E. faecalis*) (Xuan et al., 2021). Extensive resistance to erythromycin (60-100%), ciprofloxacin (23-100%), and trimethoprim-sulfamethoxazole (33-53%) is detected in some food-producing birds (chickens and turkeys), and pigs tested in 15 regions of Russia (Makarov et al., 2022). However, such resistance is considered less important from a human clinical perspective (Bortolaia et al., 2016). Resistance to streptomycin (HLS300) was detected in 12(13.04%) isolates, from which 11 were susceptible to gentamycin. Streptomycin resistance was encountered mainly in enterococci strains capable of producing the enzyme strep-

Table 2.
Antibiotic resistance of *Enterococcus* spp. isolates

Source of isolates	**Species** (No.)	**No. of isolates resistant to specific antibiotics**												
		ERY	FOS	CHL	STR	GMN	TET	DOX	CIP	MXF	QDF	LVX	NXN	FTN
Dairy products	*E. faecalis* (1)				1									
9/22 (40.9%)	*E. faecium* (8)	5	2		1						2			1
Eggs and egg powder 2/5 (20%)	*E. faecalis* (2)	2			1		1							
Beef meat	*E. faecalis* (9)	2		1	2	1	7	3						1
13/25 (52%)	*E.faecium* (3)	3												
	E. thailandicus(1)						1	1						1
Pork meat 9/19 (47.3%)	*E. faecalis*(5)	1			1		5	3						
	E. faecium (3)	3			2	1	2	2			1			
	E. thailandicus (1)						1							1
Poultry meat	*E. faecalis*(12)	7		1	3	1	11	7	4	3		3	3	
16/21(76.19%)	*E. faecium* (3)	1		1	1		3	3	1	1	1	1	1	
	E. durans(1)	1					1	1	1	1		1	1	1
	Total: 49/92 (53.3%)	**25**	**2**	**3**	**12**	**3**	**32**	**20**	**6**	**5**	**4**	**5**	**5**	**5**

Erythromycin (ERY), Fosfomycin (FOS), Chloramphenicol (CHL), Streptomycin (HLS300), Gentamicin (GMN), Tetracycline (TET), Doxycycline (DOX), Ciprofloxacin (CIP), Moxifloxacin (MXF), Quinupristin-dalfopristin (QDF), Levofloxacin (LVX), Norfloxacin (NXN), Nitrofurantoin (FTN)

Table 3.
Multidrug-resistant (MDR) *Enterococcus* spp. isolates from different food items

Food items	**Number (percent) of MDR isolates in particular food items**	**Percent out of total sample number**	**MDR Species**
Dairy products	1/22 (4.54%)	1/92 (1.08%)	*E. faecium* (n=1)
Eggs and egg powder	1/5 (20%)	1/92 (1.08%)	*E. faecalis* (n=1)
Beef meat	2/25 (8%)	2/92 (2.17%)	*E. faecalis* (n=2)
Pork meat	4/19 (21.05%)	4/92 (4.34%)	*E. faecium* (n=3) *E. faecalis* (n=1)
Poultry meat	8/21 (38.09%)	8/92 (8.69%)	*E. faecalis* (n=5) *E. faecium* (n=2) *E. durans* (n=1)
Total		**16/92 (17.39%)**	

tomycin adenyltransferase, which remained susceptible to gentamicin (Cetinkaya et al., 2000). Resistance to streptomycin occurring in enterococci may be moderate (MIC ranges from 62 mg/ml to 500 mg/ml) or high (MIC ^{3} 2,000 mg/ml) and is either ribosomally-mediated or occurs due to the synthesis of enzymes that inactivate aminoglycosides. Gentamycin resistance was confirmed in three isolates (3.26%) and quinupristin/dalfopristin in four (4.34%) isolates of *E. faecium*. Quinupristin-dalfopristin is a combination of antibiotics approved by the FDA for treating infections caused by vancomycin-resistant *E. faecium*. Quinupristin–dalfopristin-resistant *E. faecium* detected outside hospitals was considered to result from the use of virginiamycin in animals (Hammerum, 2012). The latter was banned in the EU in 1999 due to its possible selection for macrolide resistance in *E. faecium*. Transfer of gentamicin resistance in *E. faecium* and *E. faecalis* and of one of quinupristin/dalfopristin in *E. faecium* may pose serious risks, which considerably differ worldwide. This type of resistance varied from 28% to 73% in *E. faecium* isolates from poultry meat in Europe and the US (Bortolaia et al., 2016). Resistance to quinupristin-dalfopristin was confirmed in *E. faecium* isolates from turkey (54%), chicken (27%), pork (9%), and beef (18%) meat in Iowa (Hayes et al., 2003). High resistance to quinupristin-dalfopristin was detected in 79.3% *E. faecalis* strains isolated from cattle, pig, and poultry meat in Poland (Rożanska et al., 2015) and also in 28.8% of *Enterococcus* isolates from sheep, goat, and cattle carcasses in Turkey (Cebeci, 2024). *E. faecium* strains in poultry meat products may be donors of quinupristin/dalfopristin and other resistance determinants of clinical interest to the human intestinal microbiome (Bortolaia et al., 2016).

Resistance to fluoroquinolones is worrisome as it was detected in six isolates. This type of resistance depends on the breeding structure and antibiotic usage in veterinary medicine. In previous research of antimicrobial resistance in enterococci isolated in the poultry farm environment (overshoes or feces) in South Bačka and Srem, resistance to fluoroquinolones was detected in 37.5% isolates, all of which were MDR (Velhner et al., 2024). Interestingly, resistance to fluoroquinolones in the current work was confirmed only in MDR enterococci isolates from poultry meat. In this research, fluoroquinolone-resistant enterococci isolates contributed 6.5% to the total enterococci isolates, yet they accounted for as much as 28.57% of those isolated from poultry meat. This is in line with some previous research when 111 *E. faecalis* isolates from raw pork, cattle and poultry meat were checked for antimicrobial resistance, and the one to fluoroquinolones was confirmed in poultry meat isolates only (Rožanska et al., 2015). Resistance to ciprofloxacin was prevalent in *E. faecium* isolates from turkey (41%) and chicken (22%) meat (Hayes et al., 2003). Considerably higher resistance to ciprofloxacin was detected in swine fecal isolates from 61 farms throughout China: 73.6% and 66.1% in *E. faecium* and *E. fecalis*, respectively (Xuan et al., 2021). Resistance to vancomycin was confirmed neither in *Enterococcus* spp. from poultry and turkey farms in Vojvodina (Velhner et al., 2024), nor in the current work, which may be explained by the fact that avoparcin was not used as a poultry food additive in Serbia. Also, vancomycin-resistant strains of enterococci were not confirmed among 120 *E. faecalis* and 21 *E. faecium* isolates from fresh beef and pork in Slovenia (Golob et al., 2019), 1,357 enterococci isolates from raw poultry (chicken and turkey), pork and beef meats obtained from 263 stores in Iowa (Hayes et al., 2003) and 111 isolates from cattle, pig, and poultry meat sampled in slaughterhouses in Poland (Rożanska et al., 2015). In *E. faecium* and *E. faecalis* isolates in pig production in China, the resistance to vancomycin was extremely rare (Xuan et al., 2021). In Zambia, 97.3% of poultry isolates were susceptible to vancomycin (Mwikuma et al., 2023).

The spread of VRE has led to the use of new antibiotics such as linezolid, teicoplanin, and tigecycline. Study Bocella et al. (2021) showed high sensitivity of human isolates to those antibiotics. The tigecycline resistance rates in *E. faecium* and *E. faecalis* human isolates were reported as 0.7% and 0.5%, respectively, and this drug is used to treat bacteremia caused by MDR enterococci (Bocella et al., 2021).

In this research, resistance to nitrofurantoin, frequently used for urinary infection treatment, was detected in five and chloramphenicol in three enterococcus isolates obtained from meat. Chloramphenicol use has been limited in food animals for several decades (Gilmore et al., 2014). Thus, it is unsurprising that chloramphenicol resistance decreased when monitored in various human-derived samples in 2017-2020 (Rohana, Hager-Cohen, Azrad & Peretz, 2023). Chloramphenicol resistance was relatively rare (1–7%) in *E. faecium* and *E. faecalis* isolates from broilers and pigs in Denmark (Aarestrup, 2000).

Out of the 92 enterococci isolates, 16 (17.39%) were MDR, primarily those from poultry (38.09%) and pork meat (21.05%). This is in line with MDR detected in Poland, in enterococci from cattle, pig, and poultry meat (Rożanska et al., 2015), and also in *E. faecalis* (22.3%) and *E. faecium* isolates (11.1%) from sheep's and goat's milk (Gołaś-Prądzyńska, Łuszczyńska & Rola, 2022). Our results suggest that raw products of animal origin are possible reservoirs of multi-antibiotic-resistant enterococci in the food chain (Kročko et al., 2011).

All enterococci we isolated from dairy products (n=22) were identified as *E. faecium* (n=16) and *E. faecalis* (n=6), which are quite regular species in autochthonous dairy products, generally in the Western Balkans, including Serbia (Terzić Vidojević et al., 2015; Popović et al., 2018). The phenotypic resistance to antimicrobials was less prevalent in enterococci isolates from dairy products than in meat isolates. This is quite favourable since the former are consumed directly, unlike meat products that are usually heat processed before consumption, which virtually inactivates most bacteria, including enterococci (Johnston and Jaykis, 2004). Although enterococci are regularly detected in milk and meat, the sources of contamination differ. Meat is usually contaminated with *E. faecalis* and *E. faecium* from the intestines of slaughtered animals (Franz et al., 1999; Golob et al., 2019); contamination in poultry meat may reach 96% (Bortolaia et al., 2016). By contrast, fecal contamination seems insignificant for milk products (Giraffa, Carminati & Neviani, 1997; Dapkevicius, Sgardioli, Camara, Poeta & Malcata, 2021), unlike milk equipment (milking machines and bulk tanks), which are considered major sources of enterococci. The presence of enterococci in pasteurized milk products results from their thermal resistance and/or post-treatment contamination with biofilms present on milk-contact surfaces. For these reasons, enterococci are regarded as indicators of poor sanitary conditions in milk processing facilities (Giraffa et al., 1997, 2002; Jamet, 2012; Dapkevicius et al., 2021).

In the current study, 59.1% of enterococci isolates from dairy products were susceptible to all tested antibiotics, and 36.36% were resistant to only a single one, most frequently erythromycin. Resistance to macrolides, i.e. erythromycin, is acquired by important pathogenic enterococci (Gray, Stewart & Pedler, 1991), owing to the use of erythromycin in people allergic to penicillin. Erythromycin resistance has been frequently detected in enterococci obtained from dairy products: in 76.92% (Vyrostkova et al., 2021), over 44% (Terzić Vidojević et al., 2015). However, those results were obtained on cheeses traditionally produced using enterococci as starter cultures, but the isolates were found to be resistant to some other antibiotics of medical importance.

Out of 363 enterococci isolates originating from 12 locations in the Western Balkans, nearly every other (44%) was resistant to ciprofloxacin and erythromycin, and 26.2% were MDR (Terzić Vidojević et al., 2015). A considerable percent (57%) of enterococci isolates obtained from dairy products originated from Golija mountain (Serbia) and Prigorje region (Croatia) were resistant to ciprofloxacin or gentamicin; the widespread use of these antibiotics in animal husbandry in the Western Balkans implies the human influence on dairy products microbiota (Popović et al., 2018). In Slovakia, out of 52 *E. faecium*, *E. faecalis*, and *E. durans* strains obtained from sheep and goat cheeses, 84.62% were resistant to vancomycin and teicoplanin, 76.92% to erythromycin and rifampicin, and as many as 80% were MDR (Vyrostkova et al., 2021). In Poland, enterococci isolates from fermented milk products were found to be resistant to streptomycin (29.1%), erythromycin (14.3%), and tetracycline (11.6%) (Chajęcka-Wierzchowska et al., 2017). In this research, only a single isolate of *E. faecium* obtained from ice cream was MDR (resistant to ERY, FTN, QDF, and STR). However, high percentages of MDR *E. faecalis* (88.9%) and *E. faecium* (32%) were recently detected in raw milk, ice cream, mahalabia (milk pudding), and milk rice sampled in dairy shops in Assiut, Egypt (Sadek & Koriem, 2022). The (mis)use of antibiotics in humans and animals inevitably leads to the spreading and persistence of resistant microbials in animal-derived products (Van den

Bogaard & Stobberingh, 2000; Witte, 2000). Intensive animal husbandry and the long-lasting practice of continuous exposure to subtherapeutic concentrations of antibiotics as animal growth promoters have contributed to the development of antibiotic resistance mechanisms not only in pathogenic but also in commensal gut microorganisms, enterococci being the prominent example. EU legislation does not propose obligatory monitoring of antimicrobial resistance in enterococci (*E. faecalis* and *E. faecium*) isolated from animals and meat (Golob et al., 2019).

However, antimicrobial-resistant enterococci are frequently detected in raw, unfermented, fermented and ready-to-eat foods, meat and dairy products, and even strains used as probiotics (Giraffa, 2002).

Foods containing enterococci were long considered safe for human consumption (Giraffa et al., 1997). Nonetheless, it does not seem to remain so. It is significant to reduce the therapeutic use of antimicrobials in food-producing animals and administer antibiotics only when unavoidable. In addition, it is crucial to monitor antimicrobial resistance to help prevent the transmission of MDR clones to the environment, animal farms, hospitals and communities.

CONCLUSIONS

The profile of phenotypic resistance to antibiotics in enterococci isolated from food of animal origin correlates with the possible sources of contamination (faecal or environmental origin) and with the practice of antibiotic use in animal husbandry. Most common are strains with resistance to antibiotics that are particularly important for the protection of human health, and MDR strains found in poultry products. As species with exceptional evolutionary characteristics adapt to adverse environmental conditions, enterococci successfully adapt to the overuse of antibiotics. Comprehensive control of antimicrobial resistance of enterococci strains in food is not possible. To control the occurrence and spread of resistant strains, measures should start with strict monitoring and the controlled and therapeutically justified use of antibiotics in animal husbandry.

AUTHOR CONTRIBUTIONS

Conceptualization, investigation, methodology, S.V-K., J.V., S.D., and S.K.; Original draft preparation, D.M.; Editing and critically reviewing the work, N.A. All the authors have read and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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ANTIMIKROBNA REZISTENCIJA SOJEVA *ENTEROCOCCUS* SPP. IZOLOVANIH IZ NAMIRNICA ANIMALNOG POREKLA

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Sažetak: Cilj rada je ispitivanje rezistencije na antibiotike sojeva *Enterococcus* spp. izolovanih iz namirnica animalnog porekla iz maloprodajnih objekata na teritoriji A.P. Vojvodine. Primenom metoda PCR i MALDI TOF identifikovane su sledeće vrste: *E. faecalis* (51.08%), *E. faecium* (39.13%), *E. hirae* (6.52%), *E. thailandicus* (2.17%) i *E. durans* (1.08%). Kod izolovanih sojeva najčešće je ustanovljavana rezistencija na: tetraciklin (34.78%), eritromicin (27.17%), doksiciklin (21.73%) i streptomicin (13.04%). Izolati iz proizvoda od mleka su pokazali manju prevalencu fenotipske rezistencije na antibiotike u odnosu na izolate iz mesa. Od ukupno 92 izolata enterokoka, 16 (17.3%) je bilo rezistetno na tri ili više klasa antibiotika, pri čemu je najveći broj izolovan iz mesa živine (38.09%) i svinja (21.05%). Rezistencija na fluorohinolone potvrđena je kod 6 (28.57%) multiplo rezistetnih sojeva izolovanih iz mesa živine. Rezistencija na vankomicin, ampicilin, linezolid, teikoplanin i tigeciklin nije ustanovljena.

Ključne reči: *Enterococcus, rezistencija na antibiotike, namirnice animalnog porekla*

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