

Prevalence and antibiotic resistance of vancomycin-resistant enterococci in animal originated foods

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Abstract: A total of 270 animal-originated foods purchased from markets, including milk and dairy products, fresh and processed meat products, and poultry and poultry products, were analysed to determine the prevalence of vancomycin-resistant enterococci (VRE). VRE were isolated from 12 out of 270 samples (4.4%): 6 (6%) from fresh meat and meat products and 6 (8.6%) from poultry and poultry products. VRE strains were identified as *Enterococcus faecium* (6 strains), *E. avium* (4 strains), *E. durans* (1 strain) and *E. gallinarum* (1 strain). Among these strains, 11 had high levels of vancomycin resistance (2 *E. faecium* strains had minimum inhibitory concentrations [MICs] equivalent to 64 µg/mL and the others had MICs of ≥256 µg/mL), while the remaining 1 *E. faecium* had intermediate levels of vancomycin resistance (MIC = 12 µg/mL). In addition to vancomycin, all of the VRE strains were also resistant to one or more antibiotics, including teicoplanin, ampicillin, penicillin, ciprofloxacin and tetracycline.

Key words: *Enterococcus*, vancomycin-resistant enterococci, multidrug resistance, food, prevalence

1. Introduction

Enterococci are a group of bacteria belonging to the normal flora of the gastrointestinal tract of humans and animals. They are also isolated from vegetables, plant material, water, and foods, probably because of the contamination of the environment by human and animal faeces (1). Recently, they have emerged as the major cause of worldwide nosocomial infections (2) in immunocompromised patients. Among enterococci, *E. faecalis* is responsible for the majority of infections (80%–90%), followed by *E. faecium* (3).

Enterococci may acquire resistance to antimicrobial agents through transfer of plasmids and transposons, chromosomal exchange, or mutation. The major concern is the emergence of vancomycin-resistant enterococci (VRE), since vancomycin is considered one of the last alternatives when treating infections caused by gram-positive bacteria (4,5) after most other antibiotics have failed. The risk of transfer of resistance genes from VRE to other pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* is also a cause for concern (6).

VRE were isolated for the first time in 1986 in Europe and in 1987 in the United States. Since then, VRE infections have been reported all over the world (7). Different mechanisms of vancomycin resistance have been described, either of the acquired type (*vanA*, *vanB*,

vanD, *vanE*, *vanG*, and *vanL*) or the intrinsic type (*vanC*, associated with the *E. gallinarum* and *E. casseliflavus/flavescens* species) (8, 9).

Avoparcin, an analogue of the glycopeptide vancomycin, was used for several years as a growth promoter in animal husbandry. However, possible transmission of animal VRE to humans via the food chain forced authorities to ban the use of this drug in the EU in April 1997 (10). Furthermore, several reports have suggested that the use of avoparcin contributes to the development of cross-resistance against glycopeptide antibiotics (vancomycin and teicoplanin) in enterococci (11–13).

The primary reservoir and contamination source of resistant enterococci, particularly VRE, is healthcare settings. Dissemination of VRE and other resistant enterococci may be either by infected patients or by the transfer of bacteria from contaminated medical instruments and surfaces (5). Nevertheless, several studies showed the presence of VRE in farm animals (4,6,7) and animal-originated food samples in different countries (1,14–16), suggesting that the food chain would be a potential vehicle for transmission of VRE from animals to humans.

The purposes of the present study were to determine the prevalence of VRE in animal-originated foods purchased from different locations in the South Marmara

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Region of Turkey and to verify the species distribution and antibiotic resistance/susceptibility pattern of the VRE isolates obtained.

2. Materials and methods

2.1. Sample collection

Between September and December 2011, a total of 270 food samples were collected from different cities located in Turkey's South Marmara Region. The samples included milk and dairy products (20 raw milks, 60 cheeses, 10 yogurts, and 10 butters), meat and meat products (20 beef cuts, 20 raw ground meats, 20 raw meatballs, and 40 processed meats products including salami, sausage, sucuk, and pastrami), poultry products (60 raw chicken meat cut samples and 10 chicken by-products). Raw milk samples were provided from several dairy farms while the other samples were purchased from large supermarkets and smaller stores, restaurants, fast food centres, and neighbourhood bazaars. The samples were transported to the laboratory on the sampling day and processed immediately.

2.2. Isolation and phenotypic identification

For isolation of VRE (17), 25 g/mL of each sample was added to 225 mL of Enterococcosel Broth (Becton Dickinson, France) supplemented with vancomycin (final concentration of 6 µg/mL) (Oxoid SR186), and the broth was incubated at 35 ± 2 °C for 24 h. A loopful of culture was then spread on plates of Enterococcosel Agar (Becton Dickinson) supplemented with vancomycin (final concentration of 6 µg/mL). After 24 h of incubation at 35 ± 2 °C, presumptive VRE colonies (surrounded by a black halo) were randomly picked from the plates and transferred to Blood Agar Base (Oxoid CM0271). Pure cultures were kept in Brain Heart Infusion broth (Oxoid CM225B) containing 30% glycerol at -80 °C. The following tests were carried out for presumptive identification of the isolates: Gram staining; catalase and oxidase production; growth in MRS broth (Oxoid CM359B) supplemented with 6.5% NaCl, at pH 9.6 and at 10 °C and 45 °C; and growth and esculin hydrolysis on Bile Esculin Agar (Becton Dickinson). The PYR test (Oxoid ID0580) was also used to detect pyrrolidonyl aminopeptidase activity.

2.3. Species identification

The isolates were identified at the species level using the API 20 STREP system (bioMérieux, France) according to the manufacturer's instructions.

2.4. Antibiotic susceptibility testing

The antibiotic susceptibility of isolates was determined using the disc diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (18). The antibiotics tested and their concentrations in the discs were as follows: ampicillin 10 µg, penicillin 10 U, linezolid 30 µg, quinupristin/

dalfopristin 15 µg, vancomycin 30 µg, teicoplanin 30 µg, ciprofloxacin 5 µg, tetracycline 30 µg, gentamicin 120 µg, and streptomycin 300 µg (Oxoid, UK). *Staphylococcus aureus* ATCC 25923 was used as the control strain. For the experiment, the turbidity of overnight cultures in Mueller-Hinton Broth (Oxoid CM0405) was adjusted to 0.5 McFarland standards. Each suspension was spread on the surface of Mueller-Hinton Agar (MHA, Oxoid CM337) plates with a sterile stick and then the antibiotic discs were placed on the agar medium. The plates were incubated at 37 °C for 24 h. According to the inhibition zone measured, the strains were classified as susceptible, intermediate, or resistant by taking into account the criteria of the CLSI.

The minimum inhibitory concentrations (MICs) for both vancomycin and teicoplanin were determined by the agar dilution method according to the CLSI guidelines (18). Etest strips (AB Biodisk, Sweden) were used to measure MICs. A bacterial suspension equal to 0.5 McFarland turbidity standards was prepared from each VRE isolate and inoculated onto MHA plates. The Etest strips were applied to the inoculated culture plates separately, and the plates were incubated at 35 ± 2 °C for 24 h under aerobic conditions. The MICs were interpreted at the point of intersection between the inhibition zone and the Etest strip. The lowest concentration showing inhibition of growth was considered the MIC. The isolates that had MICs of ≥32 µg/mL were considered resistant, MICs of 8–16 µg/mL intermediately resistant, and MICs of ≤4 µg/mL susceptible to vancomycin. *Enterococcus faecalis* ATCC 29212 was used as the control.

3. Results

The present study was performed to determine the presence of VRE in animal-originated foods purchased from different retail markets in the South Marmara Region and the antibiotic resistance profiles of isolated strains. Selective enrichment technique in Enterococcosel Broth supplemented with 6 µg/mL vancomycin was used for isolation of VRE from the samples. Our studies evidenced that 12 (4.4%) out of 270 food samples harboured VRE, with contamination rates ranging from 8.6% of chicken samples to 6.0% of meatballs and meat cuts. These strains were identified as *E. faecium* (6 isolates), *E. avium* (4 isolates), *E. gallinarum* (1 isolate), and *E. durans* (1 isolate) using API 20 STREP test strips. Data related to the prevalence and identification at species level are presented in Table 1.

The susceptibility of 12 VRE strains to 10 antibiotics, including ampicillin, penicillin, linezolid, quinupristin/dalfopristin, vancomycin, teicoplanin, ciprofloxacin, tetracycline, gentamicin (high level), and streptomycin (high level), was evaluated by disc diffusion method and the results are presented in Table 2. All strains also exhibited

Table 1. Distribution of VRE^a strains in relation with the origin of food samples and species.

Origin of samples	Number (%) of samples with VRE	VRE isolated	
		Species	Number
Meat/meat products	6 (6%)	<i>E. faecium</i>	2
		<i>E. avium</i>	3
		<i>E. gallinarum</i>	1
		<i>E. faecium</i>	4
Poultry/poultry products	6 (8.6%)	<i>E. avium</i>	1
		<i>E. durans</i>	1
Milk/dairy products	- ^b		
Total	12 (4.4%)		12

^a: Vancomycin-resistant enterococci.

^b: Not detected.

resistance to teicoplanin, 6 strains to tetracycline, 4 strains to ciprofloxacin, 2 strains to ampicillin, and 2 strains to penicillin. The multiresistance data of VRE strains to one or more antibiotics are summarised in Table 3. On the other hand, all of the strains were found to be susceptible to linezolid, gentamicin, and streptomycin.

The MIC data of vancomycin and teicoplanin for VRE strains were obtained by Etest (Table 4). Strains with vancomycin MICs of ≤4 µg/mL were considered vancomycin-susceptible, those with MICs of 8–16 µg/mL intermediately vancomycin-resistant, and those with MICs of ≥32 µg/mL vancomycin-resistant. The results displayed that 12 strains characterised as vancomycin-resistant by disc diffusion method were resistant to vancomycin

according to the Etest. Eleven out of 12 VRE strains were observed as having high-level vancomycin resistance. All of the vancomycin-resistant strains were also resistant to teicoplanin.

4. Discussion

In this investigation, 12 samples (4.4%) from 270 animal-originated foods were found to be contaminated with VRE (Table 1). The percentage distribution of VRE strains was 8.6% (6 of 70 samples) in poultry and poultry parts (breast, thigh, wing, etc.) and 6.0% (6 of 100 samples) in meatballs and meat cuts. VRE were isolated from several foodstuffs in many other countries such as Italy (10,19), Spain (9,20), Germany (13), Korea (21), Denmark (16), and Brazil

Table 2. Antibiotic resistance profiles of VRE strains by disc diffusion method.

Sample origin	Species	Antibiotic									
		Va	Tec	Amp	P	Lin	QD	Cip	Te	Cn	S
Meatball	<i>E. gallinarum</i>	R	R	S	S	S	S	R	S	S	S
Meatball	<i>E. avium</i>	R	R	S	S	S	S	IM	S	S	S
Meatball	<i>E. faecium</i>	R	R	S	S	S	S	S	R	S	S
Meatball	<i>E. avium</i>	R	R	S	S	S	S	R	S	S	S
Veal cubes	<i>E. avium</i>	R	R	S	S	S	S	IM	S	S	S
Chicken meat	<i>E. faecium</i>	R	R	S	S	S	S	S	R	S	S
Chicken wing	<i>E. faecium</i>	R	R	S	S	S	S	S	R	S	S
Chicken meat	<i>E. faecium</i>	R	R	S	S	S	S	S	S	S	S
Chicken wing	<i>E. durans</i>	R	R	R	R	S	IM	R	R	S	S
Meatball	<i>E. faecium</i>	R	R	S	S	S	S	IM	R	S	S
Chicken thigh	<i>E. avium</i>	R	R	S	S	S	S	IM	S	S	S
Chicken breast	<i>E. faecium</i>	R	R	R	R	S	IM	R	R	S	S

Va, vancomycin; Tec, teicoplanin; Amp, ampicillin; P, penicillin; Lin, linezolid; QD, quinupristin/dalfopristin; Cip, ciprofloxacin; Te, tetracycline; Cn, gentamicin (high level); S, streptomycin (high level), R, resistant; IM, intermediately resistant; S, susceptible.

Table 3. Multidrug resistance patterns for vancomycin-resistant strains.

Species	Number of strains		Resistance pattern	Number of strains
	Resistant	Multiple resistance		
<i>E. faecium</i>	6	6	Va, Tec, Amp, P, Cip, Te	1 ^a
			Va, Tec, Te	3
			Va, Tec	1
			Va, Te	1
<i>E. avium</i>	4	4	Va, Tec	3
			Va, Tec, Cip	1
<i>E. gallinarum</i>	1	1	Va, Tec, Cip	1
<i>E. durans</i>	1	1	Va, Amp, P, Cip, Te	1
Total	12	12		12

^a: Strain having intermediate level resistance to Va and Tec.

Va, vancomycin; Tec, teicoplanin; Amp, ampicillin; P, penicillin; Cip, ciprofloxacin; Te, tetracycline.

Table 4. MIC values of vancomycin and teicoplanin for VRE strains.

Sample origin	VRE strains	Antibiotic MICs (µg/mL)	
		Va	Tec
Meatball	<i>E. gallinarum</i>	≥256	≥256
Meatball	<i>E. avium</i>	≥256	≥256
Meatball	<i>E. faecium</i>	≥256	≥256
Meatball	<i>E. avium</i>	≥256	≥256
Veal cubes	<i>E. avium</i>	≥256	≥256
Chicken meat	<i>E. faecium</i>	64	16
Chicken wing	<i>E. faecium</i>	64	32
Chicken meat	<i>E. faecium</i>	≥256	≥256
Chicken wing	<i>E. durans</i>	≥256	48
Meatball	<i>E. faecium</i>	≥256	≥256
Chicken thigh	<i>E. avium</i>	≥256	≥256
Chicken breast	<i>E. faecium</i>	12	12

(22). As shown in Table 1, out of the 12 VRE strains detected in our survey, 6 were identified as *E. faecium*, 4 as *E. avium*, 1 as *E. gallinarum*, and 1 as *E. durans*. These species are among the clinically significant species that are implicated in enterococcal infections. Although the vast majority of infections in humans are due to either *E. faecalis* or *E. faecium* (3,5), infections caused by other *Enterococcus* species including *E. durans* (23), *E. avium* (24), *E. gallinarum* (25,26), and *E. casseliflavus* (27) might also occur.

Interestingly, we did not identify *E. faecalis* among VRE strains, which is the most common enterococci species found in foods. In contrast, Koluman et al. (14) identified 4

chicken isolates as having vancomycin-resistant *E. faecalis*. Furthermore, VRE were not isolated from the milk and dairy products analysed. This result is in agreement with data obtained previously by Morandi et al. (3), in which the authors did not find vancomycin-resistant enterococci in dairy products, but not comparable with those obtained by other authors (1,21) reporting the presence of VRE in cheeses and raw milk samples.

The results of the present study indicate that consumers are exposed to VRE from several animal-originated foods. This may be either directly by consumption of contaminated foods or indirectly by cross-contamination to other foods during processing (16).

A specific concern contributing to the pathogenesis of enterococci is their resistance to a variety of antibiotics (11). The use of antibiotics for a long period of time in animal feeding as a growth promoter resulted in the spread of transferable antibiotic resistance elements in several ecosystems. Food chains may also have a possible role in the transmission route of resistant enterococci (15).

By disc assay, in addition to vancomycin, all of the VRE strains isolated in the present study displayed resistance to at least one other antibiotic tested (Table 2). Multiple-antibiotic resistance patterns among VRE strains are shown in Table 3. Among 12 vancomycin-resistant strains, 6 were also resistant to tetracycline, 4 to ciprofloxacin, 2 to penicillin, and 2 to ampicillin. These results are in good agreement with those of previously published studies on VRE from food products in different countries, indicating the worldwide importance of antibiotic resistance (9,10,21,22). Robredo et al. (20) reported resistance to quinupristin/dalfopristin among vancomycin-resistant *E. faecium* strains isolated from chicken in Spain. In comparison to that study, we observed that 3 (2 *E. faecium* and 1 *E. durans*) of the VRE strains had intermediate resistance to quinupristin/dalfopristin. None of the VRE strains from the present survey exhibited resistance to gentamicin and streptomycin. A similar observation was made by Gomes et al. (22), who isolated vancomycin-resistant but gentamicin-susceptible *E. faecium* strains from several animal-originated foods. All the tested strains exhibited susceptibility to linezolid, which is a new synthetic oxazolidinone antibiotic active against gram-positive bacteria, including VRE (28).

Vancomycin and teicoplanin MICs of VRE strains, as determined by Etest, are shown in Table 4. Among these VRE, 11 strains exhibited high-level resistance, 2 of which had a MIC value equivalent to 64 µg/mL, while 9 had MICs

higher than 256 µg/mL. VRE having MIC values higher than 64 µg/mL were reported for the isolates from Danish meat products by Wegener et al. (16) and from Italian cheeses by Giraffa et al. (1). Another study carried out by Pedonese et al. (29) in Tuscany revealed that *E. faecium* isolated from meat products had MIC values of ≥ 256 µg/mL. In the present study, 1 *E. gallinarum* specimen isolated from a meatball exhibited a MIC value of ≥ 256 µg/mL. Intermediate-level resistance of *E. gallinarum* strains (MIC of 8 µg/mL) was reported in cows' milk with mastitis, chicken, and turkey litter in the United States (4).

As presented in Table 4, 12 vancomycin-resistant strains were also resistant to teicoplanin. MICs of teicoplanin for 10 VRE strains varied from 32 µg/mL to ≥ 256 µg/mL. However, 2 strains, 1 of which was with high-level resistance to vancomycin, had intermediate teicoplanin MICs (12 and 16 µg/mL, respectively). MICs of teicoplanin for vancomycin-resistant *E. faecium* from chicken and meat products were reported to change between 16 and 128 µg/mL by Chen et al. (30) and ≥ 128 µg/mL by Pedonese et al. (29).

The present study reports the contamination of animal-originated foods with VRE in the South Marmara Region. Our findings confirm the presence of VRE in foods and highlight their potential in the dissemination of vancomycin resistance in nature. Since vancomycin is the last resort of antibiotics used to control enterococcal infections, special attention has to be given to routine analysis of VRE in animal-originated foods and to the controlled use of antimicrobials in veterinary medicine and animal husbandry.

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