An Acad Bras Cienc (2022) 94(1): e20201765 DOI 10.1590/0001-3765202220201765
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

MICROBIOLOGY

Antimicrobial resistance of enterococci isolated from food in South Brazil: Comparing pre- and post-RDC 20/2011

LETÍCIA DA F.X. COSTA, DAIANE A. FALCÃO, TIELA T. GRASSOTTI, FRANCIELE D.P. CHRISTIANO, JEVERSON FRAZZON & ANA PAULA G. FRAZZON

Abstract: Antimicrobial resistance has been attributed to the overuse of antibiotics. To control the use of antibiotics, Brazil adopted the RDC 20/2011. A comparison the antibiotic-resistance profile of bacterial has provided important insights into resistance evolution. Enterococci are ubiquitous bacteria recommended to be used as a sentinel organism, in national surveillance systems, for tracking antimicrobial resistance through the food chain. The present study aimed to evaluate the diversity and antimicrobial resistance of enterococci collected from food in South Brazil in 2017 (pos-RDC 20/11) for comparison with isolated in 2007 (pre-RDC 20/11). A total of 310 enterococci were isolated from vegetables and products of animal origin, identified by PCR and MALDI-TOF, tested for antimicrobial susceptibility and screened for resistance genes. *Enterococcus casseliflavus* was dominant in vegetables and *E. faecalis* in products of animal origin. Enterococcal isolates in 2017 were mostly sensitive to ampicillin, gentamicin, chloramphenicol, and vancomycin when compared to isolated collected in 2007. While resistance levels to most compounds remained relatively stable, multidrug resistance decreased by 24% during this period. Our results suggest that RDC 20/11 had a positive outcome in controlling the spread of antimicrobial resistance. This study provides baseline data to measure future changes in the prevalence of resistant enterococci.

Key words: Enterococcal isolates, vegetable and products of animal origin, antibiotic resistance, RDC 20/2011.

INTRODUCTION

Antimicrobials are widely used to treat bacterial infections in humans and animals. Moreover, they are also used as growth promoters in animal husbandry and aquaculture. The misuse and overuse of antimicrobials are the main driving forces for the selection of resistant bacterial populations in various environments (World Health Organization 2020). The impact of the presence of antimicrobial agents or resistant bacteria in all environments is a growing global public health concern. Antibiotic-resistant bacteria can reach humans directly through the consumption of contaminated food and/or indirectly through environmental pollution containing human or animal excrements (Kristiansson et al. 2011, Marathe et al. 2013).

In terms of transmission through food, antimicrobial resistant bacteria can contaminate animal products during slaughter and processing. Moreover, fruits and vegetables can be contaminated by direct contact with soil, water, or fertilizer containing insufficiently treated material (e.g., animal feces-based fertilizers or human sewage). Several studies have identified resistant bacteria in food products and animal samples, such as

methicillin-resistant *Staphylococcus aureus* in livestock (Price et al. 2012), extended spectrum-beta-lactamase *Escherichia coli* on vegetables, raw chicken, raw egg-surfaces, and raw meat (Rasheed et al. 2014), and antibiotic-resistant *enterococci* in various foods (McGowan et al. 2006, Gomes et al. 2008, Frazzon et al. 2009, Riboldi et al. 2009, Ben Said et al. 2015). Studies have suggested that antimicrobial-resistant bacteria and their resistance genes can be transmitted from food to humans via the food chain (Leverstein-van Hall et al. 2011, Verraes et al. 2013).

Enterococci are ubiquitous microorganisms found in the gastrointestinal tracts of humans and animals, as well as in plants, sewage, water, soil, and foods (Frazzon et al. 2009, Lebreton et al. 2014, Grassotti et al. 2018, Costa et al. 2019, Bhardwaj 2019). *Enterococcus* spp. exhibit greater environmental persistence and are regarded as robust organisms capable of tolerating a wide range of temperatures and pH levels that can grow in the presence of 6.5 % sodium chloride (NaCl) or 40% of bile salts. Additionally, they can exchange resistance determinants through gene transfer by plasmids and transposons, among themselves and with other genera (Lebreton et al. 2014). Due to their ubiquity, resilience and ability to acquire antibiotic resistance, enterococci have been recommended for integration into antimicrobial resistance-monitoring systems as sentinel bacteria (World Health Organization 2013).

Some enterococci species have been used in food and feed, such as starter-culture and probiotics, respectively (Giraffa 2002, Franz et al. 2003). However, despite their beneficial effects in foods, they can also be involved in food spoilage and their presence may also serve as an indicator of microbial contamination of fecal origin (Hanchi et al. 2018). Furthermore, enterococci are among the most important MDR microorganisms associated with health-associated infection worldwide, causing a wide range of infections in immunocompromised and hospitalized patients (Lebreton et al. 2014, Bhardwaj 2019). Nonetheless, resistant enterococci are not restricted to clinical samples, since strains have been isolated from various environments, including food, animals, and environmental samples (Frazzon et al. 2009, Lebreton et al. 2014, Grassotti et al. 2018, Costa et al. 2019, Bhardwaj 2019).

Interventions to promote practical antibiotic use are essential to reduce the emergence and spread of antimicrobial-resistant bacteria in human healthcare and food production. Many countries have been improving the prudent use and dispensing of antimicrobials (Roca et al. 2015). Notably, the World Health Organization (WHO) has created a set of strategies to combat rising antibiotic resistance, which include, colon improving sanitation and hygiene to reduce overall infection rates, and optimizing the use (and preventing the overuse) of antibiotics in humans and animals (World Health Organization 2013). In this sense, in 2010, the Brazilian Health Surveillance Agency published the RDC nº 20/2011 (RDC 20/11) to facilitate the dispensation and control of antimicrobial use (Brasil 2011).

Studies comparing antibiotic resistance patterns in collections of clinical strains have been conducted to address the antibiotic resistance in community settings (Ventola 2015, Massot et al. 2016, Kulik et al. 2019). However, few studies have compared antibiotic-resistant strains from food collections (Alonso-Hernando et al. 2012, Tyson et al. 2017). To date, no study in Brazil has compared antibiotic-resistant enterococci isolated from food products, since the RDC 20/11 was implemented. Therefore, the present study aimed to compare the species distribution and antibiotic resistance patterns of enterococci isolated from food products in

South Brazil in 2017 (pos-RDC 20/11) with those collected and isolated in 2007 (pre-RDC 20/11).

MATERIALS AND METHODS

Enterococci from foods

Isolation: A total of 30 food samples comprising: vegetables (cassava, beetroot, potato, sweet potato, parsley, carrot and cabbage), and food of animal sources (raw chicken meat, colonial cheese type and soft cheese) were purchased from different popular markets in the Porto Alegre, South Brazil in 2017. The enterococci isolation was performed according to protocol described by Riboldi et al. (2009).

Previous collection: *Enterococcus faecalis* (n=27), *E. faecium* (n=23), *E. mundtii* (n=1) and *Enterococcus* sp. (n=5)- isolated under the same conditions in 2007 (Frazzon et al. 2009, Riboldi et al. 2009) were used to compare species distribution and antibiotic resistance patterns.

Genus and species identification

DNA extraction: Genomic DNA was extracted by boiling method, as described by Depardieu et al. (2004). Genus-specific polymerase chain reaction (PCR) assays, which targeted the *tuf* gene, performed as described by Ke et al. (1999) (Table I). The *E. faecalis* ATCC 29212 and *E. faecium* SS1274 (D'Azevedo et al. 2006) were used as positive control.

PCR assay: Isolates were screened with the species-specific PCR assay for *E. faecalis*, *E. faecium*, *E. casseliflavus* and *E. hirae* (Table I). Amplifications were carried out in a total volume of 25 µL containing: 100 ng of template DNA, 1 X reaction buffer (Ludwig Biotechnology), 0.4 µM of each primer (Ludwig Biotechnology), 1.5 mM MgCl2, 200 µM of dNTPs (Ludwig Biotechnology), 1 U *Taq* DNA polymerase (Ludwig Biotechnology), and MilliQ water. PCR amplifications were performed in the conventional thermocycler (Applied Biosystems 2720 Thermal Cycler) according to the following program: 94 °C for

Table I. Primers used in the PCR reactions carried out for identification of *enterococci* genus and species.

		Nucleotide sequence (5'-3')	Size[1] (bp)	AT[2] (°C)	References
Genus					
		TACTGACAAACCATTCATGATG	112	55	Ke et al. (1999)
		AACTTCGTCACCAACGCGAAC			
Species					
	E. casseliflavus	TAGGATGTTACGTCTGCGTG	139	58	Medeiros et al. (2016)
		TTGTTGGTTTGGGCTTTTCCCG			
	E. faecalis	CCGAGTGCTTGCACTCAATTGG	136	66	Sedgley et al. (2005)
		CTCTTATGCCATGCGGCATAAAC			
	E. faecium	TTGAGGCAGACCAGATTGACG	172	62	Medeiros et al. (2016)
		CGGAAGTGATGCTTCCTACTG			
	E. hirae	TTATGTCCCWGTWTTGAAAAATCAA	94	62	Medeiros et al. (2016)
		TATTGATAAGCTAATGCAAGCGC			

1: bp, base pairs; 2: AT, annealing temperatures.

5 min followed by 35 cycles of 94 °C for 1 min, appropriate annealing temperature for each primer for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The DNA fragments amplified were analyzed in 1.5% (w/v) agarose gels stained with SYBR® Safe DNA Gel, and visualized on a photo-documenter.

Isolates not identified by PCR were submitted to matrix-assisted laser desorption and ionization time-of-flight technique (MALDI-TOF), according to Sauget et al. (2017).

Antimicrobial susceptibility analysis

Determination of antimicrobial susceptibility was performed by disk diffusion method, according to Clinical and Laboratory Standards Institute (CLSI 2017). Eleven antibiotics commonly used in clinical and veterinary medicine were evaluated: ampicillin (AMP - 10 µg), ciprofloxacin (CIP - 5 µg), chloramphenicol (CHL - 30 µg), erythromycin (ERY - 15 µg), gentamicin (GEN - 120 µg), nitrofurantoin (NIT - 300 µg), norfloxacin (NOR - 10 µg), streptomycin (STR - 300 µg), tetracycline (TET - 30 µg), rifampicin (RIF - 5 µg) and vancomycin (VAN - 30 µg). The minimum inhibitory concentration (MIC) for streptomycin, gentamicin and vancomycin was performed using the microdilution method according to CLSI (2017). Strains resistant to three or more unrelated antibiotics were considered as multidrug resistant (MDR) (Schwarz et al. 2010).

Detection of antibiotic resistance genes by PCR

Erythromycin-, gentamicin- and tetracycline-resistant strains were tested by PCR for presence resistance genes commonly associated with clinical and environmental enterococci. The *erm*(A), *erm*(B), *erm*(C) genes (Sutcliffe et al. 1996), which encode resistance to erythromycin from the modification of the target preventing the binding of macrolides, and the *msrC* gene (Werner et al. 2001), which produces low-level resistance to erythromycin through the efflux pump mechanism were tested in all erythromycin-resistant strains. To gentamicin-resistant strains, the *aac*(6')-*aph*(2') gene (Jia et al. 2014), which encode an enzymatic modification was evaluated. The *tet*(M) and *tet*(S) genes (Aarestrup et al. 2000) that encode for ribosome protection, and *tet*(L) gene (Frazzon et al. 2009) that encodes for efflux proteins were evaluated in all tetracycline-resistant strains. The primers used are listed in Table II.

Amplifications were carried out in a total volume of 25 µL containing: 100 ng of template DNA, 1 X reaction buffer (Ludwig Biotechnology), 0.4 µM of each primer (Ludwig Biotechnology), 1.5 mM MgCl2, 200 µM of dNTPs (Ludwig Biotechnology), 1 U Taq DNA polymerase (Ludwig Biotechnology), and MilliQ water. PCR amplifications were performed in the conventional thermocycler (Applied Biosystems 2720 Thermal Cycler) according to the following program: 94 °C for 5 min followed by 35 cycles of 94 °C for 1 min, appropriate annealing temperature for each primer for 1 min, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The DNA fragments amplified were analyzed in 1.5% (w/v) agarose gels stained with SYBR® Safe DNA Gel, and visualized on a photo-documenter.

Statistical analysis

The results obtained here were used to compare species distribution and antimicrobial resistant profile with our enterococcal collection isolated pre-RDC 20/11. The results obtained were submitted to statistical analysis, which was performed using Statistic Package of the Social Science (SPSS) software 18th edition, using Pearson's chi-square test (χ^2) ($p \leq 0.05$) and Fisher's exact test as evaluation parameters.

Table II. Primers used in the PCR reactions carried out for detection of resistance genes.

Resistance	gene	Nucleotide sequence (5'-3')	Size[1] (bp)	AT[2] (°C)	References
Erythromycin	*erm*(A)	TCTAAAAAGCATGTAAAAGAA	420	52	Sutcliffe et al. (1996)
		CTTCGATAGTTTATTAATATTAGT			
	erm(B)	GAAAAGGTACTCAACCAAATA	547	52	Sutcliffe et al. (1996)
		AGTAACGGTACTTAAATTGTTTAC			
	erm(C)	TCAAAACATAATATAGATAAA	837	52	Sutcliffe et al. (1996)
		GCTAATATTGTTTAAATCGTCAAT			
	msrC	AAGGAATCCTTCTCTCTCCG	343	52	Werner et al. (2001)
		GTAAACAAAATCGTTCCCG			
Gentamycin	*aac(6')-aph(2')*	CACTATCATAACCACTACCG	220	56	Jia et al. (2014)
		CCAAGAGCAATAAGGGCATA			
Tetracycline	*tet*(L)	ACTCGTAATGGTGTAGTTGC	625	58	Frazzon et al. (2009)
		TGTAACTCCGATGTTTAACACG			
	tet(M)	GTTAAATAGTGTTCTTGGAG	657	52	Aarestrup et al. (2000)
		CTAAGATATGGCTCTAACAA			
	tet(S)	TGGAACGCCAGAGAGGTATT	720	58	Aarestrup et al. (2000)
		ACATAGACAAGCCGTTGACC			

1: bp, base pairs; 2: AT, annealing temperatures.

RESULTS AND DISCUSSION

Enterococcus species diversity in food

Among the 360 Gram-positive and catalase-negative cocci isolated, 310 (86.1%) were confirmed as enterococci. *E. faecalis* (57.1%) was the most abundant species detected in both groups of analyzed food, followed by *E. casseliflavus* (33.2%), *E. hirae* (5.5%), *E. faecium* (1.9%), and *E. durans* (1.0%) (Table III). Four isolates (1.3%) could not be identified at the species level and were classified as *Enterococcus* sp. Considering the ubiquitous natural distribution of enterococci, the presence of these species in food is consistent with previous studies (McGowan et al. 2006, Gomes et al. 2008, Frazzon et al. 2009, Riboldi et al. 2009, Kim et al. 2020).

In the vegetables group, *E. casseliflavus* (50.99%) and *E. faecalis* (43.56%) were the most frequent species detected in cassava, beetroot, potato, sweet potato, parsley, and cabbage. *Enterococcus hirae* (2.47%), and *Enterococcus faecium* (0.99%) were identified in low rates. In all foods of animal origin, *E. faecalis* was more frequently found (82.4%), while *E. hirae* (11.11%), *E. faecium* (3.7%) and *E. durans* (2.77%) were only isolated in soft cheeses samples, and none of the samples contained *E. casseliflavus*. Although some differences in enterococcal distribution were detected among food samples from distinct origins, not significant differences could be verified ($p > 0.05$).

The predominance of *E. casseliflavus* in vegetables is consistent with previous studies (McGowan et al. 2006, Gomes et al. 2008, Kim

Table III. Distribution of *Enterococcus* species among food samples.

Source	Food samples (n)	Number (%) of species isolated					
		E. casseliflavus	***E. durans***	***E. faecalis***	***E. faecium***	***E. hirae***	***Enterococcus* sp.**
Vegetables	Sweet potato (35)	21 (6.8) [c]	0 [a]	12 (3.9) [a]	1 (0.3) [a]	0 [a]	2 (0.65) [a]
	Potato (36)	19 (6.1) [c]	0 [a]	17 (5.5) [b]	0 [a]	0 [a]	0 [a]
	Beetroot (24)	24 (7.7) [c]	0 [a]	0 [a]	0 [a]	0 [a]	0 [a]
	Carrot (3)	0 [a]	0 [a]	3 (1.0) [b]	0 [a]	0 [a]	0 [a]
	Cabbage (33)	5 (1.6) [a]	0 [a]	26 (8.4) [c]	1 (0.3) [a]	1 (0.3) [b]	0 [a]
	Parsley (34)	22 (7.1) [c]	0 [a]	6 (1.9) [a]	0 [a]	4 (1.3) [b]	2 (0.65) [a]
	Cassava (36)	12 (3.9) [b]	0 [a]	24 (7.7) [b]	0 [a]	0 [a]	0 [a]
	Sub-total (202)	103 (50.99)	0	88 (43.56)	2 (0.99)	5 (2.47)	4 (1.98)
Animal	Raw chicken meat (36)	0 [a]	0 [a]	36 (11.6) [c]	0 [a]	0 [a]	0 [a]
	Colonial cheese (36)	0 [a]	0 [a]	36 (11.6) [c]	0 [a]	0 [a]	0 [a]
	Soft cheese (36)	0 [a]	3 (1.0) [a]	17 (5.5) [b]	4 (1.3) [a]	12 (3.9) [c]	0 [a]
	Sub-total (108)	0	3 (2.77)	89 (82.4)	4 (3.7)	12 (11.11)	0
	Total (310)	103 (33.2)	3 (1.0)	177 (57.1)	6 (1.9)	17 (5.5)	4 (1.3)

Same downcase letters on the same column means there is no statistical difference between samples (p>0.05).

et al. 2020). The presence of *E. casseliflavus*, *E. faecium*, and *E. hirae* in vegetables samples can be associated with untreated wastewater used for agriculture (mainly irrigation) and/or raw animal manure (organic fertilizers) used on vegetable crops. This finding is supported by the published study of Ben Said et al. (2015) who isolated the same species from vegetables, soil, and irrigation water samples from farms in Tunisia.

The occurrence of *E. faecalis, E. hirae, E. faecium,* and *E. durans* is consistent with the results of McGowan et al. (2006), Gomes et al. (2008), Frazzon et al. (2009), Campos et al. (2013), Camargo et al. (2014), Kim et al. (2018) and Tyson et al. (2017), who also noted these species were predominant in meats and cheese samples. The higher occurrence of *E. faecalis* in raw chicken meat, can be justified by the manipulation and elevated susceptibility of these products to contamination with feces from slaughtered animals during the processing (Hölzel et al. 2018). The presence of *E. faecalis*, *E. faecium, E. durans,* and *E. hirae* in cheese samples may be related to the raw material used in cheese making process. Different enterococci species were found in artisanal cheeses made from raw or pasteurized milk from goats, sheep, buffaloes, and cows (Gelsomino et al. 2002). In cheeses, enterococci represent a major part of the microbiota and are the predominant microorganisms in the ripened cheeses (Lebreton et al. 2014).

Upon comparing the enterococci diversity observed in this study with the enterococcal collection isolated in 2007, some differences were observed. For example, in this study *E.*

casseliflavus and *E. hirae* was detected in vegetables, while Riboldi et al. (2009) and Frazzon et al. (2009) isolated only *E. faecalis* and *E. faecium* from vegetables. This difference can be related to numerous sources beyond the soil sources, fertilization and irrigation used in vegetable production (e.g., handling and storage) (Regitano & Leal 2010). On the other hand, no differences in the proportion of enterococci isolates from foods of animal origin (e.g., raw meat and colonial and soft cheeses) were observed upon isolates from 2007 and 2017. In both studies, *E. faecalis* was the most frequently isolated species.

Antimicrobial susceptibility patterns of enterococci isolated from food samples

Among the 310 strains isolated in this study, 33 (10.6%) were susceptible to all antimicrobials tested, while 277 (89.4%) showed resistance to at least one antimicrobial (Table IV). Resistance to rifampicin (54.8%) was the most commonly observed profile, followed by resistance to erythromycin (47.7%), tetracycline (31.3%), and ciprofloxacin (20%). A lower rate was found to streptomycin (14.8%), norfloxacin (12.6%), nitrofurantoin (9.3%), gentamicin (3.5%), chloramphenicol (2.3%), and ampicillin (0.6%). No resistance to vancomycin was observed. These findings are consistent with published data that also reported the presence of resistant enterococci in foods of animal and vegetable origin (McGowan et al. 2006, Ben Said et al. 2015, Ngbede et al. 2017, Kim et al. 2018).

Enterococci isolated from vegetables showed elevated rates of resistance to rifampicin (125/202, 61.88%) and erythromycin (82/202, 40.6%) were detected. Moderate rates of resistance were observed for ciprofloxacin (38/202, 18%), and tetracycline (34/202, 16.83%). Moreover, low rates of resistance were detected for norfloxacin (29/202, 14.3%), nitrofurantoin (26/202, 12.8%), streptomycin (25/202, 12.3%), and chloramphenicol (6/202, 2.9%). Several studies conducted with vegetables have isolated antimicrobial resistant enterococci (McGowan et al. 2006, Ben Said et al. 2015, Kim et al. 2017, Hölzel et al. 2018). Ben Said et al. (2015) evaluated the antimicrobial resistance of enterococci isolated from vegetables sourced from farms and markets in Tunisia, and noted resistance for ciprofloxacin, erythromycin, tetracycline, chloramphenicol, and streptomycin. Resistant strains were also observed in vegetables from grocery stores in Athens, Georgia, USA from 2000 to 2001 (McGowan et al. 2006). Furthermore, Ngbede et al. (2017) detected ampicillin- and aminoglycosides-resistant *E. faecium* strains isolated from vegetables in Nigeria. The occurrence of erythromycin-resistant enterococci in vegetable samples observed in our study might be associated with the application of untreated irrigation water and natural fertilizers from animal manure (Regitano & Leal 2010). Erythromycin is excreted as an active metabolite via the manure or urine of livestock and remains stable in the environments such as soil and water (Gothwal & Shashidhar 2014, Dizavandi et al. 2017). Biological fluids (e.g., urine and feces) contaminated with antimicrobials or antimicrobial-resistant strains are released into the environment, especially soil, water and wastewater. Kim et al. (2017) compared in Korea fecal samples with fresh vegetable samples in Korea and observed that the vegetables can be contaminated by human and animal fecal material via environmental sources.

Regarding enterococci isolated from foods of animal origin in the present study, high rates of resistance to erythromycin (66/108, 61.11%), tetracycline (63/108, 58.3%), and rifampicin (45/108, 41.66%) were observed, while moderate rates of resistance were observed for ciprofloxacin (24/108, 22.2%), and streptomycin

Table IV. Antimicrobial resistance profiles among enterococci isolated from different type of food.

Food samples	Species (n)	Number of the resistant strains to*										
		AMP	CIP	CHL	ERY	STR	GEN	NIT	NOR	RIF	TET	MDR**
Vegetables												
Sweet potato	*E. casseliflavus* (21)	-	1 [a]	-	5 [a]	-	-	7 [b]	7 [a]	18 [c]	1 [a]	6
	E. faecalis (12)	-	-	-	-	-	-	3 [b]	-	12 [c]	-	-
	E. faecium (1)	-	-	-	-	-	-	-	-	1 [c]	-	-
	Enterococcus sp. (2)	-	-	-	-	-	-	1 [b]	1 [a]	1 [c]	-	1
Potato	*E. casseliflavus* (19)	-	-	-	1 [a]	-	-	-	-	7 [b]	-	-
	E. faecalis (17)	-	-	-	-	-	-	-	-	17 [b]	-	-
Beetroot	*E. casseliflavus* (24)	-	4 [b]	1 [a]	11 [b]	5 [a]	-	-	-	23 [c]	1 [a]	3
Carrot	*E. faecalis* (3)	-	-	-	1 [b]	-	-	2 [b]	1 [a]	3 [b]	-	1
Cabbage	*E. casseliflavus* (5)	-	3 [c]	-	4 [c]	-	-	-	-	5 [b]	-	2
	E. faecalis (26)	-	15 [c]	4 [b]	19 [c]	13 [b]	-	-	7 [a]	8 [b]	12 [b]	17
	E. faecium (1)	-	1 [c]	-	1 [c]	1 [b]	-	-	-	-	1 [b]	1
	E. hirae (1)	-	-	-	1 [c]	-	-	-	-	1 [b]	-	-
Parsley	*E. casseliflavus* (22)	-	2 [b]	-	6 [a]	-	-	3 [a]	4 [a]	8 [a]	11 [b]	5
	E. faecalis (6)	-	3 [b]	-	1 [a]	-	-	3 [a]	3 [a]	6 [a]	-	4
	E. hirae (4)	-	-	-	-	-	-	-	-	-	-	-
	Enterococcus sp. (2)	-	-	-	-	-	-	-	-	-	2 [b]	-
Cassava	*E. casseliflavus* (12)	1 [a]	4 [b]	1 [a]	12 [c]	4 [a]	1[a]	7 [b]	1 [a]	3 [b]	3 [a]	7
	E. faecalis (24)	-	5 [b]	-	20 [c]	2 [a]	-	-	5 [a]	12 [b]	3 [a]	5
	Sub-total (202)	1	38	6	82	25	1	26	29	125	34	52
Animals												
Raw chicken meat	*E. faecalis* (36)	-	6 [b]	-	21 [b]	9 [a]	10 [b]	1 [a]	7 [a]	9 [a]	24 [c]	21
Colonial cheese	*E. faecalis* (36)	1 [a]	7 [b]	1 [a]	33 [c]	12 [b]	-	-	1 [a]	25 [b]	23 [c]	22
	E. durans (3)	-	1 [b]	-	2 [b]	-	-	2 [a]	-	-	2 [b]	2
Soft cheese	*E. faecalis* (17)	-	7 [b]	-	2 [b]	-	-	-	2 [a]	3 [a]	4 [b]	2
	E. faecium (4)	-	3 [b]	-	4 [b]	-	-	-	-	-	-	-
	E. hirae (12)	-	-	-	4 [b]	-	-	-	-	8 [a]	10 [b]	2
	Sub-total (108)	1	24	1	66	21	10	3	10	45	63	49
	Total (%)	2 (0.6)	62 (20)	7 (2.3)	148 (47.7)	46 (14.8)	11 (3.5)	29 (9.3)	39 (12.6)	170 (54.8)	97 (31.3)	101 (33.1)

***Antimicrobials: AMP: ampicillin; ERY, erythromycin; CIP, ciprofloxacin; NOR, norfloxacin; RIF, rifampicin; STR, streptomycin; GEN, gentamicin; NIT, nitrofurantoin; CHL, chloramphenicol; TET, tetracycline. ** Profile: MDR, multidrug-resistance. Intermediate and resistant strains were considered in a single category and classified as resistant strains; Same downcase letters on the same column means there is no statistical difference between samples (p>0.05). The statistics were divided between the different foods, and not regarding the species of each food.**

(21/108, 19.4%). Moreover, low rates of resistance were detected for gentamicin (10/108, 9.2%), and norfloxacin (10/108, 9.2%). Resistant strains have also been reported in animal food sources (McGowan et al. 2006, Lebreton et al. 2014, Vaz Jr 2016). McGowan et al. (2006) isolated bacitracin, lincomycin, tetracycline and streptomycin-resistant enterococci from meat samples in Athens, Georgia in 2000 and 2001. Notably, relatively high rates of resistance for erythromycin and tetracycline were observed in our study. These profiles might be related to antibiotics that are used to treat animal infections. Although tetracycline was banned as a growth promoter in Brazil in 1998, this antibiotic class continues to be widely used to treat *Salmonella*-related infections in poultry and represents one of the main classes of antibiotics marketed and applied to the Brazilian livestock (Vaz Jr 2016). Erythromycin is also a narrow-spectrum antibiotic used by veterinarians to treat specific types of bacterial infections in animals (Dinos 2017).

Enterococci antimicrobial profiles from the present study were compared to those of the enterococcal collection from 2007. The results showed a reduction in the prevalence of ampicillin, gentamicin, chloramphenicol, and vancomycin-resistant strains (Figure 1). Resistance to ampicillin decreased from 6.7% in 2007 to 0.6% in 2017, while gentamicin resistance decreased from 16.1% in 2007 to 3.5% in 2017, chloramphenicol resistance decreased from 23.65% in 2007 to 2.3% in 2017, and vancomycin resistance decreased from 5.4% in 2007 to 0.0% in 2017. These reductions in resistant enterococci observed in food samples over the 10-years study period might be associated with the control of the antimicrobial use under the RDC 20/11.

Resistance to erythromycin, tetracycline, ciprofloxacin, norfloxacin, and nitrofurantoin remained the same throughout the evaluated

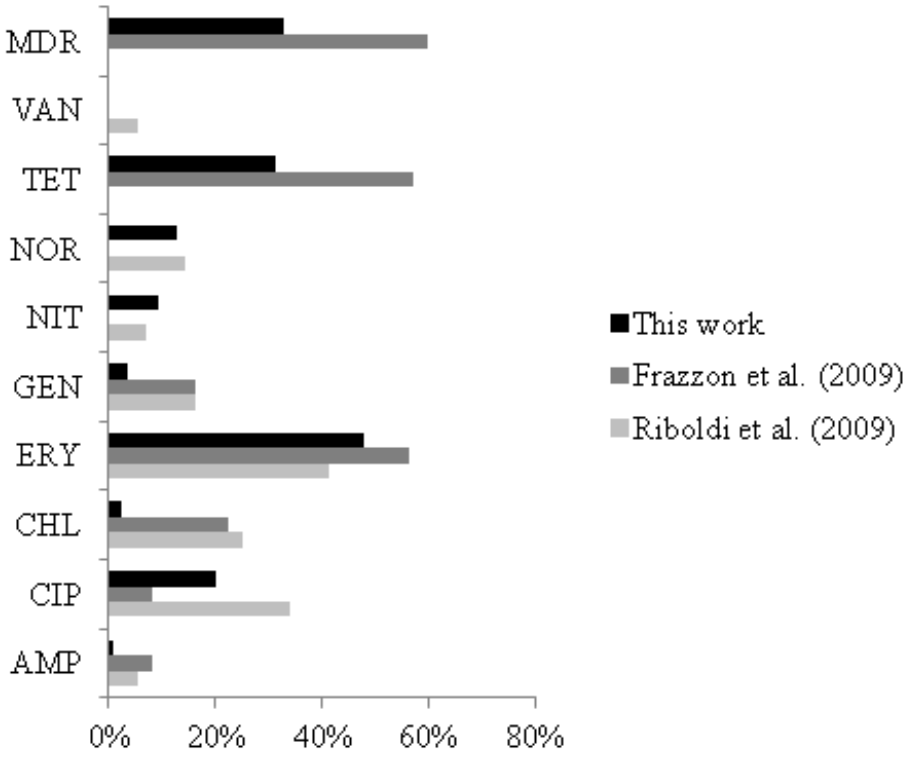

Figure 1. Distribution of resistant enterococci isolated from foods comparing pre-RDC 20/2011 (Riboldi et al. 2009, Frazzon et al. 2009) with post-RDC 20/2011 (our data). Antimicrobials: AMP: ampicillin, CIP: ciprofloxacin, CHL: chloramphenicol, ERY: erythromycin, GEN: gentamicin, NIT: nitrofurantoin, NOR: norfloxacin, TET: tetracycline, VAN: vancomycin, MDR: Multidrug resistance profile.

period. This result might be explained by the fact that these drugs being widely used in veterinary medicine. Veterinary drugs are primarily used in avian production and dairy cattle management mainly for therapy and disease prophylaxis of diseases (Novaes et al. 2017). Additionally, in Brazil there is not a controlling antimicrobial used in veterinary medicine in Brazil.

In the present study, MDR strains were identified in foods of vegetable and animal origin. A total of 101 enterococci (33.1%) strains had an MDR profile (Table II). High rates of MDR were observed in strains isolated from cabbage (20/33, 66.66%), colonial cheese (22/36, 61.11%), and chicken meat (21/36, 58.33%). Among these strains, an MDR profile was more frequently in *E. faecalis* (71.3%), followed by *E. casseliflavus* (22.8%), *E. hirae* (1.9%), *E. durans* (1.9%), *E. faecium* (0.9%), and *Enterococcus* sp. (0.9%). MDR enterococci have previously been previously isolated from food (McGrowan et al. 2007, Frazzon et al. 2009, Soares-Santos et al. 2015, Kim et al. 2017). Multidrug resistance decreased from 57.1% of the isolates in 2007 to 33.1% in 2017 (Figure 1). However, it is important to highlight that the emergence of MDR appears to be driven by the ubiquitous nature of *Enterococcus* spp., the plasticity of their genomes, and the widespread use of antibiotics (Prieto et al. 2016).

Frequency of antibiotic resistance genes

Of the 95 tetracycline-resistant strains, 76 (78.3%) were positive for the *tet*(M) gene and 23 (23.7%) were positive for the *tet*(L) gene. Among the 123 erythromycin-resistant strains, 63 (42.6%) were positive for the *erm*(B) gene and 7 (4.7%) were positive for the *msr*C gene. Moreover, the *aac*(6')-*aph*(2') gene was detected in 10 (90.9%) gentamicin-resistant strains isolated from raw chicken meat. Similar results have previously been observed by Frazzon et al. (2009) among tetracycline-resistant strains isolated from beetroot, raw chicken meat, potato, cassava, and colonial and soft cheeses in southern Brazil in 2009. Ben Said et al. (2015) also discovered *tet*(M) and/or *tet*(L), *erm*(B) and *aac*(6')-*aph*(2') genes in enterococci isolates from vegetables, soil, and irrigation water in Tunisia. According to the same authors, the potential presence of other antibiotic resistance genes cannot be ignored.

Cooking is an effective step that can be used to kill most harmful bacteria. Currently, there is a growing demand for ready-to-eat or ready-to-use products. Minimally processed fruits and vegetables are fresh fruits and vegetables processed to preserve their nutritional value and obtain their maximum benefits (Panja 2017). In the present study, we primarily focused on analyzing mainly ready-to-eat products since they are usually eaten raw, without washing or other decontamination procedures. Recently, Cruz et al. (2019) showed that minimally processed vegetables may contain pathogenic bacteria and therefore could represent a risk for consumers. Notably, the presence of antibiotic-resistant bacteria in this type of food could pose a risk to consumer. As reported by Hölzel et al. (2018), three additional situations can be connected to antimicrobial-resistant microorganisms in vegetables: (1) foodborne infectious disease due to obligate or opportunistic pathogens, (2) foodborne microbial intoxication, and (3) foodborne colonization, which could be followed by opportunistic disease after a considerable period of time. Studies have noted the transfer of antibiotic resistance genes between species, which are often passed to humans (Leverstein-van Hall et al. 2011, Nawaz et al. 2011).

CONCLUSION

In the present study, *E. casseliflavus* was predominant in vegetables, while *E. faecalis* was dominant in food sources of animal origin. Upon comparing the antimicrobial resistance profiles of enterococci recovered from food samples between 2007 and 2017, a reduction in the prevalence of resistant isolates associated with some antimicrobials used to treat human infections was observed. These results suggest that RDC 20/11 had a positive outcome in controlling the spread of antimicrobial resistance in the environment. Thus, the present study highlights that there is scope for improvement in food security through advancing food safety knowledge and practices. Therefore, it is recommended that the population should be educated on food safety and safer food practices. Ultimately, effective precautionary measures must be taken to prevent the transmission of enterococci through food. This study provides baseline data to measure future changes in the prevalence of resistant enterococci in Brazil.

Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico - Brazil, CNPq #401714/2016-0 and #302574/2017-4 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

AARESTRUP FM, AGERSO Y, GERNER-SMIDT P, MADSEN M & JENSEN LB. 2000. 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 37: 127-137.

ALONSO-HERNANDO A, PRIETO M, GARCÍA-FERNÁNDEZ C, ALONSO-CALLEJA C & CAPITA R. 2012. Increase over time in the prevalence of multiple antibiotic resistance among isolates of *Listeria monocytogenes* from poultry in Spain. Food Control 23: 37-41.

BEN SAID L, KLIBI N, DZIRI R, BORGO F, BOUDABOUS A, SLAMA KB & TORRES C. 2015. Prevalence, antimicrobial resistance and genetic lineages of *Enterococcus* spp. from vegetable food, soil and irrigation water in farm environments in Tunisia. J Sci Food Agric 96: 1627-1633.

BHARDWAJ SB. 2019. Enterococci: An Important Nosocomial Pathogen. In: KIRMUSAOGLU S (Ed), Pathogenic Bacteria, IntechOpen. Rijeka, Croatia, p. 1-17.

BRASIL. 2011. Ministério da Saúde, Agência Nacional de Vigilância em Saúde (ANVISA). Resolução da Diretoria Colegiada – RDC nº 20, de 5 de maio de 2011. Dispõe sobre o controle de medicamentos à base de substâncias classificadas como antimicrobianos, de uso sob prescrição, isoladas ou em associação. Diário Oficial da União, Brasília: Ministério da Saúde, 2011.

CAMARGO CH, BRUDER-NASCIMENTO A, LEE SHI, JÚNIOR AF, KANENO R & RALL VLM. 2014. Prevalence and phenotypic characterization of *Enterococcus* spp. isolated from food in Brazil. Braz J Microbiol 45: 111-115.

CAMPOS ACFB, SOUZA NR, SILVA PHC & SANTANA AP. 2013. Resistência antimicrobiana em *Enterococcus faecalis* e *Enterococcus faecium* isolados de carcaças de frango. Pesq Vet Bras 33: 575-580.

CLSI - CLINICAL AND LABORATORY STANDARDS INSTITUTE. 2017. M100 Performance Standards for Antimicrobial Susceptibility Testing, 26ª ed., informational supplement M100.

COSTA LFX, GRASSOTTI TT, CANANI CR, DE LIRA AD, DE MOURA TM, CAMPOS AAS, FRAZZON J & FRAZZON APG. 2019. Diversidade, perfis de resistência e virulência de *Enterococcus* spp. em fezes de morcegos urbanos *Tadarida brasiliensis* (Brazilian free-tailed bats). Braz J Biosci 17: 43-52.

CRUZ MRG, LEITE YJBS, MARQUES JL, PAVELQUESI SLS, OLIVEIRA LRA, SILVA ICR & ORSI DC. 2019. Microbiological quality of minimally processed vegetables commercialized in Brasilia, DF, Brazil. Food Sci Technol 39: 498-503.

D'AZEVEDO PA, DIAS CAG & TEIXEIRA LM. 2006. Genetic diversity and antimicrobial resistance of enterococcal isolates from southern regions of Brazil. Rev Inst Med Trop São Paulo 48: 11-16.

DEPARDIEU F, PERICHON B & COURVALIN P. 2004. Detection of the van alphabet and identification of enterococci and staphylococci at the species level by multiplex PCR. J Clin Microbiol 42: 5857-5860.

DIZAVANDI ZR, ALIAKBAR A & SHEYKHAN M. 2017. A novel Pb-poly aminophenol glassy carbon electrode for determination of tetracycline by adsorptive differential

pulse cathodic stripping voltammetry. Electrochim Acta 227: 345-356.

DINOS GP. 2017. The macrolide antibiotic renaissance. Br J Pharmaco 174: 2967-2983.

FRANZ CMAP, STILES ME, SCHLEIFER KH & HOLZAPFEL WH. 2003. Enterococci in foods – a conundrum for food safety. Int J Food Microbiol 88: 105-122.

FRAZZON APG, GAMA BA, HERMES V, BIERHALS CG, PEREIRA RI, GUEDES AG, D'AZEVEDO PA & FRAZZON J. 2009. Prevalence of antimicrobial resistance and molecular characterization of tetracycline resistance mediated by *tet*(M) and *tet*(L) genes in *Enterococcus* spp. isolated from food in Southern Brazil. World J Microbiol Biotechnol 26: 365-370.

GELSOMINO R, VANCANNEYT M, COGAN TM, CONDON S & SWINGS J. 2002. Source of enterococci in a farmhouse raw-milk cheese. Appl Environ Microbiol 68: 3560-3565.

GIRAFFA G. 2002. Enterococci from foods. FEMS Microbiol Rev 26: 163-171.

GOMES BC, ESTEVES CT, PALAZZO ICV, DARINI ALC, FELIS GE, SECHI LA, FRANCO BDGM & MARTINIS ECP. 2008. Prevalence and characterization of *Enterococcus* spp. isolated from Brazilian foods. Food Microbiol 25: 668-675.

GOTHWAL R & SHASHIDHAR T. 2014. Antibiotic pollution in the environment: A Review. CLEAN - Soil, Air, Water 43: 479-489.

GRASSOTTI TT, ZVOBODA DA, COSTA LFX, ARAÚJO AJG, PEREIRA RI, SOARES RO, WAGNER PGC, FRAZZON J & FRAZZON APG. 2018. Antimicrobial resistance profiles in *Enterococcus* spp. isolates from fecal samples of wild and captive black capuchin monkeys (*Sapajus nigritus*) in South Brazil. Front Microbiol 9: 2366.

HANCHI HM, MOTTAWEA W, KHALED SE & HAMMAMI R. 2018. The Genus Enterococcus: Between Probiotic Potential and Safety Concerns-An Update. Front Microbiol 9: 1791.

HÖLZEL CS, TETENS JL & SCHWAIGER K. 2018. Unraveling the role of vegetables in spreading antimicrobial-resistant bacteria: a need for quantitative risk assessment. Foodborne Pathog Dis 15: 671-688.

JIA W, LI G & WANG W. 2014. Prevalence and antimicrobial resistance of *Enterococcus* species: a hospital-based study in China. Int J Environ Res Public Health 11: 3424-3442.

KE D, PICARD FJ, MARTINEAU F, MENARD C, ROY PH, OUELLTTE M & BERGERON MG. 1999. Development of a PCR assay for rapid detection of Enterococci. J Clin Microbiol 37: 3497-3503.

KIM MC, CHA MH, RYU JG & WOO GJ. 2017. Characterization of vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from fresh produces and human fecal samples. Foodborne Pathog Dis 14: 195-201.

KIM NH, KIM HW, PARK SM, SEO GH, CHO TJ, YU HR, KIM SH, HWANG JH, CHOI C & RHEE MS. 2020. Virulence patterns and prevalence of seven *Enterococcus* species isolated from meats and leafy vegetables in South Korea. Food Control 108: 106867.

KIM YJ, PARK JH & SEO KH. 2018. Comparison of the loads and antibiotic-resistance profiles of *Enterococcus* species from conventional and organic chicken carcasses in South Korea. Poult Sci 97: 271-278.

KRISTIANSSON E, FICK J, JANZON A, GRABIC R, RUTGERSSON C, WEIJDEGARD B, SODERSTROM H & LARSSON DG. 2011. Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. PLoS ONE 6: e17038.

KULIK EM, THURNHUEER T, KARYGIANNI L, WALTER C, SCULEAN A & EICK S. 2019. Antibiotic susceptibility patterns of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* strains from different decades. Antibiotics 8: 253.

LEBRETON F, WILLEMS RJL & GILMORE MS. 2014. *Enterococcus* diversity, origins in nature, and gut colonization. In: In: Gilmore MS, Clewell DB, Ike Y & Shankar N (Eds), Enterococci from commensals to leading causes of drug resistance infection: Eye and Ear Infirmary, Boston, p. 1-59.

LEVERSTEIN-VAN HALL MA, DIERIKX CM, COHEN STUART J, VOETS GM, VAN DEN MUNCKHOF MP, VAN ESSEN-ZANDBERGEN A, PLATTEEL T & FLUI AC. 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect 17: 873-880.

MARATHE NP, REGINA VR, WALUJKAR SA, CHARAN SS, MOORE ERB, LARSSON DGJ & SHOUCHE YS. 2013. A treatment plant receiving waste water from multiple bulk drug manufacturers is a reservoir for highly multi-drug resistant integron-bearing bacteria. PLoS ONE 8: e77310.

MASSOT M ET AL. 2016. Phylogenetic, virulence and antibiotic resistance characteristics of commensal strain populations of *Escherichia coli* from community subjects in the Paris area in 2010 and evolution over 30 years. Microbiol 162: 642-650.

MCGOWAN LL, JACKSON CR, BARRETT JB, HIOTT LM & FEDORKA-CRAY PJ. 2006. Prevalence and antimicrobial resistance of enterococci isolated from retail fruits, vegetables and meats. J Food Prot 69: 2976-2982.

MEDEIROS AW, AMORIM DB, TAVARES M, MOURA TM, FRANCO AC, D'AZEVEDO PA, FRAZZON J & FRAZZON APG. 2016. *Enterococcus*

species diversity in fecal samples of wild marine species as determined by real-time PCR. Can J Microbiol 63: 129-136.

NAWAZ M, WANG J, ZHOU A, CHAOFENG M, WU X, MOORE JE, MILLAR BC & XU J. 2011. Characterization and transfer of antibiotic resistance in lactic acid bacteria from fermented food products. Curr Microbiol 62: 1081-1089.

NGBEDE EO, RAJI MA, KWANASHIE CN, KWAGA JKP, ADIKWU AA, MAURICE NA & ADAMU AM. 2017. Characterization of high-level ampicillin- and aminoglycoside-resistant enterococci isolated from non-hospital sources. J Med Microbiol 66: 1027-1032.

NOVAES SF, SCHREINER LL, SILVA IP & FRANCO RM. 2017. Residues of veterinary drugs in milk in Brazil. Cienc Rural 47: e20170215.

PANJA P. 2017. Minimal Processing of Fruits and Vegetables. In: Deb P, Bhowmick N, Munsi PS & Ghosh SK (Eds), Innovative Horticulture: Concepts for Sustainable Development, Recent trends: New Delhi Publishers, p. 217-226.

PRICE LB ET AL. 2012. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. MBio 3: e00305-e00311.

PRIETO AMG, VAN SCHAIK W, ROGERS MRC, COQUE TM, BAQUERO F, CORANDER J & WILLEMS RJL. 2016. Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones? Front Microbiol 7: 788.

RASHEED MU, THAJUDDIN N, AHAMED P, TEKLEMARIAM Z & JAMIL K. 2014. Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. Rev Inst Med Trop São Paulo 56: 341-346.

REGITANO JB & LEAL RMP. 2010. Comportamento e impacto ambiental de antibióticos usados na produção animal brasileira. Rev Bras Ciênc Solo 34: 601-616.

RIBOLDI GP, FRAZZON J, D'AZEVEDO PA & FRAZZON APG. 2009. Antimicrobial resistance profile of *Enterococcus* spp. isolated from food in Southern Brazil. Braz J Microbiol 40: 125-128.

ROCA I ET AL. 2015. The global threat of antimicrobial resistance: science for intervention. New Microbes New Infect 6: 22-29.

SAUGET M, VALOT B, BERTRAND X & HOCQUET D. 2017. Can MALDI-TOF mass spectrometry reasonably type bacteria? Trends Microbiol 25: 447-455.

SCHWARZ S, SILLEY P, SIMJEE S, WOODFORD N, VAN DUIJKEREN E, JOHNSON AP & GAASTRA W. 2010. Assessing the antimicrobial susceptibility of bacteria obtained from animals. Vet Microbiol 141: 1-4.

SEDGLEY CM, NAGEL AC, SHELBURNE CE, CLEWELL DB, APPELBE O & MOLANDER A. 2005. Quantitative real-time PCR detection of oral *Enterococcus faecalis* in humans. Arch Oral Biol 50: 575-583.

SOARES-SANTOS V, BARRETO AS & SEMEDO-LEMSADDEK T. 2015. Characterization of Enterococci from food and food-related settings. J Food Prot 78: 1320-1326.

SUTCLIFFE J, GREBE T, TAIT-KAMRADT A & WONDRACK L. 1996. Detection of erythromycin-resistant determinants by PCR. Antimicrob Agents Chemother 40: 2562-2566.

TYSON GH, NYIRABAHIZI E, CRAREY E, KABERA C, LAM C, RICE-TRUJILLO C, MCDERMOTT PF & TATE H. 2017. Prevalence and Antimicrobial Resistance of Enterococci Isolated from Retail Meats in the United States, 2002 to 2014. Appl Environ Microbiol 84: e01902-17.

VAZ JR S. 2016. Erratum to: Sorption behavior of the oxytetracycline antibiotic to two Brazilian soils. Chem Biol Technol Agric 3: 14.

VENTOLA CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. P&T 40: 277-283.

VERRAES C ET AL. 2013. Antimicrobial resistance in the food chain: a review. Int J Environ Res Public Health 10: 2643-2669.

WERNER G, HILDEBRANDT B & WITTE W. 2001. The newly described *msrC* gene is not equally distributed among all isolates of *Enterococcus faecium*. Antimicrob Agents Chemother 45: 3672-3673.

WORLD HEALTH ORGANIZATION (WHO). 2013. Integrated surveillance of antimicrobial resistance: guidance from a WHO Advisory Group. World Health Organization, Geneva, Switzerland.

WORLD HEALTH ORGANIZATION (WHO). 2020. Integrated surveillance of antimicrobial resistance: guidance from a WHO Advisory Group. World Health Organization, Geneva, Switzerland. https://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance.

How to cite
COSTA LFX, FALCÃO DA, GRASSOTTI TT, CHRISTIANO FDP, FRAZZON J & G. FRAZZON APG. 2022. Antimicrobial resistance of enterococci isolated from food in South Brazil: Comparing pre- and post-RDC 20/2011. An Acad Bras Cienc 94: e20201765. DOI10.1590/0001-3765202220201765.

Manuscript received on November 11, 2020;
accepted for publication on March 15, 2021

LETÍCIA DA F.X. COSTA[*1]
https://orcid.org/0000-0001-8636-6449

DAIANE A. FALCÃO[*1]
https://orcid.org/0000-0001-8590-2414

TIELA T. GRASSOTTI[1]
https://orcid.org/0000-0003-1166-514X

FRANCIELE D.P. CHRISTIANO[2]
https://orcid.org/0000-0002-6231-3470

JEVERSON FRAZZON[3]
https://orcid.org/0000-0002-0209-1466

ANA PAULA G. FRAZZON[*1,2]
https://orcid.org/0000-0002-1029-5776

[1]Programa de Pós-Graduação em Microbiologia Agrícola e do Ambiente, Instituto de Ciências Básicas da Saúde, UFRGS, Rua Sarmento Leite, 500, 90050-170 Porto Alegre, RS, Brazil

[2]Instituto de Ciências Básicas da Saúde, UFRGS, Departamento de Microbiologia, Imunologia e Parasitologia, Rua Sarmento Leite, 500, 90050-170 Porto Alegre, RS, Brazil

[3]Instituto de Ciência e Tecnologia de Alimentos, UFRGS, Avenida Bento Gonçalves, 9500, 91501-970 Porto Alegre, RS, Brazil

Correspondence to: **Ana Paula Guedes Frazzon**
E-mail: ana.frazzon@ufrgs.br

*Contributed equally to the work

Author contributions

Jeverson Frazzon and Ana Paula Guedes Frazzon designed the study. Daiane Acosta Falcão and Franciele Dalla Porta Christiano carried out the sampling work. Letícia da Fontoura Xavier Costa and Tiela Trapp Grassotti analyzed data and drafted the manuscript. All authors have read and approved the final manuscript.