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ANTIMICROBIAL RESISTANCE PROFILE OF *ENTEROCOCCUS* SPP ISOLATED FROM FOOD IN SOUTHERN BRAZIL

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ABSTRACT

Fifty-six *Enterococcus* spp. strains were isolated from foods in Southern Brazil, confirmed by PCR and classified as *Enterococcus faecalis* (27), *Enterococcus faecium* (23) and *Enterococcus* spp (6). Antimicrobial susceptibility tests showed resistance phenotypes to a range of antibiotics widely administrated in humans such as gentamycin, streptomycin, ampicillin and vancomycin.

Keywords: *Enterococcus* spp, antibiotic resistant enterococci, food

Enterococcus spp. are Gram-positive bacteria able of growing and surviving under harsh conditions. They are ubiquitous in nature being present in soil, waters, raw plant and animal products. Enterococci are natural component of foods, representing important role in ripening and flavor enhancement of cheese and sausage. They have been used as probiotics to improve the microbial balance of the intestinal tract in humans and animals. Despite the fact that the *Enterococcus* genus is responsible for beneficial effects in foods, they are not considered "generally recognized as safe" (GRAS), due to its use as an indicator of fecal contamination, and the frequent association with food-borne illnesses by biogenic amines production (6,14). An important clinical feature of *Enterococcus* spp. is the resistance to a wide range of antimicrobial agents as demonstrated in clinical, food and water isolates strains (4,7,8). In addition, these bacteria are able to acquire resistance determinants through gene transference by plasmids and transposons. The use of antimicrobials in animal feed as growth promoters have created large reservoirs of transferable antibiotic resistance genes in several ecosystems, and consequently a possible route transmission of resistant *Enterococcus* spp. via food chain could be suggested (17). In this way, the aim of the present study was to determine the species distribution and the antibiotic resistance patterns of enterococci isolated from *in natura* food and dairy products in Porto Alegre, Southern Brazil.

The enterococcal strains were isolated from cassava, beetroot, potato, sweet potato, parsley, cabbage, raw meat, pasteurized milk and dairy products, such colonial cheese type and soft cheese. Food samples were purchased from different popular markets in Porto Alegre, Brazil. The *Enterococcus* spp. isolation, characterization and identification methodology was carried out as previously described by Domig *et al*. (2). The first isolation step consisted of inoculation of 25 g of food in sterile buffered peptone water (225 ml) and incubation at 37ºC for 16 h. From this suspension, 125 µl were plated on brain heart infusion (BHI) agar, supplemented with 0.02% azide and 6,5% NaCl (w/v) and incubated at 37ºC for 72 h. Phenotypic criteria (such as size/volume, shape, color, hemolytic profile) were used for strains isolation and colony picking of presumable streptococcal/enterococcal strains. Phenotype tests were used to separate the enterococci group and the non-enterococcal strains. Colonies with typical enterococci morphology were identified to genus level by the following methods: Gram staining, catalase production and esculin hydrolysis tests in combination with resistance to bile salts, production of

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pyrrolidonylarylamidase (PYR test) and growth at 10ºC and 45ºC. The selected strains were stored in BHI containing 50% glycerol at -20ºC. Fifty six previously isolated *Enterococcus* spp. were submitted to Polimerase Chain Reaction (PCR) using genus-specific primer pair of the *tuf* gene, which targeted and amplified a 112 base pair (bp) DNA fragment. Reactions were carried out as described by Ke *et al*. (12). Genomic DNA was extracted by the boiling method, as described by Hagen *et al.* (9). Strains isolated were subjected to species identification by phenotype characterization according to the protocol proposed by Facklam *et al.*, (5). The trial assay included: presence of arginine and pyruvate dehydrolases; acid production from 1% L-arabinose, raffinose, mannitol, α-methyl-D-glucopyranoside, sorbitol and L-sorbose; motility and pigmentation characteristics. *Enterococcus faecalis* ATCC 51299 and *Staphylococcus aureus* (ATCC 25923) were used as control strains. Antimicrobial susceptibility was determined using the disk diffusion, according to the recommendations of NCCLS (16). Twelve antibiotics commonly used in the treatment of clinical infection and agricultural procedures were tested (concentrations are expressed in μg ml^{-1}): ampicillin-10 (AMP), vancomycin-30 (VAN), erythromycin-15 (ERI), tetracycline-30 (TET), ciprofloxacin-5 (CIP), norfloxacin-10 (NOR), nitrofurantoin-300 (NIT), cloramphenicol- 30 (CLO), gentamycin-120 (GEN), streptomycin-300 (ET), quinupristin-dalfopristin-15 (QD), bacitracin - 0,04UI (BC) and lincomycin-15 (MY). The appropriate antibiotic dosage was purchased from Oxoid (Hampshire, United Kingdom), bioMérieux (Marcy-l'Etoile, France) and Difco (Detroit, USA). Inhibition zone diameters were measured and strains were classified according to the criteria from NCCLS (for AMP, VAN, ERI, TET, CIP, NOR, NIT, CLO, GEN, ET, QD) and Hart *et al*. (10) and Moyaert *et al.* (15) (for BAC and MY).

A total of 150 Gram-positive and catalase negative cocci were isolated from vegetables, raw meat, pasteurized milk and dairy products during March to July, 2005. Among these isolates, 82 strains were classified as *Enterococcus* spp. by phenotypic methods, and to grow at 45ºC and in the presence of 6.5% NaCl, after 48 h of incubation. All 82 strains were tested using a genus-specific primer for the *tuf* gene. Fifty-six isolates were confirmed as *Enterococcus* genus by PCR amplification. Species characterizations were performed and the strains were classified as *E. faecalis* (27 strains), *Enterococcus faecium* (23 strains), *Enterococcus* spp (6 strains) (Fig. 1). In the vegetables group *E. faecium* was the most abundant species detected, mainly in beetroot and potato (100 %) and parsley (80%), whereas *E. faecalis* was observed in cabbage (65%). These data's are in agreement which has been described by Abriuoel *et al*., (1). Only cassava and sweet potato did not present any *Enterococcus* spp.

In raw meat and colonial cheese type strains of *E. faecalis* was the most prevalent species, which could be explained by the fact that these foods are manipulated by hands suggesting a contamination during the manufacturing process. Otherwise,

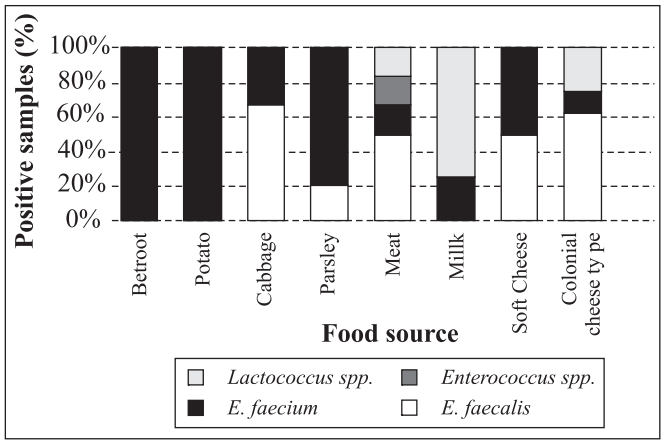

Figure 1. Percentage distribution of *Enterococcus* species isolated from food in Porto Alegre, Brazil.

in pasteurized milk nearly all microorganisms get (around 80%) was *Lactococcus spp.* go behind by *E. faecium*. The presence of the bacteria *E. faecium* in processed milk is explained by often capable of these microorganisms survives during pasteurization process (18).

Antibiotic susceptibility tests of *Enterococcus* spp. isolated are shown in Table 1. Almost all enterococcus strains displayed resistance to at least one antibiotic tested, with the exception for milk isolates where all strains were susceptible. Resistance to antimicrobials commonly used in agriculture such as bacitracin (34 strains) and lincomycin (12 strains) showed relatively high frequency of occurrence. Resistance to nitrofurantoin, antibiotic used for treatment of genitourinary tract infections, was observed among isolates strains from soft cheese and cabbage. However, none of the *Enterococcus* spp. strains was resistant to norfloxacin; drug used in urinary tract infection treatments. Enterococcus isolated from cabbage showed the resistance to several of antimicrobials used in human medicine such as tetracycline, ciprofloxacin, nitrofurantoin and quinupristin/ dalfopristin. Elevated amount of High-Level of Aminoglycosides Resistance (HLAR) was observed in both *E. faecalis* and *E faecium* strains from all foods samples analyzed.

Three *E. faecalis* strains isolated from cheese and meat showed ampicillin-resistant (AMPR) pattern, a clinical relevant antibiotic since ampicillin remains the drug of choice for the treatment of enterococcal infections. Furthermore, a combination of cell-wall active agent and aminoglycoside (e.g. ampicillin + gentamicin) is the selective treatment for enterococcal endocarditis (13). Moreover, in colonial cheese type it was detected the presence of one *E. faecalis* vancomycin-resistant (VRE) strain. This finding is the most relevant in this study and serves as warning to authorities of public healthy since vancomycin is the last antibiotic alternative utilized for treatment of nosocomial infections.

Table 1. Antibiotic resistant phenotypes of *Enterococcus* species isolated from food.

Antimicrobial agent	*E. faecalis* (n=27)			*E. faecium* (n=23)			*Enterococcus spp.* (n=6)		
	S(%)	I (%)	R (%)	S (%)	I (%)	R (%)	S(%)	I (%)	R(%)
AMP (a)	88,9	0	11,1	23	0	0	100	0	0
VAN (a)	88,9	7,4	3,7	23	0	0	100	0	0
ERI (a)	77,8	11,1	11,1	52,2	34,8	13	0	66,6	26,4
TET (a)	66,67	0	33,33	82,6	8,7	8,7	100	0	0
CIP (a)	63	29,6	7,4	78,3	21,7	0	33,3	50	16,7
NOR (a)	85,2	14,8	0	87	13	0	83,3	16,7	0
NIT (a)	100	0	0	87	0	13	83,3	0	16,7
CLO (a)	88,9	3,7	7,4	70	30	0	33,3	33,3	33,3
QD (a)	-[2]	-[2]	-[2]	91,3	0	8,7	60	40	0
GEN (a)[1]	77,8	0	22,2	87	0	13	100	0	0
ET (a)[1]	77,8	3,7	18,5	87	3,7	9,3	100	0	0
BC (b)	0	40,7	59,3	0	39	61	0	16,7	83,3
MY (b)	48,1	0	51,9	91,3	0	8,7	100	0	0

Ampicillin (AMP), vancomycin (VAN), erythromycin (ERI), tetracycline (TET), ciprofloxacin (CIP), norfloxacin (NOR), nitrofurantoin (NIT), cloranphenicol (CLO), quinupristin-dalfopristin (QD), gentamycin (GEN), streptomycin (ET), bacitracin (BC) and lincomycin (MY). (a) NCCLS *(14)*; (b) Hart *et al. (8)*; Moyaert *et al. (13)*; [1]High level resistance; [2]Not tested due to the intrinsic resistance of the species against the substance. Resistance profile: S: susceptible; I: intermediate; R: resistant.

Finally, one reason that could explain the emergence of antibiotic resistant enterococci in food samples would be the massive use of antibiotic in agriculture (e.g., avoparcin as animal growth promoters). Some studies have shown that the same resistance gene was found in bacteria isolated from both food samples and patients (3,4). Experimental investigations showed that resistance plasmid pAMβ1 has been transferred among *E. faecalis*, *E. faecium*, and *Lactobacillus reuteri* in the digestive tracts of mice, and this plasmid has also been transferred between *L. curvatus* strains in fermenting sausages (14). These observations support the hypothesis that resistant enterococci can contaminate food, enter the human gastrointestinal tract and colonize humans and/or pass their resistance genes to commensal bacteria present in the human intestinal tract (7). Emergence of enterococci antimicrobial resistance and its spreading in food suggest a situation of risk for community, and also a possible correlation between strains present in hospitals with those isolated from food must be considered. Here, we described the first report of antibiotic resistant enterococci isolated from foods in Porto Alegre, Southern Brazil.

RESUMO

Perfil de resistência antimicrobiana de *Enterococcus spp* isolados de alimentos no Sul do Brasil

Cinqüenta e seis cepas de *Enterococcus* spp. foram isoladas de alimentos no Sul do Brasil, confirmados por PCR e classificadas como *Enterococcus faecalis* (27), *Enterococcus faecium* (23) e *Enterococcus* spp. (6). Testes de susceptibilidade aos antimicrobianos demonstraram fenótipos de resistência a uma gama de antibióticos administrados em humanos, como gentamicina, estreptomicina, ampicilina e vancomicina.

Palavras-chave: *Enterococcus* spp., enterococos resistentes a antimicrobianos , alimentos

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