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MICROBIOLOGY

Resistant enterococci isolated from raw sheep's milk and cheeses from South region of Brazil

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ABSTRACT: Enterococci have been used as sentinel organisms for monitoring antimicrobial resistance in food, humans, and other animals. In this sense, the present study evaluated the antimicrobial susceptibility profile and the presence of genes associated with resistance to erythromycin (*msr*C and *erm*B) and tetracycline [*tet*(M) and/or *tet*(L)] in enterococci isolated from raw sheep's milk and cheeses (colonial, feta-, and pecorino-type) from South region of Brazil. A total of 156 enterococci were isolated from milk (n=80) and cheese (n=76) samples, identified by MALDI-TOF. *Enterococcus faecalis* (50.6%; n=79) was the most frequent species isolated from both samples. According to *in vitro* susceptibility tests, enterococci strains were not susceptible to the most commonly antimicrobial agents used in human and veterinary medicine. The frequency of MDR strains in enterococci isolated from milk (53.7%) was higher than those from cheese (24.2%). The *tet*(M) gene was the most commonly detected among tetracycline not-susceptible strains. The present study provided the first evidence of antimicrobial not-susceptible enterococci in raw sheep's milk and cheeses in South Brazil. Drug-resistant strains, particularly those that are MDR, constitute a One Health issue.
Key words: *Enterococcus*, dairy products, ewe's milk, antibiotic resistance, One Health.

Enterococos resistentes isolados de leite cru de ovelha e queijos da região Sul do Brasil

RESUMO: Os enterococos têm sido usados como organismos sentinela para monitorar o padrão de suscetibilidade a antimicrobianos em alimentos, humanos e outros animais. Neste sentido, o presente estudo objetivou avaliar o perfil de susceptibilidade a antimicrobianos e os genes associados com a resistência a eritromicina (*msr*C and *erm*B) e à tetraciclina [*tet*(M) and/or *tet*(L)] em enterococos isolados de leite cru de ovelha e queijos (colonial, tipo-feta e tipo-pecorino) do Sul do Brasil. Um total de 156 enterococos foram isolados de leite (n=80) e queijo (n=76), identificados por MALDI-TOF. *Enterococcus faecalis* (50,6%; n=79) foi a espécie mais frequentemente isolada de ambas as amostras. De acordo com o teste de suscetibilidade *in vitro,* as cepas de enterococos não foram susceptíveis aos agentes antimicrobianos mais comumente utilizados na clínica humana e veterinária. A frequência de cepas de enterococos MDR isoladas do leite (53,7%) foi superior à do queijo (24,2%). O gene *tet*(M) foi o mais comumente detectado entre as cepas não susceptíveis à tetraciclina. O presente estudo fornece as primeiras evidências de enterococos não susceptíveis aos antimicrobianos em leite cru de ovelha e queijos no Sul do Brasil. Cepas resistentes a drogas, particularmente as que são MDR, representam uma preocupação de Saúde Única.
Palavras-chave: *Enterococcus*, produtos lácteos, leite de ovelha, resistência a antibióticos, Saúde única.

INTRODUCTION

Global milk production reached nearly 906 million tonnes in 2020, up 2% from 2019. The annual production of sheep's milk in the world is about 10.5 million tonnes and the major producers are Turkey, China, and Greek (FAO, 2021). In Brazil, the production and industrial processing of sheep's milk are still not very significant, corresponding to only 0.0019% of the total milk produced in the country, being the South and Southeast regions with the largest sheep raising for milk production flock (MERLIN JUNIOR et al., 2015). Despite low shares of production, this is an important economic activity for small and medium farmers (FERNANDES et al., 2017).

Milk microbial communities contribute greatly to the organoleptic properties, flavour ripening, taste, aroma, shelf life, and safety of resultant products (PAPADAKIS et al., 2021). Sheep's milk (raw or pasteurized) is used to make different types

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of cheeses with special flavors, like Feta (Greece), Roquefort (France), Manchego (Spain), Pecorino Romano, Ricotta (Italy), and Colonial (Southern Brazil) (PINEDA et al., 2021). In the last few years, the bacterial community of milk and cheese from sheep have been evaluated and it consisted of starter and non-starter lactic acid bacteria (LAB), probiotics, fecal and pathogenic bacteria, such as *Bifidobacterium* spp., *Corynebacterium* spp., *Enterococcus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., *Phyllobacterium* spp., and *Propionibacterium* spp. (QUIGLEY et al., 2013; ACURCIO et al., 2014; ENDRES et al., 2021).

Enterococci is a part of the lactic acid bacteria (LAB) of importance in food. They often present on animal source foods, such as meats, fermented and cooked meats, as well as cheese and dairy products. They play an important role in modulating organoleptic properties as a starter culture, contributing to aroma and flavor development in cheeses, and as probiotics in humans and slaughter animals (DAPKEVICIUS et al., 2021). Despite the beneficial effects, enterococci can also be implicated in spoilage of foods and are commonly monitored as hygiene indicators in other types of food matrices. In the last decades, they are also related to health-associated infection (urinary tract infections, bacteremia, or endocarditis) in immunocompromised and hospitalized patients (LEBRETON et al., 2017). Enterococci are used as markers of animal fecal contamination of human foods and are employed as sentinel organisms for tracking trends in resistance to antimicrobial agents with activity against Gram-positive bacteria, in humans, retail meats and food animals (WHO, 2017).

Antimicrobial-resistant enterococci strains have been isolated from various environments, including clinical, food, animals, soil, and water samples (RIBOLDI et al., 2009; LEBRETON et al., 2017; SANLIBABA & SENTURK, 2018; PRICHULA et al., 2016; HECK et al., 2021; DAPKEVICIUS et al., 2021; VÝROSTKOVÁ et al., 2021). Several studies have described the occurrence of antimicrobial-resistant enterococci in milk, cheeses and yogurts from cows, buffalos, and other mammals (RIBOLDI et al., 2009; PRICHULA et al., 2013; DAPKEVICIUS et al., 2021); however, few have focused on sheep (DUCKOVÁ et al., 2009, ACURCIO et al., 2014; VÝROSTKOVÁ et al., 2021).

The impact of the presence of antibiotic-resistant bacteria in food constitutes a One Health issue. One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems (WHO, 2022). Resistant microorganisms can reach humans directly through consumption of food or food products and/or contact with colonized animals or even as indirectly through environmental pollution containing human and/or animal excrement (ROBINSON et al., 2016). In this sense, this study detected and evaluated the antimicrobial susceptibility profile and the presence of genes associated with resistance to erythromycin (*msr*C and *erm*B) and tetracycline [*tet*(M) and/or *tet*(L)] in *Enterococcus* spp. present in raw sheep's milk and cheeses from South Brazil. The data generated here also aims to contribute to the One Health antimicrobial resistance Network.

MATERIALS AND METHODS

Isolation and identification of enterococci in raw milk and cheese samples of sheep

Seventeen samples comprising raw milk (n=9) and cheeses (Colonial, Feta-type, and Pecorino-type) (n=8) of sheep were collected from January 2017 to August 2019 in sheep's farming from Rio Grande do Sul and Santa Catarina states, Brazil (Table 1).

Cheeses were made from pasteurized milk with the addition of autochthonous starter cultures according to the regulations approved by Ministério da Agricultura, Pecuária e Abastecimento (MAPA), Brazil. Colonial cheeses were prepared by using a starter culture of *Lactobacillus helveticus*. Feta- and Pecorino-type cheeses were prepared using *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *L. helveticus*, and *Lactobacillus casei* as a starter culture. All samples were stored at 4 °C for transport. Due to the nature of the samples, ethical approval was not required for this study.

The enterococci isolation followed the methodology described by RIBOLDI et al. (2009) and PRICHULA et al. (2016). Samples were resuspended in 10 mL of saline 0.85 % and maintained under agitation (100 rpm) at 37 °C for 24 h. One mL was inoculated in 9 mL of selective medium Azide Dextrose Broth (Himedia, Mumbai, India) for 24 h at 37 °C, remaining under agitation (100 rpm). Aliquots of 1 mL were placed in 9 mL of sterile water, and the initial samples were further diluted 10-fold to obtain a final dilution factor of 1/1000. From each dilution, 100 µL were plated in triplicate on a Brain Heart Infusion Agar (Himedia, Mumbai, India) supplemented with 6.5 % NaCl, being incubated as described. The pure colonies were stored at -20 °C in a 10 % (w/v) solution of skim milk (Difco, Sparks, MD, USA) and 10 % (v/v) glycerol (Neon ComercialLtda,

Table 1 - Information of samples collected from raw milk and cheese of sheep's in South Brazil.

Sample ID	Collection date	Maturationdays	Expiration date	Location
----------Milk*----------				
M 1	08/28/2018		N.A	Santa Catarina
M 2	04/26/2019		N.A	Santa Catarina
M 3	04/29/2019		N.A	Santa Catarina
M 4	09/10/2018		N.A	Rio Grande do Sul
M 5	09/10/2018		N.A	Rio Grande do Sul
M 6	09/11/2018		N.A	Rio Grande do Sul
M 7	09/19/2018		N.A	Santa Catarina
M 8	09/20/2018		N.A	Santa Catarina
M 9	09/21/2018		N.A	Santa Catarina
----------Cheese-type----------				
C 1 (Feta)	02/28/2018	0	11/28/2018	Rio Grande do Sul
C 2 (Feta)	12/18/2018	0	09/18/2018	Rio Grande do Sul
C 3 (Feta)	12/22/2017	0	09/22/2018	Rio Grande do Sul
C 4 (Colonial)	01/25/2018	20	07/25/2018	Santa Catarina
C 5 (Colonial)	04/18/2018	20	10/18/2018	Santa Catarina
C 6 (Colonial)	04/25/2018	20	10/25/2018	Santa Catarina
C 7 (Pecorino)	04/17/2018	45	04/17/2019	Rio Grande do Sul
C 8 (Pecorino)	06/11/2018	45	06/11/2019	Rio Grande do Sul

N.A: not applicable. Cheeses were not manufactured using the milks samples tested.

São Paulo, BR). Species were identified by matrix assisted laser desorption and ionization time-of-flight mass spectrometry method (MALDI-TOF) according to the protocol described by SAUGET et al. (2017). MALDI-TOF analysis was performed using a LT Bruker microflex mass spectrometer (Bruker Daltonik GmbH) and spectra were automatically identified using BrukerBioTyperTM 1.1 software. The identification by MALDI-TOF MS is based on the score value released by the equipment. A higher or similar 2.3 value indicated that the identifications of genus and species are reliable. The 2.0–2.29 values showed that the genus is reliable, and the species is probable, and the 1.7–1.99 values indicated that the identification of genus is probable.

Antimicrobial susceptibility tests

Antimicrobial susceptibility was determined by Kirby-Bauer disk diffusion method, according to Clinical and Laboratory Standards Institute (CLSI, 2019) and Brazilian Committee on Antimicrobial Susceptibility Testing – BrCAST (2019). Twelve antimicrobial agents commonly used in human 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), linezolid (LIN- 10 µg-BrCast), and vancomycin (VAN - 30 µg). Commercially prepared paper disks containing the appropriate antibiotic dosage were purchased from Laborclin (São Paulo, BR).

Colonies were suspended in peptone water until it reached 0.5 McFarland standard. The dilution was then streaked across a Mueller-Hinton Agar plate (Himedia, Mumbai, India), and the 12 antimicrobial disks were placed onto the surface of the agar. The plates were incubated for 24 h at 37^0C. Zone diameters were recorded after a 24 h incubation period. Strains were classified as susceptible or not susceptible (intermediate susceptible or resistant) according to criteria from CLSI (2019) and BrCAST (2019) for linezolid. *Enterococcus faecalis* ATCC 29212 was used as a positive control.

Strains were classified as multidrug-resistant (MDR) when showed not susceptibility to three or more antimicrobial classes (EFSA & ECDC, 2013).

Detection of antimicrobial resistance genes associated with mobile genetic elements by polymerase chain reaction (PCR)

Presence of erythromycin (*erm*B and *msr*C), and tetracycline [*tet*(M) and *tet*(L)] genes were determined by PCR. The DNA was isolated from enterococcal strains according to RIBOLDI et al. (2009). The primers are listed in table 2. Amplifications were carried out in a total volume of 25 µL containing: 100 ng of template DNA, 1 X reaction buffer (Ludwig Biotechnology, Porto Alegre, BR), 0.4 µM of each primer (Exxtend Biotechnology Ltda, São Paulo, BR), 1.5 mM MgCl2 (Ludwig Biotechnology, Porto Alegre, BR), 200 µM of dNTPs (Ludwig Biotechnology, Porto Alegre, BR), 1 *U* Taq DNA polymerase (Ludwig Biotechnology), and MilliQ water. *Enterococcus durans* (PRICHULA et al., 2013), *Enterococcus hirae* MP1-1 (PRICHULA et al., 2016), *Enterococcus faecium* MP1-10 (PRICHULA et al., 2016), *E. faecalis* T3R2-198 (CASSENEGO et al., 2011), *Enterococcus gallinarum* F3 (SANTESTEVAN et al., 2015) were used as positive control. PCR amplifications were performed in the conventional thermocycler (Applied Biosystems 2720 Thermal Cycler) as follows: 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.

RESULTS AND DISCUSSION

Enterococci species isolated from raw milk and cheese of sheep

A total of 156 enterococci were isolated, 80 (51.3%) from raw milk and 76 (48.7%) from cheeses. The most frequently isolated species was *Enterococcus faecalis* (50.6%; n=79), followed by *E. faecium* (20.5%; n=32), *E. hirae* (14.8%; n=23), *E. durans* (13.5%; n=21), and *E. gallinarum* (0.6%; n=1). According to the results shown in table 3, the most common species identified in milk samples were *E. faecalis* (58.8%; n = 47), *E. hirae* (28.8%; n = 23), and *E. faecium* (12.5%; n = 10) isolated from milk samples. As well as in other studies, *E. faecalis* was dominating species in sheep milk samples. DUCKOVÁ et al. (2009) identified 70% of *E. faecalis* in sheep´s milk samples in Slovakia. ARIZCUN et al. (1997) also found *E. faecalis* as a dominating species in ewe's milk in the Autonomous Region of Navarre in Spain. It is important to highlight that *E. faecalis, E. hirae,* and *E. faecium* occur in the gastrointestinal tract of sheep, and their presence in the raw sheep's milk might be associated with fecal contamination during milking and/or handling of animals (DAPKEVICIUS et al., 2021).

Out of 76 strains isolated from cheeses, 29 (38.2%) were collected from Colonial, 29 (38.2%) from Feta-type and 18 (23.7 %) from Pecorino-type. According to the results shown in table 3, differences in the species distributions were detected amongst cheese samples. *Enterococcus durans* (13.5%; n = 21) was found in Colonial and Feta-type cheeses; and *E. faecium* (75.9 %; n = 22) and *E. gallinarum* (3.5%; n = 1) were only isolated from Colonial cheese samples. These results are in line with VÝROSTKOVÁ et al. (2021), that reported different strains of enterococci in sheep cheeses samples. *Enterococcus faecalis* and *E. faecium* were also the most frequent species detected in artisanal cheeses produced with sheep, goat, buffalo, and cow pasteurized or raw milk (GELSOMINO et al., 2002; RIBOLDI et al., 2009; PRICHULA et al., 2013).

Table 2 - Primers used in the PCR reactions carried out for detection of resistance genes.

Gene	Nucleotide sequence (5'-3')	Size (bp)[1]	AT (°C)[2]	Reference
*erm*B	GAAAAGGTACTCAACCAAATA AGTAACGGTACTTAAATTGTTTAC	547	52	SUTCLIFFE et al. (1996)
*msr*C	AAGGAATCCTTCTCTCTCCG GTAAACAAAATCGTTCCCG	343	52	WERNER et al. (2001)
tet(L)	ACTCGTAATGGTGTAGTTGC TGTAACTCCGATGTTTAACACG	625	58	FRAZZON et al. (2009)
tet(M)	GTTAAATAGTGTTCTTGGAG CTAAGATATGGCTCTAACAA	657	52	AARESTRUP et al. (2000)

[1]bp: base pairs. [2] AT: annealing temperature.

Table 3 - Distribution of *Enterococcus* species among sheep's milk and cheeses-types samples collected in South Brazil from 2017 to 2019.

Sample (n)	Number (%) ofspecies				
	E. faecalis	*E. faecium*	*E. hirae*	*E. durans*	*E. gallinarum*
Milk (80)	47 (58.8)	10 (12.5)	23 (28.8)	0	0
Cheese (76)					
Colonial (29)	4 (13.8)	22 (75.9)	0	2 (6.9)	1 (3.5)
Feta-type (29)	10 (34.5)	0	0	19 (65.5)	0
Pecorino-type (18)	18 (100)	0	0	0	0
Total (156)	79 (50.6)	32 (20.5)	23 (14.8)	21 (13.5)	1 (0.6)

Starter lactic acid bacteria, like enterococci, are naturally present in milk or beneficial contaminants originated from cheese production environments. The occurrence of *E. faecalis* in sheep's cheeses might be related to the development of aromatic and organoleptic features, since this species is able to produce acetaldehyde and diacetyl (ARIZCUN et al., 1997). Furthermore, some enterococci strains (such as *E. durans, E. faecium,* and *E. faecalis*) present in the raw milk and cheeses of sheep's are recognized to produce bacteriocins, with ability to killing or inhibits important foodborne pathogen, like *Listeria monocytogenes* (PRICHULA et al., 2021).

Antimicrobial resistance profile of enterococci isolated from raw milk and cheese of sheep's

To not overestimate the data referring to antimicrobial not susceptible profiles, strains isolated from the same sample with similar phenotypic and genotypic profiles, which could indicate clonal strains, were grouped. Thus, 156 strains were grouped into 74 strains (41 from milk, and 33 from cheese). Among the 74 strains, 65 (87.8%) strains were not susceptible to at least one antimicrobial agent evaluated, being this profile more frequently observed in strains collected from milk than those from cheese samples (Figure 1). All enterococci strains were susceptible to ampicillin, linezolid, and vancomycin. Single, double, and MDR profiles were observed in 21.6% (n = 16), 25.7% (n = 19), and 40.5% (n = 30) of the strains, respectively (Figure 1) (Table 4). The frequency of MDR strains isolated from milk (53.7%; n=22) were higher compared to cheese (24.2%; n=8).

Strains isolated from raw milks (n=41) were not susceptible to tetracycline (58.5%; n=24), rifampicin (56.1%; n=23), erythromycin (46.3%; n=19), aminoglycosides (gentamicin/ streptomycin) (39 %; n=16), and nitrofurantoin (31.7%; n=13). Strains were also not susceptible to quinolones (ciprofloxacin/norfloxacin) (12.2%;

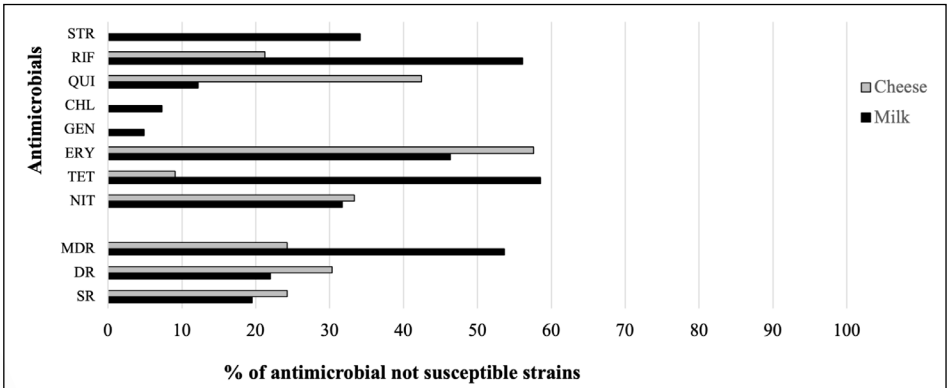

Figure 1 - Antimicrobial susceptibility profile of enterococci isolated from South Brazil sheep's milk and cheeses. Antimicrobial:STR: Streptomycin; RIF: Rifampicin; QUI, quinolones (ciprofloxacin or norfloxacin); CHL: Chloramphenicol; GEN: Gentamicin; ERY: Erythromycin; TET: Tetracycline; NIT: Nitrofurantoin. SR, single resistant; DR, doubleresistant; MDR, multidrug-resistant.

Table 4 - Antibiotic resistance patterns among enterococci recovered from raw milk and cheeses-types of sheep in South Brazil.

		----------Number of resistant strains to*								--------Profiles**		
Samples	Species (n)	NIT	TET	ERY	GEN	CHL	QUI	RIF	STR	SR	DR	MR
Milk	*E. faecium* (6)	2	0	5	0	0	4	5	0	0	1	5
	E. faecalis (22)	3	16	14	2	3	1	16	14	2	2	18
	E. hirae (13)	8	8	0	0	0	0	2	0	6	6	0
	Subtotal (41)	13	24	19	2	3	5	23	14	8	9	22
Cheese	*E. faecium* (14)	6	0	12	0	0	10	0	0	3	5	5
	E. faecalis (11)	1	2	6	0	0	2	7	0	2	3	3
	E. durans (6)	4	0	1	0	0	1	0	0	2	2	0
	E. gallinarum (1)	0	1	0	0	0	0	0	0	1	0	0
	Subtotal (33)	11	3	19	0	0	14	7	0	8	10	8
	Total (74)	25	27	38	2	3	19	30	14	16	19	30

*NIT: Nitrofurantoin (300 µg); TET: Tetracycline (30 µg); ERY: Erythromycin (15 µg); GEN: Gentamicin (120 µg); LIN: Linezolid (10 µg-BrCast); CHL: Chloramphenicol (30 µg); QUI: Quinolones (ciprofloxacin - 5 µg ornorfloxacin -10 µg); RIF: Rifampicin (5 µg); STR: Streptomycin (300 µg). **Profiles: SR, single resistant; DR, double resistant; MDR, multidrug-resistant.

n=5), and chloramphenicol (7.3%; n=3) (Figure 1). Resistant enterococci have been isolated from the raw milk of cows, goats, and buffalos around the world (PRICHULA et al., 2013; PALMERI et al., 2020; KANG et al., 2021). Ampicillin, erythromycin, gentamicin, and tetracycline resistant enterococci were isolated from raw sheep milk samples in Slovakia (DUCKOVÁ et al., 2009). In Brazil, previous studies showed resistant enterococci in raw buffalo and goat milk samples (PRICHULA et al., 2013; PERIN et al., 2014). PRICHULA et al. (2013) recovered from raw buffalo milk samples enterococci strains showing resistance to nitrofurantoin, tetracycline, erythromycin, norfloxacin, chloramphenicol, and streptomycin. PERIN et al. (2014) reported strains resistant to vancomycin, gentamicin, chloramphenicol, ampicillin, and rifampicin in goat milk samples from farms located in Minas Gerais state, Brazil.

The elevated frequency of tetracycline, rifampicin, erythromycin, and nitrofurantoin not susceptible strains in raw sheep milk, may be related to the fact that these drugs are widely used in veterinary medicine for the prophylaxis and treatment of infections (NOVAES et al., 2017). Tetracycline and streptomycin have been used to treat intramammary infections in sheep (LOLLAI et al., 2016; RÓŻAŃSKA et al., 2019). Another source of not susceptible strains in raw milk maybe been related to the milking equipment, since this genus is well known to survive desiccation, starvation, and disinfection, and are able to develop biofilms (ORTIGOSA et al., 2008; LEBRETON et al., 2017; PEREIRA et al., 2017). In addition, these resistance profiles observed might be also associated with environmental resistome (SURETTE & WRIGHT, 2017).

Among the 33 enterococci obtained from cheese samples, 19 (57.6%) strains were not susceptible to erythromycin, 14 (42.4%) to quinolones (ciprofloxacin/norfloxacin), 11 (33.3%) to nitrofurantoin, 7 (21.2%) to rifampicin, and 3 (9.1%) to tetracycline. All strains isolated from cheese were susceptible to aminoglycosides (gentamicin/streptomycin), linezolid, vancomycin, and chloramphenicol (Table 4). Recently, VÝROSTKOVÁ et al. (2021) isolated *Enterococcus* spp. resistant to vancomycin, teicoplanin, erythromycin, doxycycline, minocycline, ciprofloxacin, levofloxacin, nitrofurantoin, and rifampicin in traditional sheep cheeses. SANLIBABA & SENTURK (2018) evaluated 215 traditional Turkish cheese samples in Ankara (Turkey) and identified a high frequency of nalidixic acid (100%), kanamycin (98.6%), rifampicin (78.4%), ampicillin (48.8%), and ciprofloxacin (45.5%) resistance in enterococcal isolates, and low frequency to erythromycin (18.8%), tetracycline (11.7%), penicillin G (5.6%), chloramphenicol (4.2%), gentamicin (3.8%), and streptomycin (1.4%).

In regard to MDR strains isolated from milk, three *E. faecalis* strains were not susceptible to at least five antimicrobial agents (TET/ERI/GEN/RIF/EST; NIT/TET/ERI/QUI/RIF and TET/ERI/CLO/RIF/EST). Concerning MDR strains isolated from cheese samples, one *E. faecium* (NIT/ERI/QUI) and one *E. faecalis* (TET/ERI/QUI/RIF) showed not susceptible to three and four antimicrobial agents, respectively (Table 5). Antimicrobial not susceptible

Table 5 - Main phenotypic multidrug-resistant profiles observed among enterococci isolates from raw milk and cheeses-types of sheep´s in South Brazil.

Species	Multidrugresistant profile[1]	----------Number of enterococci by sample----------	
		Cheese	Milk
E. faecalis	TET, ERY, STR		4
	TET, ERY, RIF	1	1
	TET, RIF, STR		2
	CHL, RIF, STR		1
	ERY, QUI, RIF	1	1
	GEN, CHL, RIF		1
E. faecium	NIT, ERY, QUI	5	
	ERY, QUI, RIF		2
E. faecalis	TET, ERY, RIF, STR		3
	TET, NIT, ERY, RIF		1
	TET, ERY, RIF, QUI	1	
E. faecium	NIT, ERY, QUI, RIF		2
E. faecalis	TET, ERY, GEN, RIF, STR		1
	TET, NIT, ERY, RIF, STR		1
	TET, NIT, ERY, RIF, QUI		1
	TET, ERY, CHL, RIF, STR		1

[1]QUI: Quinolones (ciprofloxacin - 5 µg ornorfloxacin - 10 µg); TET: Tetracycline (30 µg); STR: streptomycin (300 µg); ERY: Erythromycin (15 µg); GEN: Gentamicin (120 µg); NIT: Nitrofurantoin (300 µg); CHL: Chloramphenicol (30 µg); RIF: Rifampicin (5 µg).

strains in food, particularly those that are MDR, constitute a One Health issue, since they might pose a potential health risk to the consumer.

Frequency of tetracycline and erythromycin resistance genes between enterococci isolated from raw milks and cheese of sheep

Among the 24 tetracycline not susceptible strains isolated from raw milks, 12 (50%) harbored the *tet*(M), 10 (41.7%) *tet*(M) and *tet*(L), and 1 (4.2%) only the *tet*(L). In contrast, all (n=4) tetracycline not susceptible strains collected from cheeses presented the *tet(*M), and none of them was positive for the *tet*(L). Erythromycin not susceptible strains (n=20) collected from milks, displayed two resistance genotypes: 6 (30%) carried the *mrs*C and 6 (30%) the *erm*B genes. Of the 21 strains not susceptible to erythromycin isolated from cheeses, 9 (42.9%) harbored the *mrs*C gene and 1 (4.8%) the *erm*B gene. These results are in agreement with other studies that evaluated these genes in enterococci isolated from milk and cheese samples (KANG et al., 2019; SILVETTI et al., 2019; VÝROSTKOVÁ et al., 2021).

Resistance to macrolides and tetracyclines in enterococci has been assumed to be related to the presence of a conjugative transposon. This mechanism is an important factor for spreading of tetracycline resistance between enterococci and other Gram-positive bacteria (RIZZOTTI et al., 2009). The occurrence of antimicrobial resistance genes in our samples, especially in cheeses, is a very important issue since these genes can be transmitted by conjugative transposons, facilitating horizontal genetic exchange and, therefore, promote the acquisition and spread of resistance genes.

CONCLUSION

The present study provided the first report of resistant enterococci in raw sheep's milk and cheeses in South Brazil. However, other points need to be explored to understand the origin, emergence, and dissemination of antimicrobial not susceptible and MDR enterococci in sheep samples. The dissemination of antimicrobial-resistant bacteria between human–animal–environment interface has been recognized as a One Health issue.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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