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Original Article

Phenotypic and Molecular Antibiotic Resistance Profile of *Enterococcus faecalis* and *Enterococcus faecium* Isolated from Different Traditional Fermented Foods

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Abstract

A collection of 55 enterococci (41 *Enterococcus faecium* and 14 *E. faecalis* strains) isolated from various traditional fermented foodstuffs of both animal and vegetable origins, and water was evaluated for resistance against 15 antibiotics. Lower incidence of resistance was observed with gentamicin, ampicillin, penicillin and teicoplanin. However, a high incidence of antibiotic resistance was detected for rifampicin (12 out of 14 of isolates), ciprofloxacin (9/14), and quinupristin/dalfopristin (8/14) in *E. faecalis* strains. *Enterococcus faecium* isolates were resistant to rifampicin (25/41), ciprofloxacin (23/41), erythromycin (18/41), levofloxacin (16/41), and nitrofurantoin (15/41). One *Enterococcus faecalis* and two *E. faecium* strains were resistant to vancomycin (MIC > 16 μg/mL). Among 55 isolates, 27 (19 *E. faecium* and eight *E. faecalis*) were resistant to at least three antibiotics. High level of multidrug resistance to clinically important antibiotics was detected in *E. faecalis* strains (57% of *E. faecalis* versus 46% of *E. faecium*), which showed resistance to six to seven antibiotics, especially those isolated from foods of animal origin. So, it is necessary to re-evaluate the use of therapeutic antibiotics in stock farms at both regional and international levels due to the high number of multiple resistant (MR) bacteria. Fifty-six MR *E. faecalis* and *E. faecium* strains selected from this and previous studies (Valenzuela *et al*., 2008, 2010) were screened by polymerase chain reaction for antibiotic resistance genes, revealing the presence of *tet*(L), *tet*(M), *ermB, cat, efrA, efrB, mphA,* or *msr*A/B genes. The ABC Multidrug Efflux Pump EfrAB was detected in 96% of *E. faecalis* strains and also in 13% of *E. faecium* strains; this is the first report describing EfrAB in this enterococcal species. The efflux pump–associated *msr*A/B gene was detected in 66.66% of *E. faecium* strains, but not in *E. faecalis* strains.

Introduction

Enterococci have emerged as important nosocomial pathogens over the past decade (Dupre *et al*., 2003; Vankerckhoven *et al*., 2004). Their ubiquitous nature determines their frequent finding in food as contaminants (Giraffa, 2002); they are implicated in food spoilage (Franz *et al*., 1999), intoxication (Giraffa *et al*., 1997), and the spreading of antibiotic resistance through the food chain (Leavis *et al*., 2006). At the same time, enterococci possess many technological applications in the food industry as starter cultures (Foulquie-Moreno *et al*., 2006), as potential biopreservatives (enterocin producers), and as probiotics (Stiles and Holzapfel, 1997); they are also responsible for the sensory characteristics of some fermented foods. The dualistic aspects of enterococci pose a great challenge concerning their presence in food products.

Enterococci have both an intrinsic and acquired resistance to antibiotics, making them important nosocomial pathogens (Murray *et al*., 1990; Klare *et al*., 2002, 2003; Hummel *et al*., 2007). Vancomycin-resistant enterococci (VRE) are of major concern (Hershberger *et al*., 2005; de Jong *et al*., 2009). Enterococci readily acquire resistance genes (Dever, 2000) and are also capable of transferring resistance genes to other bacteria (Shepard and Gilmore, 2002; Lester *et al*., 2006). Clinical practices and animal husbandry are important foci of selective antibiotic pressure, and the food chain has been shown to act as a reservoir of antibiotic resistance determinants to be spread to humans via various routes (Witte, 2000; Kojima *et al*., 2010).

The present work aimed to study the antimicrobial resistance profiles and the incidence of genetic determinants of antimicrobial resistance in enterococci isolated from different traditional fermented foods including fermented milk

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(Morocco and Spain), meat (Morocco), and vegetable products (Morocco, Spain, and Republic of Congo).

Materials and Methods

Bacterial strains and media

A total of 55 enterococci strains—26 strains isolated in the present study and 29 strains isolated by Valenzuela *et al.* (2008, 2010) from traditional foods and water used for traditional food preparation—belonging to the species of *Enterococcus faecium* and *E. faecalis* were used in this study (Table 1). The fermented milk products from Morocco that served as sources of enterococci were commercial butter, traditional cheese (Jben), commercial goat cheese (Valenzuela *et al.*, 2008), and fermented milk. Enterococci from goat milk cheeses of Southern Spain (qE isolates in Table 1) were also included. All fermented food products were traditional foods without starter cultures added. All strains were maintained and stored in brain-heart infusion (BHI) broth (Scharlab, Barcelona, Spain) containing 20% glycerol at −80°C. For routine use, enterococcal isolates were cultivated on BHI broth at 37°C.

Identification of E. faecium *and* E. faecalis *strains*

Presumptive identification of isolates was carried out with the following tests: observation of colony characteristics and cell morphology, Gram staining, catalase, growth at 10°C and 45°C, growth in the presence of 6.5% NaCl, at pH 9.6, as well as growth and esculin hydrolysis on bile–esculin agar (Scharlab). The isolates identified as presumptive *Enterococcus* sp. were further identified at species level by species-specific polymerase chain reaction (PCR) to detect the *ddlE.faecalis* and *ddlE.faecium* genes. The primers used were 5′CAAACTG TTGGCATTCCACAA3′ and 5′TGGATTTCCTTTCCAGTC ACTTC3′ (*E. faecalis* forward and reverse primers respectively); and 5′GAAGAGCTGCTGCAAAATGCTTTAGC3′ and 5′GCGCGCTTCAATTCCTTGT3′ (*E. faecium* forward and reverse primers respectively), as described elsewhere (Abriouel *et al.*, 2005).

Antibiotic resistance

The antibiotic susceptibility of isolates was determined by using ATB ENTEROC 5 strips (BioMérieux, Marcy-l'Etoile, France). The tests were performed by using the antibiotics described in Table 2 and following the manufacturer's instructions. Results were recorded after 24 h of incubation at 37°C and were evaluated according to the manufacturer's instructions (Table 2).

PCR amplification for the detection of antibiotic resistance genes

PCR amplification of well-known structural genes of antibiotic resistance—erythromycin (*erm*A,B,C; *mef*A,E; *msr*A,B; and *ere*A,B), tetracycline (*tet*[M], *tet*[O], *tet*[S], *tet*[K], and *tet*[L]), and chloramphenicol (*cat*)—was performed as reported by Hummel *et al.* (2007). PCR of *efrA* and *efrB* genes was done according to Lee *et al.* (2003).

Table 1. Enterococci Isolated from Different Traditional Food Products

Enterococci species	*Cheese and butter*	*Fermented meat*	*Vegetable foods and water*
E. faecalis	$n=9$	$n=1$	$n=4$
E. faecium	$n=18$	$n=6$	$n=17$

Table 2. Antibiotic Resistance of Enterococci Isolated from Different Foods

Antibiotic	*Antibiotic MIC breaking points*[a] *(mg/L)*	*Incidence of antibiotic resistance*	
		E. faecalis	E. faecium
Ampicillin	8	0/14	1/41
Penicillin	8	0/14	1/41
Erythromycin	4	6/14	18/41
Tetracycline	8	5/14	6/41
Chloramphenicol	16	2/14	0/41
Rifampicin	2	12/14	25/41
Ciprofloxacin	2	9/14	23/41
Levofloxacin	4	1/14	16/41
Vancomycin	16	1/14	2/41
Teicoplanin	16	1/14	2/41
Nitrofurantoin	64	1/14	15/41
Gentamycin	500	0/14	0/41
Streptomycin	1000	2/14	3/41
Quinupristin/ dalfopristin	2	8/14	5/41

[a]Values determined according to ATB ENTEROC 5 strips used in this study.

Results and Discussion

Enterococci strains isolated from different traditional fermented foods (of both animal and vegetable origins) and water were used in this study. Food samples were obtained from various regions: Spain, Morocco, and Republic of Congo. All isolates showed phenotypic properties typical of *E. faecium* and *E. faecalis* strains, i.e., they were Gram-positive cocci, catalase-negative, and hydrolized esculin in the presence of 40% bile salt. They grew at 10°C and 45°C, in the presence of 6.5% NaCl, at pH 9.6, and survived at 70°C for 30 min. According to PCR amplification with *E. faecium* and *E. faecalis* species-specific primers, 14 isolates were identified as *E. faecalis* and 41 were identified as *E. faecium*. Previous studies also showed a higher incidence of *E. faecium* in fermented capers (nine *E. faecium* versus four *E. faecalis* strains) (Pérez-Pulido *et al.*, 2006) and Slovak Bryndza Cheese (178 *E. faecium* versus 49 *E. faecalis* strains) (Jurkovic *et al.*, 2006). *E. faecium* is also one of the lactic acid bacteria (LAB) species that can be found in relatively high numbers during meat fermentation (Hugas *et al.*, 2003). In Argentinean artisanal dry fermented sausages, 56% of enterococcal isolates were identified as *E. faecium*, followed by *E. faecalis* (17%) and other species (*Enterococcus durans*, *Enterococcus casseliflavus,* and *Enterococcus mundtii*) (Fontana *et al.*, 2009). However, Trivedi *et al.* (2011) showed a high predominance of *E. faecalis* (127 strains) followed by *E. faecium* (77 strains) in foodstuffs of all origins (raw and pasteurized milk samples, cheeses of different varieties, ready-to-eat meat products, and various fruits and vegetables).

Antibiotic sensitivity

The antibiotic susceptibility profile of isolates is summarized in Table 2. According to ATB ENTEROC 5 test, a lower

incidence of resistance was seen with clinically relevant antibiotics such as gentamicin, ampicillin, and penicillin (Table 2) in both *Enterococcus* species. However, a high incidence of antibiotic resistance was detected among *E. faecalis* isolates to rifampicin (12 out of 14 of isolates), ciprofloxacin (9/14), and quinupristin/dalfopristin (8/14). Furthermore, *E. faecalis* isolates showed an intermediate resistance to erythromycin (6/14) and tetracycline (5/14), and to a lesser extent to streptomycin (2/14), chloramphenicol (2/14), nitrofurantoin (1/14), levofloxacin (1/14), teicoplanin (1/14), and vancomycin (1/14). All *E. faecalis* isolates were susceptible to three antibiotics (ampicillin, penicillin, and gentamicin). On the other hand, *E. faecium* isolates were resistant to rifampicin (25/41), ciprofloxacin (23/41), erythromycin (18/41), levofloxacin (16/41), and nitrofurantoin (15/41). In addition, two isolates were resistant to vancomycin and teicoplanin, and one isolate was resistant to ampicillin and penicillin. All *E. faecium* isolates were susceptible to gentamycin and chloramphenicol. The results obtained in the current study were in accordance with those obtained in food isolates reported by Ben Omar *et al.* (2004), Pérez-Pulido *et al*. (2006), and Valenzuela *et al*. (2008) regarding resistance to rifampicin, ciprofloxacin, and erythromycin. Only *E. faecium* H2OP3 (a strain isolated from water used for preparation of food at Republic of Congo) was resistant to penicillin and ampicillin, and also to vancomycin and teicoplanin. Resistance of enterococci to glycopeptide antibiotics such as vancomycin and teicoplanin and to aminoglycosides (Kacmaz and Aksoy, 2005) is well documented. In this study, few *Enterococcus* strains were resistant to teicoplanin and simultaneously to vancomycin (Table 3), which could pose a great concern in clinical treatment, by increasing treatment failure by 20% and mortality from 27% to 52%, as reported by Brown *et al*. (2006). However, our results indicated an almost complete (97.6–100%) susceptibility of enterococcal isolates to ampicillin or penicillin as cell wall active agents and gentamycin (aminoglycoside). Thus, strains with resistance to vancomycin and/or teicoplanin are completely susceptible to ampicillin and penicillin, which is of great importance in health care because of their synergistic bactericidal effects against enterococci (Filipová *et al*., 2006).

Multiple resistant (MR) enterococci

Analysis of antibiotic resistance pattern of enterococci isolates revealed multiple antibiotic resistant strains in food isolates in the same way as reported by Peters *et al*. (2003). Among the total of 55 strains analyzed, 27 strains (19 *E. faecium* and eight *E. faecalis*) were resistant to at least three antibiotics (Table 3). Furthermore, 29 MR enterococci of food origin previously evaluated by Valenzuela *et al*. (2008, 2010) were included in the present study for molecular screening of resistance genes.

In this study, MR *E. faecalis* strains showed various levels of antibiotic resistance: resistant to seven antibiotics (strain qE-29) and six antibiotics (strains qE-12 and qE-14). Similarly, some *E. faecalis* strains analyzed by Valenzuela *et al*. (2008, 2010) and included here for molecular studies showed resistance to eight antibiotics (strain Mz2), seven antibiotics (strains J3, J39 and J41), or six antibiotics (strain CM5). It is noteworthy that *E. faecalis* strains with resistance to six to eight antibiotics were isolated from foods of animal origin (Table 3). As shown in Table 3, VR *E. faecalis* qE-29 and Mz2 isolates were also resistant to erythromycin, tetracycline, rifampicin, ciprofloxacin, levofloxacin, nitrofurantoin, and quinupristin/dalfopristin. Tetracycline and erythromycin resistance in foods of animal origin is likely related to the wide use of these classes of antibiotics in husbandry activities (Šustačkova *et al*., 2004; Kročko *et al*., 2011). The vancomycin-resistant *E. faecalis* qE-29 was also resistant to teicoplanin (Table 3). Regarding antibiotic resistance in *E. faecium*, we detected resistance to five to six antibiotics in eight strains analyzed in this study (*E. faecium* H2OP3, KAA1, KAA3, KAA4, YA 2, qE-11, qE-18, and qE-23), all of them of animal origin except for H2OP3 (water) and only in three strains (*E. faecium* H2, Mz1B, and S1) reported previously by Valenzuela *et al*. (2008) and included in the current study. The presence of MR bacteria in foods may have a negative impact on treatment outcomes as well as increased treatment costs (Roberts *et al*., 2009; Wassenberg *et al*., 2010) due to their transmission to human via the food chain, especially foods of animal origin such as cheese and meat. The frequent occurrence of MR enterococci in farm animals and food products was reported by several authors (Peters *et al*., 2003; Leclercq, 2009; Vignaroli *et al*., 2011), highlighting the role of nonhuman reservoirs as sources of resistance genes. In this study, the percentage of MR *E. faecalis* (57% of *E. faecalis*) was higher than *E. faecium* (46% of *E. faecium*); this may be due to the presence of resistance genes in mobile genetic elements associated with *E. faecalis*.

Antibiotic resistance determinants

Detection of antibiotic resistance determinants was carried out by PCR amplification of known genes in 56 MR *E. faecalis* and *E. faecium* strains. The genetic basis of the observed tetracycline resistance (TetR) was investigated by PCR amplification of *tet* genes: *tet*(L), *tet*(K), *tet*(M), *tet*(S), and *tet*(O). All of the TetR strains carried either *tet*(L) or *tet*(M), or a combination of both determinants (Table 3). The gene *tet*(M) was the most common among the enterococci strains studied in a similar way as reported previously in food isolates (Aarestrup *et al*., 2000; Huys *et al*., 2004; Wilcks *et al*., 2005), whereas *tet*(L) was the second-most common (Table 3); the combination of both determinants was mostly present among *E. faecium* isolates (13.33% in *E. faecium* versus 3.8% in *E. faecalis* strains). However, we could not detect by PCR the presence of *tet*(O), *tet*(S), and *tet*(K) genes in a manner similar to that reported by Wilcks *et al*. (2005) and Huys *et al*. (2004). In those cases, the incidence of *tet*(O) and *tet*(S) was very low ($<10\%$); therefore, such low incidences may suggest that the chance of isolating enterococci bearing these resistances would be higher when the sample size is larger. Both *tet*(M) and *tet*(S) were found in raw products, whereas only *tet*(M) was found after fermentation, as reported by Teuber *et al*. (1999) and Gevers *et al*. (2003) in meat products.

All chloramphenicol resistant (CmR) isolates carried the *cat* gene, as reported in other studies (Werner *et al*., 2000; Teuber *et al*., 2003; Huys *et al*., 2004). We determined that four CmR *E. faecalis* strains were also resistant to tetracycline. The occurrence of CmR among food enterococci has been frequently reported at various incidences (Teuber *et al*., 1999; Franz *et al*., 2001). *Cat* genes are located usually on plasmids such as the conjugative and mobilizing, multi-resistance plasmid pRE25 from *E. faecalis* (Schwarz *et al*., 2001; Teuber *et al*., 2003), the conjugative plasmid pUW1965 (Werner *et al*.,

Table 3. Incidence of Antibiotic Resistance in Enterococcal Isolates from Different Foods

Isolate	*Food source*	*Antibiotic resistance (identified resistance determinants)*
E. faecalis Ac 8-2	Olives	RFA, CIP, QDA (*efrA, efrB*)
E. faecalis Oli1	Olives	ERY, TET, CMP, RFA, QDA[a] (*ermB, cat, efrA) efrB, tetM*)
E. faecalis Oli2	Olives	TET, RFA, QDA[a] (*efrA, efrB*)
E. faecalis Oli3	Olives	TET, RFA, QDA[a] (*efrA, efrB, tetM*)
E. faecalis Oli4	Olives	ERY, TET, RFA[a] (*ermB, efrA, efrB, tetM*)
E. faecalis Mz2	Cow meat salami	ERY, TET, RFA, CIP, LVX, VAN, FUR, QDA[a] (*ermB, mphA,efrB, tetM)*
E. faecalis Mz4	Cow meat salami	RFA, CIP, QDA (*efrA, efrB*)
E. faecalis GM2	Goat milk	TET, RFA, CIP, LVX, FUR[a] (*efrA, efrB) tetM*)
E. faecalis GM43	Goat milk	TET, RFA, CIP, FUR, STH[a] (*efrA, efrB) tetM*)
E. faecalis CM5	Cow milk	TET, RFA, CIP, LVX, FUR, QDA[a] (*efrA, efrB) tetM*)
E. faecalis CM11	Cow milk	CIP, LVX, FUR[a]
E. faecalis Mq7.1	Butter	TET, RFA, CIP, QDA[a] (*efrA, efrB, tetM*)
E. faecalis Mq7.2	Butter	TET, RFA, CIP, QDA[a] (*ermB, efrA, efrB*)
E. faecalis Mq7.4	Butter	TET, RFA, CIP, LVX[a] (*efrA, efrB, tetM*)
E. faecalis qE-12	Cheese	ERY, TET, CMP, RFA, STH, QDA (*ermB, cat) efrA, efrB, tetM*)
E. faecalis qE-14	Cheese	ERY, TET, CMP, RFA, STH, QDA (*ermB, cat) efrA, efrB, tetM*)
E. faecalis qE-20	Cheese	RFA, CIP, QDA (*ermB, efrB*)
E. faecalis qE-21	Cheese	RFA, CIP, QDA (*efrA, efrB*)
E. faecalis qE-27	Cheese	TET, RFA, CIP (*ermB, efrA, efrB, tetL*)
E. faecalis qE-29	Cheese	ERY, RFA, CIP, LVX, VAN, TEC, QDA (*efrA, efrB*)
E. faecalis FB1	Cheese	ERY, TET, CMP, RFA, CIP[a] (*cat, efrA, efrB*)
E. faecalis FB2	Cheese	ERY, TET, RFA[a] (*efrA, efrB*)
E. faecalis FB3	Cheese	TET, RFA, CIP, FUR, QDA[a] (*efrA, efrB) tetM*)
E. faecalis J3	Cheese	TET, RFA, CIP, LVX, FUR, STH, QDA[a] (*efrA) efrB, tetM, tetL*)
E. faecalis J39	Cheese	TET, RFA, CIP, LVX, FUR, STH, QDA[a] (*efrA) efrB, tetM*)
E. faecalis J41	Cheese	TET, RFA, CIP, LVX, FUR, STH, QDA[a] (*efrA) efrB, tetM*)
E. faecium H2OP3	Water	ERY, RFA, VAN, TEC, QDA (*msr*A/B, *efrA) efrB*)
E. faecium Tg6	Ginger beer	ERY, RFA, CIP, QDA (*msr*A/B, *efrA, efrB*)
E. faecium VP3 01	Palm wine	ERY, RFA, CIP, LVX (*msr*A/B)
E. faecium VP3 02	Palm wine	RFA, CIP, LVX
E. faecium H1	Ground pepper	ERY, CIP, LVX, FUR[a] (*msr*A/B, *ermB*)
E. faecium H2	Ground pepper	ERY, RFA, CIP, LVX, FUR[a] (*msr*A/B, *ermB) efrB*)
E. faecium H3	Ground pepper	ERY, CIP, LVX, FUR[a] (*msr*A/B)
E. faecium H4	Ground pepper	ERY, CIP, LVX, FUR[a] (*msr*A/B, *ermB*)
E. faecium PE 2-2	Fish	RFA, CIP, LVX, FUR[b]
E. faecium Sln 1	Blood sausage	TET, RFA, CIP, LVX (*tetM, tetL*)
E. faecium Sln2	Blood sausage	TET, CIP, LVX, FUR (*tetL*)
E. faecium KAA 1	Blood sausage	ERY, TET, CIP, FUR, STH (*msr*A/B, *ermB) tetM, tetL*)
E. faecium KAA 3	Blood sausage	TET, RFA, CIP, LVX, FUR, STH (*tetM, tetL*)
E. faecium KAA 4	Blood sausage	ERY, TET, RFA, CIP, LVX (*msr*A/B, *ermB)*
E. faecium Mz1B	Cow meat salami	ERY, RFA, CIP, LVX, VAN, TEC[a] (*msr*A/B) *ermB*)
E. faecium Mz3B	Cow meat salami	CIP, VAN, TEC[a]
E. faecium S1	Turkey salami	ERY, TET, RFA, CIP, LVX, FUR[a] (*msr*A/B) *ermB, tetM, tetL*)
E. faecium YA 2	Fermented milk	ERY, RFA, CIP, LVX, QDA (*msr*A/B, *ermB*)
E. faecium YA 6	Fermented milk	ERY, CIP, LVX (*msr*A/B, *ermB*)
E. faecium YA 9-A	Fermented milk	ERY, CIP, LVX (*msr*A/B, *ermB*)
E. faecium YA 9-B	Fermented milk	ERY, CIP, LVX (msrA/B, *ermB*)
E. faecium Mq1	Butter	ERY, CIP, LVX, FUR[a] (*msr*A/B,)
E. faecium Mq2	Butter	ERY, CIP, LVX, FUR[a] (*msr*A/B, *ermB*)
E. faecium Mq3	Butter	ERY, CIP, LVX, FUR[a] (*msr*A/B, *ermB)*
E. faecium qE-11	Cheese	ERY, RFA, CIP, LVX, FUR, QDA
E. faecium qE-17	Cheese	RFA, CIP, FUR
E. faecium qE-18	Cheese	ERY, RFA, CIP, LVX, FUR (*msr*A/B)
E. faecium qE-23	Cheese	ERY, RFA, CIP, LVX, FUR (*msr*A/B, *ermB*)
E. faecium qE-24	Cheese	VAN, TEC, FUR (*efrA, efrB*)
E. faecium qE-26	Cheese	RFA, CIP, FUR

[a,b]Antibiotic resistance phenotypes were determined previously by Valenzuela *et al.* (2008, 2010), respectively.

2000), and pRUM, a non-conjugative, multidrug-resistance plasmid from *E. faecium* (Grady and Hayes, 2003). The sequences of the enterococcal *cat* genes found on plasmids usually contain genetic segments of small staphylococcal or streptococcal (i.e., pIP501) plasmids, indicating that these genes were originally obtained from *Streptococcus* or *Staphylococcus* spp. (Pepper *et al.*, 1986; Grady and Hayes, 2003; Klare *et al.*, 2003).

Twenty-nine (52%) of the MR enterococci strains were resistant to erythromycin (EmR). These strains were tested for

the presence of *erm*(A), *erm*(B), *erm*(C), *msr*A/B, *ere*A/B, *mef*A/E, and *mph*A genes. 66.66% of *E. faecium* strains (which includes 20 of the 21 EmR MR *E. faecium*) yielded positive results for the PCR with primers for the efflux pump–associated *msr*A/B gene. This gene has also been detected previously in EmR *E. faecium* from foods but not in *E. faecalis* (Portillo *et al*., 2000; Hummel *et al*., 2007; Toomey *et al*., 2010), which is in accordance with the results obtained in the present study. In fact, none of the EmR *E. faecalis* strains harbored *msr*A/B genes. However, clinical *E. faecalis* isolates showed the presence of *msr*A/B genes, as reported by Chouchani *et al*. (2012). None of the EmR strains tested positive for *erm*(C), *ere*A/B, *mef*A/E, and *mph*A genes. The gene *erm*(B) was detected in eight EmR *E. faecium* and five *E. faecalis* strains. These data are in accordance with the studies of Jensen *et al*. (1999) and Khan *et al*. (2002), who found that *erm*(B) was the dominating EmR gene among enterococci. Jensen *et al*. (1999) observed that only 88% of EmR isolates contained *erm*(B), while *erm*(A) and *erm*(C) genes were not detected, indicating that other resistance mechanisms must also occur in enterococci. This was confirmed by Portillo *et al*. (2000) and Hummel *et al*. (2007). Furthermore, *erm*(B) was also detected in three additional *E. faecalis* strains for which erythromycin resistance was not detected by phenotype; those results were also obtained by McBride *et al*. (2007) in *E. faecalis*. This data could be explained by the fact that the *erm*(B) gene could be a silent gene or may lack functionality. The *erm*(B) genes are well known to occur on either conjugative plasmids such as pAMb1 (Martin *et al*., 1987), pRE25 (Teuber *et al*., 2003), and pUW1965 (Werner *et al*., 2000), or on transposons such as Tn917 (Shaw and Clewell, 1985), Tn1545 (Courvalin and Carlier, 1987), Tn5384, and Tn5385 (Bonafede *et al*., 1997), often linked with other antibiotic resistance determinants. Some EmR strains did not show amplification of *erm*A,B,C, *ere*A/B, *mph*A, *mef*A/E, and *msr*A/B genes, and thus, the mechanism and associated genes for this observed EmR are still unclear.

Regarding the multidrug efflux pump EfrAB, the results obtained in this study indicated that 96% of *E. faecalis* strains possess *efr*A and/or *efr*B genes, whereas only 13% of *E. faecium* strains carried *efr*A and *efr*B genes. This is the first work describing the presence of the ABC Multidrug Efflux Pump in *E. faecium* previously reported in *E. faecalis* (Lee *et al*., 2003), which suggests the possibility of co-transfer of resistance genes between both species in foods.

Conclusion

Our results suggest that fermented foods of both animal and vegetable origins are possible reservoirs of multi-drug-resistant enterococci. The isolates were resistant to three or more antibiotics simultaneously, so enterococci strains should come under tighter control. Due to the high number of MR *E. faecium* and *E. faecalis* strains with resistance to common antibiotics, it is necessary to re-evaluate the use of therapeutic antibiotics in stock farms at both regional and international levels.

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Disclosure Statement

No competing financial interests exist.

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