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Macrolide Resistance and *In Vitro* Selection of Resistance to Antibiotics in *Lactobacillus* Isolates

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Spreading of resistance to antibiotics is of great concern due to the increasing rate of isolation of multiresistant pathogens. Since commensal bacteria may transfer determinants of resistance to pathogens, studies on development of resistance should include also lactobacilli. Resistance to macrolides, penicillins and tetracycline was determined in 40 isolates of *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus crispatus*, and *Lactobacillus casei* isolated from faeces of apparently healthy volunteers. Frequency of mutation and changes in susceptibility after serial exposure to these antibiotics at concentrations of 4× and 8× MIC were evaluated in susceptible isolates. Acquired resistance was defined as an increment in MIC values of at least four times in respect to the pre-selection values. Resistance to macrolides and/or tetracycline was identified in 14 and 4 isolates, respectively. *ermB* gene and A2058G mutation in 23S rRNA were detected in macrolide resistant isolates. Frequencies of mutation of susceptible isolates (n=26) were lower for ampicillin and erythromycin than for tetracycline. Serial exposure to antibiotics led to selection of resistant mutants. However, acquired resistance was rather unstable and was lost after subcultures in antibiotic-free medium in most mutants. Resistance to erythromycin was associated to a A2058G mutation in 23S rRNA. In conclusion, results indicate that resistance to macrolides and tetracycline is present among intestinal lactobacilli. Decrease in susceptibility following serial exposure to antibiotics might occur in lactobacilli, in a strain- and antibiotic-dependent way. Since lactobacilli are often used as probiotics, their ability to acquire resistance should be evaluated for isolates candidate to be included in probiotics based products.

Keywords: lactobacilli, antibiotics, selection of resistance

Since their introduction in medicine, antibiotics have led to a marked improvement in public health, being the mainstay for treatment of infections. However their efficacy is threatened by the continual spread of resistance in pathogenic bacteria. Exposure to antibiotics may lead to emergence of resistant mutants, as widely demonstrated both *in vitro* and *in vivo* (Hawkey and Jones, 2009; Smirnova *et al.*, 2009; Drago *et al.*, 2010). Therefore in development of antibiotics the ability to select for resistance in pathogenic bacteria should be carefully evaluated.

Intestinal microflora, including lactobacilli, plays a pivotal role in maintaining intestinal homeostasis, which, if altered, is known to affect not only intestinal function but has been also linked to a wide variety of pathological conditions (Macfarlane and Cummings, 2002). Administration of antimicrobial agents, including those that are administered parenterally and excreted in the bile, can cause several adverse effects on the intestinal microflora. Emergence of resistance among bacteria in the normal flora and distribution of resistant genes by transfer of DNA in the microbial community can contribute to an increased load of resistant, potentially pathogenic microorganisms (Sullivan *et al.*, 2001). Recently, some studies have speculated that commensal bacteria may act as reservoirs of antibiotic resistance genes similar to those found in human pathogens and thus may contribute to dissemination of antimicrobial resistance determinants (Perreten *et al.*, 1997; Teuber, 2001; Gevers *et al.*, 2003b). Prevalence of *tetM* and *ermB* genes has been estimated to be higher than 60% in lactobacilli of human and dairy origin (Temmermann *et al.*, 2002).

However, since commensal bacteria have been shown to transfer determinants of resistance to pathogens (Le *et al.*, 2009), studies on development of resistance should include also lactobacilli. Drug resistance has been shown to be likely acquired during transit in the intestinal tract where, in some cases, resistant transconjugants arose at relatively high frequencies and could persist in the digestive environment (Cataloluk and Gogebakan, 2004; Mater *et al.*, 2008).

For this reason, this study reports data on ability of different species of lactobacilli isolated from human faeces to develop resistance to some antibiotics, currently used in therapy of common infections.

Materials and Methods

Lactobacilli isolates

Isolates of *Lactobacillus acidophilus* (n=13), *Lactobacillus plantarum* (n=9), and *Lactobacillus crispatus* (n=6) and of *Lactobacillus casei* (n=12) were used in this study.

Lactobacilli were isolated from faeces of apparently healthy volun-

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Table 1. Primers used in this study

	Forward and reverse primers (5′→3′)
L. casei	CAGACTGAAAGTCTGACGG GCGATGCGAATTTCTTTTTC
L. plantarum	GCCGCCTAAGGTGGGACAGAT TTACCTAACGGTAAATGCGA
L. crispatus	GTAATGACGTTAGGAAAGCG ACTACCAGGGTATCTAATCC
L. acidophilus	AGCTGAACCAACAGATTCAC ACTACCAGGGTATCTAATCC
mefA/E	AGTATCATTAATCACTAGTGC TTCTTCTGGTACTAAAAGTGG
ermA	TCTAAAAAGCATGTAAAAGAA CTTCGATAGTTTATTAATATTAGT
ermB	CATTTAACGACGAAACTGGC GGAACATCTGTGGTATGGCG
ermC	TCAAAACATAATATAGATAAA GCTAATATTGTTTAAATCGTCAAT
23S rRNA gene:	
amplification	TGCGTAATAGCTCAC ACCGCCCCAGTCAAACT
sequencing	AGCTCCATGGGGTCTTTCCG

teers declaring to have not consumed yoghurt or probiotic based products in the two weeks before bacterial isolation. Faeces were plated, after dilution in sterile saline, on homofermentative-hetero-fermentative differential (HHD) agar (McDonald *et al.*, 1987) and plates were incubated for 48-72 h in anaerobiosis.

Lactobacilli were initially identified by means of biochemical assays (API50 CHL, bioMérieux, France). Their identification was further confirmed by a PCR method as described by Walter *et al.* (2000); primers are indicated in Table 1. Isolates were grown at 37°C in CO_2 enriched atmosphere on de Man Rogosa Sharpe (MRS) agar plates and stored at -80°C in MRS broth supplemented with 10% glycerol, until use.

Only isolates susceptible to ampicillin, erythromycin, and tetracycline were used to evaluate selection of resistance.

Antibiotics

Antibiotics representative of different classes were tested: a β-lactam (ampicillin), a macrolide (erythromycin), and a tetracycline (tetracycline). Selection of resistance was tested at antibiotic concentrations equal to 4× and 8× MIC (Minimum Inhibitory Concentration).

Evaluation of susceptibility to the tested antibiotics

Determination of MICs was performed by means of microdilution broth method (microwell method). Briefly, serial twofold dilutions of a starting antibiotic concentration were inoculated into wells of a microtiter plate containing MRS broth, so that each well contained about 5×10^5 CFU/ml. MICs were considered as the lowest antibiotic concentration able to inhibit visible bacterial growth after 18-20 h of incubation at 37°C in a 10% CO_2 enriched atmosphere.

Susceptibility was determined in accordance to breakpoints established by EFSA (ampicillin, erythromycin, and tetracycline) (European Commission, 2002; EFSA, 2008). For *L. crispatus*, breakpoints established for *L. acidophilus* group were used.

Mutational frequency

Bacterial suspension of about 10^{10}-10^{11} CFU/ml were obtained from 48-72 h cultures on MRS agar. The frequency of spontaneous single-step mutations was determined by spreading 0.1 ml from each bacterial suspension on antibiotic-free agar plates (after proper dilution) and on antibiotic-containing agar plates (undiluted inoculum). Colonies grown after 72 h of incubation at 37°C in CO_2 enriched atmosphere were counted. Frequency of mutation was calculated as the number of grown colonies per inoculum. MIC values of colonies grown on antibiotic containing agar were determined as described above.

Multi-step selection of resistance

The ability to select resistant bacteria was evaluated by serially subculturing the chosen isolates onto agar plates containing a linear gradient of each antibiotic. Gradients were prepared in agar plates providing linear antibiotics concentration from 0 to either 4× MIC or 8× MIC. An inoculum of 10^{10} CFU/ml, verified by plating on MRS agar, was homogenously spread on each plate, and incubated for 48 h at 37°C in a 10% CO_2 enriched atmosphere. Colonies growing at the highest antibiotic concentration were sampled, checked for purity, grown overnight into antibiotic free broth, and replated on new antibiotic gradient plates. Bacteria were exposed to ten consecutive passages on the antibiotic gradient-plates and subsequently to ten passages on antibiotic-free plates. MIC values were determined after the first, the second, the fifth and the last passage on antibiotic-containing agar and after the first, the fifth and the last passage on antibiotic-free plates by means of broth microdilution technique, as described above. Acquisition of resistance was defined as >4-fold increase in MIC in respect to the pre-exposure values.

Characterization of macrolide resistance

To evaluate mechanisms of resistance to erythromycin, PCR amplification of genes associated with macrolide resistance (*ermA*, *ermB*, *ermC*, and *mefA/E*) was performed. DNA from each resistant isolate was extracted by means of a spin column chromatography method (Bacterial genomic DNA isolation kit, Norgen, Canada). For amplification, conditions described by Hummel *et al.* (2007) were used primers are indicated in Table 1. The PCR products were subjected to electrophoresis on 1.8% agarose gels, and they were visualized by staining with ethidium bromide.

The presence of point mutations in the 23S rRNA gene was also investigated. A 1.2 kb fragment of the 23S rRNA gene was amplified as previously described (Begovic *et al.*, 2009). Purified amplicons were sequenced by means of Pyrosequencing (PSQ96RA, Diatech,

Table 2. Susceptibility of the tested isolates

	MIC Range (mg/L)			Macrolide determinants of resistance *erm/mef*	Isolates with A2058G mutations
	Ampicillin	Erythromycin	Tetracycline		
L. acidophilus (n=13)	0.125-0.5	0.125-256	0.5-2	3/0	2
L. plantarum (n=9)	0.125-0.5	0.25-128	4-16	2/0	1
L. casei (n=12)	0.125-0.5	0.06-128	0.25-1	2/0	2
L. crispatus (n=6)	0.125-0.5	0.125-64	0.5-2	1/0	1

Table 3. Frequencies of mutation of the tested isolates at 4× MIC

	Frequency of mutation		
	Ampicillin	Erythromycin	Tetracycline
L. acidophilus (n=8)			
Mean	8.91×10^{-9}	3.54×10^{-8}	1.66×10^{-6}
Range	5.13×10^{-7} – $<10^{-11}$	4.79×10^{-6} – $<10^{-11}$	6.91×10^{-5} – 1.09×10^{-9}
L. plantarum (n=6)			
Mean	2.30×10^{-10}	7.78×10^{-9}	2.21×10^{-6}
Range	3.33×10^{-7} – $<10^{-11}$	1.88×10^{-7} – $<10^{-11}$	3.55×10^{-5} – 6.55×10^{-8}
L. casei (n=8)			
Mean	6.51×10^{-9}	8.53×10^{-8}	9.54×10^{-7}
Range	4.15×10^{-7} – $<10^{-11}$	9.11×10^{-6} – $<10^{-11}$	2.32×10^{-5} – 3.33×10^{-9}
L. crispatus (n=4)			
Mean	1.01×10^{-9}	4.29×10^{-9}	6.57×10^{-6}
Range	2.78×10^{-6} – $<10^{-11}$	8.35×10^{-7} – $<10^{-11}$	1.22×10^{-5} – 2.96×10^{-8}

Italy); sequences of 60-80 bp were obtained. All primers are indicated in Table 1.

Results

Antimicrobial susceptibility

Susceptibility of the tested isolated to the antibiotics under evaluation are reported in Table 2. Five isolates of *L. acidophilus*, 3 of *L. plantarum*, 2 of *L. crispatus*, and 4 of *L. casei* resulted resistant to erythromycin, 3 isolates of *L. acidophilus* and 1 of *L. crispatus* were found also resistant to tetracycline.

These isolates were not included in the second part of the study on the ability to acquire resistance during exposure to antibiotics.

Characterization of macrolide resistance in the intestinal isolates

Mechanism of resistance to erythromycin was evaluated by a PCR method targeted to detect *erm* and *mef* genes. PCR evidenced the presence of *ermB* gene in 3, 2, 1, and 2 isolates of *L. acidophilus*, *L. plantarum*, *L. crispatus*, and *L. casei*, respectively. *mefA/E* genes were never detected in resistant isolates (Table 2). Mutations A2058G (following *Escherichia coli* numbering) were identified in 2, 1, 2, and 1 isolates of *L. acidophilus*, *L. plantarum*, *L. casei*, and *L. crispatus*, respectively.

Mutational frequency

Frequencies of mutation for *L. acidophilus*, *L. casei*, *L. plantarum*, and *L. crispatus* are reported in Tables 3 and 4. At 4× MIC an extremely variable behaviour was observed among the tested isolates with frequencies ranging from $<10^{-11}$ to 10^{-5}, with no marked differences among antibiotics and/or bacterial species (Table 3). At 8× MIC, all isolates showed mutational frequencies quite similar for the same antibiotic and apparently lower than at 4× MIC, though presenting some differences among the tested antibiotics (Table 4). Generally, tetracycline showed higher frequencies of mutations in respect to the comparators at both 4× and 8× MIC.

Multi-step selection of resistance

Tables 5 and 6 show the number of isolates grown after multi-step selection, and MIC values after 1, 5, and 10 passages on antibiotic-gradient plates and after the subsequent 10 passages on antibiotic-free medium. After multi-step selection a general increment in MICs was observed for all the tested isolate with all antibiotics. The entity of MIC increment was widely variable among isolates belonging to the same species. Subcultures onto antibiotic free agar led to a reduction in MIC values, though evidencing the same variability, observed during exposure to antibiotic containing plates. Although after subcultures onto antibiotic-free plates a decrease in MICs was observed, some isolates recovered basal susceptibility, while

Table 4. Frequencies of mutation of the tested isolates at 8× MIC

	Frequency of mutation		
	Ampicillin	Erythromycin	Tetracycline
L. acidophilus (n=8)			
Mean	4.69×10^{-10}	2.39×10^{-9}	1.86×10^{-6}
Range	4.33×10^{-9} – $<10^{-11}$	1.33×10^{-8} – $<10^{-11}$	6.45×10^{-5} – 8.79×10^{-9}
L. plantarum			
Mean	1.43×10^{-9}	6.21×10^{-9}	3.23×10^{-6}
Range	3.21×10^{-9} – $<10^{-11}$	4.99×10^{-7} – $<10^{-11}$	4.88×10^{-4} – 1.55×10^{-9}
L. casei			
Mean	1.21×10^{-9}	4.35×10^{-10}	4.54×10^{-7}
Range	2.44×10^{-9} – $<10^{-10}$	7.24×10^{-9} – 3.71×10^{-11}	5.32×10^{-5} – 8.41×10^{-10}
L. crispatus			
Mean	6.89×10^{-10}	7.85×10^{-9}	9.55×10^{-7}
Range	4.64×10^{-9} – $<10^{-11}$	5.31×10^{-8} – 9.22×10^{-10}	6.37×10^{-5} – 4.91×10^{-9}

Table 5. Selection of resistance in lactobacilli isolates at 4× MIC

	MIC ranges (mg/L) and number of isolates (n) with MIC≥4 times basal MIC after:			
	1 step	5 steps	10 steps	10 steps w/o antibiotic
L. acidophilus (n=8)				
Ampicillin	0.125-0.5 (0)	0.25-0.5 (2)	0.5-1 (4)	0.125-1 (3)
Erythromycin	0.125-1 (0)	0.25-2 (2)	0.25-4 (5)	0.125-2 (3)
Tetracycline	0.5-2 (0)	1-4 (2)	2-8 (6)	0.5-4 (2)
L. plantarum (n=6)				
Ampicillin	0.125-0.5 (0)	0.125-1 (1)	0.25-4 (3)	0.125-1 (2)
Erythromycin	0.5-1 (0)	1-4 (4)	2-8 (4)	0.25-4 (3)
Tetracycline	4-32 (1)	16-64 (2)	16-128 (4)	4-64 (2)
L. casei (n=8)				
Ampicillin	0.125-0.25 (0)	0.25-1 (2)	0.5-4 (3)	0.125-1 (2)
Erythromycin	0.125-0.5 (0)	0.25-2 (3)	0.5-4 (5)	0.25-1 (3)
Tetracycline	0.25-2 (0)	0.5-4 (4)	1-4 (4)	0.5-4 (2)
L. crispatus (n=4)				
Ampicillin	0.125-0.5 (0)	0.25-0.5 (0)	0.25-1 (1)	0.125-1 (1)
Erythromycin	0.25-0.5 (0)	0.25-2 (2)	0.25-2 (2)	0.125-1 (1)
Tetracycline	1-2 (0)	1-2 (1)	2-4 (2)	1-2 (1)

for the rest of the tested bacteria MICs remained 4-16 times higher than the pre-selection level.

Characterization of macrolide resistance acquired during selection

Sequencing of a fragment of the 23S rRNA gene detected a A to G transition at position A2058 (following *E. coli* numbering) in 4 resistant mutants, 2 *L. acidophilus*, and 1 *L. plantarum* selected after exposure to erythromycin at 8× MIC and 1 *L. plantarum* selected after exposure to erythromycin at 4× MIC. Bacteria harbouring this mutation were those showing the highest MICs for erythromycin (4-8 mg/L).

Discussion

In the last years the increasing use of lactobacilli as probiotics has led to evaluation of antimicrobial pattern of resistance of these bacteria isolated from food or other commercial products (Ammor *et al.*, 2008; Ouoba *et al.*, 2008), while less is known about the possibility to acquire resistance by commensal lactobacilli. At our best knowledge, this is the first time that selection of resistance by commonly used antibiotics has been evaluated in lactobacilli of human origin. The isolates tested in this study were obtained from faeces of apparently healthy volunteers, who declared to have not consumed probiotics products in the two weeks before sample collection. Therefore the isolates were likely components of the gastrointestinal microflora and not isolates used for preparation of probiotics products, although, due to the large use of lactobacilli in food industry, it can not be excluded that they could have been introduced with diet.

The choice of antibiotics and of concentrations used for

Table 6. Selection of resistance in lactobacilli isolates at 8× MIC

	MIC ranges (mg/L) and number of isolates (n) with MIC≥4 times basal MIC after:				
	Basal	1 step	5 steps	10 steps	10 steps w/o antibiotic
L. acidophilus (n=8)					
Ampicillin	0.125-0.25	0.125-0.5 (0)	0.25-1 (2)	0.5-2 (5)	0.125-1 (3)
Erythromycin	0.125-0.5	0.125-1 (1)	0.5-4 (3)	1-16 (7)	0.125-8 (4)
Tetracycline	0.5-2	0.5-2 (0)	1-8 (3)	2-32 (6)	0.5-16 (3)
L. plantarum (n=6)					
Ampicillin	0.125-0.5	0.125-1 (0)	0.5-2 (2)	0.5-4 (4)	0.25-2 (2)
Erythromycin	0.25-0.5	0.5-1 (0)	0.5-4 (3)	1-8 (4)	0.5-4 (3)
Tetracycline	4-16	8-32 (1)	16-128 (3)	32-128 (5)	8-64 (3)
L. casei (n=8)					
Ampicillin	0.25-0.5	0.25-0.5 (0)	0.5-4 (4)	1-8 (5)	0.5-4 (3)
Erythromycin	0.06-0.25	0.125-0.5 (0)	0.25-2 (3)	1-8 (6)	0.25-2 (4)
Tetracycline	0.25-1	0.25-2 (0)	0.5-8 (5)	1-16 (6)	0.5-8 (3)
L. crispatus (n=4)					
Ampicillin	0.125-0.25	0.125-0.5 (0)	0.5-1 (1)	0.5-2 (2)	0.25-2 (1)
Erythromycin	0.125-0.5	0.25-0.5 (0)	0.25-2 (2)	0.5-4 (3)	0.25-1 (2)
Tetracycline	0.5-2	1-2 (0)	2-4 (1)	2-8 (2)	1-4 (1)

selection of resistance was arbitrary and based on the willingness to assess some among most commonly prescribed antibiotics. Concentrations tested were chosen with the aim to evaluate antibiotics' levels which might occur during antibiotic therapy.

In this study, more than one third of the isolates were resistant to macrolides before the *in vitro* exposure to erythromycin, while resistance to tetracycline seemed to be less common among lactobacilli of human origin, although it is often reported among strains used in food industry (Huys *et al.*, 2008; Zonenschain *et al.*, 2009). Resistance to macrolides was associated in most cases to the presence of an *ermB* gene. Our results are concordant with those reported by other authors, who evidenced the prevalence of methylation genes in macrolides resistant lactobacilli (Zonenschain *et al.*, 2009). Recently *ermA* has been identified for the first time in members of *L. acidophilus* group (Mayrhofer *et al.*, 2010), while, as other authors, we observed a prevalence of *ermB* genes. In a minor number of isolates an A to G transition in the 23S rRNA at position A2058 (following *E. coli* numbering) was found. This mutation has been associated to macrolide resistance in many bacterial species (Vester and Douthwaite, 2001) and it has been the only point mutation detected in erythromycin-resistant lactobacilli until now (Begovic *et al.*, 2009).

Frequencies of mutations observed for the tested isolates were lower for ampicillin and erythromycin than for tetracycline, which seemed more prone to favour occurrence of resistant mutants than comparators.

Results from multi-step selection of resistance confirm data obtained in *in vitro* studies on pathogenic bacteria showing that development of resistance seems to depend on antibiotic, isolate and duration of exposure to antibiotics (Smirnova *et al.*, 2009; Drago *et al.*, 2010). However, for lactobacilli resistance acquired after serial exposure to antibiotics seemed to be rather unstable, since a part of the tested isolates tended to recover pre-selection level of susceptibility when subcultured in absence of antibiotics. It is worthy to underline that acquisition of resistance was defined as an increment of at least 4 times in MIC values, and therefore it was not dependent on the method and culture conditions used to measure MIC and on available breakpoints.

Since isolates harbouring *erm* genes were excluded from assessment of selection of resistance, alternative mechanisms of resistance should be involved in macrolide resistance in mutants obtained after serial exposure to erythromycin. In 4 resistant mutants an A to G transition in the 23S rRNA at position A2058 (following *E. coli* numbering) was detected after serial exposure to erythromycin. However, the presence of not characterised mutations in other regions cannot be excluded.

The lack of data on molecular determinants of resistance to tetracyclines may be a potential limitation of the study. We limited molecular characterization only to macrolides since they are more frequently used in clinical practice and, consequently, resistance to them is more common than resistance to the other antibiotics tested in this study. Therefore resistance to macrolides was used to evaluate if the acquired resistance was due to determinants common to other bacterial species, such as pathogen bacteria. Evaluation of transferability of acquired resistance to other bacteria was beyond the purpose of this study, which aimed to evaluate the ability to develop resistance after serial exposure to some antibiotics.

Lactobacilli play a crucial role in the production of fermented foods like vegetables, meats, and particular fermented products. In recent years, scientific understanding of physiology of lactobacilli has led to increase range of industrial dairy applications of lactobacilli as starters and adjunct starters/cultures, including their use as probiotics (Parvez *et al.*, 2006; Ouoba *et al.*, 2008). Since often lactobacilli used as probiotics are of human origin, it seems reasonable to extend data obtained in this study to probiotic lactobacilli. In this context, the use of lactobacilli as probiotics has recently raised concerns about safety aspects, one of them being the nature of acquiring and spreading resistance (Courvalin, 2006).

In 2005 and subsequently, EFSA stated that intrinsic resistance and resistance due to mutation of chromosomal genes present a low risk of horizontal dissemination, and such strains should be acceptable for food consumption. By contrast, acquired resistance mediated by added genes may present a risk for public health and strains with such resistance should not be used (EFSA, 2008). Antibiotic resistance in European probiotic products has been detected in 68.4% of the isolates tested (Temmerman *et al.*, 2002) and lactobacilli isolated from fermented dry sausages have been reported to be able to harbour tetracycline resistance gene and to transfer macrolide resistance from *Lactobacillus* to enterococci *in vivo* (Kleinschmidt *et al.*, 1993; Gevers *et al.*, 2003a; Jacobsen *et al.*, 2006).

Although in recent years a number of initiatives has been launched across the world to address biosafety of probiotic microorganisms, few systematic studies investigating acquired antibiotic resistance in probiotic strains are currently available (Mathur and Singh, 2005; Ammor *et al.*, 2008; Ouoba *et al.*, 2008; Zonenschain *et al.*, 2009). Most of knowledge on antibiotic resistance of probiotic regards intrinsically encoded resistance to common antibiotics, and, in a small part, the ability to transfer such resistance to other bacteria, either commensal or pathogens. In this scenario, attention of researchers has been mainly addressed to evaluation of antibiotic resistance in genera including pathogenic bacteria, such as enterococci, while lactobacilli have been less investigated. Although not included in the definition of QPS, evaluation of the ability of bacteria to become resistant when exposed to antibiotics could provide further information about the safety of probiotics.

In conclusion, results of this study suggest that changes in susceptibility following exposure to antibiotics might occur in some lactobacilli leading to decreased susceptibility. Consequently evaluation of the ability to acquire resistance to common antibiotics should be performed in parallel with investigations on the presence of resistance determinants for strains intended for human and animal use and extended to other antibiotics such as fluoroquinolones. Acquisition and retransfer of resistance genes should be addressed in the safety evaluation of probiotics.

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