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VIRULENCE FACTOR GENES POSSESSING *ENTEROCOCCUS FAECALIS* STRAINS FROM RABBITS AND THEIR SENSITIVITY TO ENTEROCINS

POGÁNY SIMONOVÁ M., LAUKOVÁ A.

Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésovej 4-6, 04001 Košice, Slovakia.

Abstract: Information concerning the virulence factor genes and antibiotic resistance of rabbit enterococci is limited, so in this study we tested the virulence factor genes in *Enterococcus faecalis* strains from rabbits. Moreover, their resistance/sensitivity to antibiotics and sensitivity to enterocins was also tested, with the aim of contributing to our enterocin spectra study and to indicate the possibility of enterocin application in prevention or contaminant elimination in rabbit husbandry. A total of 144 rabbit samples were treated using a standard microbiological method. Thirty-one pure colonies of the species *Enterococcus faecalis* were identified, using the MALDI-TOF identification system and confirmed using phenotyping, among which 15 strains were virulence factor gene absent. The *gelE* gene was the most detected (42%); however, the expression of gelatinase phenotype did not always correlate with the detection of *gel*E. Strains did not show ß-haemolysis and were mostly resistant to tested antibiotics, but sensitive to enterocins (Ent), mainly to Ents EK13=A (P), 2019 and Ent M. Rabbit *E. faecalis* strains displayed antibiotic resistant traits and the presence of expressed and silent virulence genes, but they showed high levels of sensitivity to natural antimicrobials-enterocins, which indicates the possible prevention of multidrug and virulent enterococcal contaminants by enterocins.

Key Words: *Enterococcus faecalis*, virulence factors, resistance, antimicrobials sensitivity, rabbits.

INTRODUCTION

Enterococci are common bacteria of the gastrointestinal tract in human and animals, but they are also widely distributed in the environment, soil, water and plants (Devriese *et al.,* 1991; Lauková *et al.,* 1993; De Vuyst *et al.,* 2003; Giraffa, 2002). Some enterococci are used in the food industry as starter cultures due to their lipolytic and proteolytic activities, their ability to produce bacteriocins and to adapt to different environmental conditions (De Vuyst *et al.,* 2003; Foulquie Moreno *et al.,* 2006). Moreover, some enterococci possess probiotic characteristics and can improve the microbial balance of the intestine and prevent gastrointestinal diseases in humans and animals (Franz *et al.,* 1999; Foulquie Moreno *et al.,* 2006; Strompfová *et al.,* 2006; Pogány Simonová *et al.,* 2009) which means they have to be individually evaluated. Besides their beneficial effects, enterococci are often recognised as nosocomial pathogens because of their increased resistance to antibiotics and the presence of virulence factor genes which ease bacterial adherence to the host cell membranes (Franz *et al.,* 1999; Foulquie Moreno *et al.,* 2006; Özmen Toğay *et al.,* 2010; Semedo-Lemsaddek *et al.,* 2013). Although a number of enterococcal virulence factors have been described, the range is still incomplete. The most important and intensively studied are haemolysin, hyaluronidase, serine protease, gelatinase, aggregation substance, cytolysins, extracellular surface protein and other adhesins (Semedo *et al.,* 2003; Fischer and Philips, 2009). It is well known that the virulence of an organism is regulated with virulence coding genes present on the genome in special regions. The aggregation substance (Agg), encoded by the *agg* gene, is a pheromone-inducible surface glycoprotein of *E. faecalis* involved in the conjugative transfer of plasmids (Upadhyaya *et al.,* 2009). The extracellular surface protein (Esp), encoded by the *esp* gene, is a cell-wall associated protein serving adhesin, which colonises and evades the immune system, playing a role in antibiotic resistance and

Correspondence: A. Lauková, laukova@saske.sk. Received *May 2016* - Accepted *November 2016.*
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contributing to enterococcal biofilm formation (Foulquie Moreno *et al.,* 2006; Fisher and Philips, 2009). The adhesion to eukaryotic cells is also enabled by the *E. faecalis* (EfaAfs) cell wall adhesin, encoded by the *efaAfs* gene (Eaton and Gasson, 2001). Gelatinase (GelE) – *gelE* gene encoding – is a zinc metalloprotease able to hydrolyse gelatin, casein, haemoglobin and other bioactive compounds (Fisher and Philips, 2009).

The antibiotic resistance of enterococci is well documented. Enterococcal infections have increased in frequency mainly in humans, and although animals are not affected by enterococcal infections, they act as a reservoir of pathogenic and resistant strains, which disseminate into the environment and can infect humans by direct contact and/or through the food-chain. The emergence of enterococci in the last decade has resulted in their acquired antimicrobial resistance, especially to ß-lactams, aminoglycosides and glycopeptides (vancomycin and teicoplanin) and the resulting possible occurrence of multi-drug resistant strains (Peeters *et al.,* 2003). However, the dramatic increase of antibiotic resistant bacteria and residue in products has reinforced the research into natural substances such as bacteriocins, lactic acid and plant extracts (Smith-Palmer *et al.,* 1998; Lauková *et al.,* 2003).

The purpose of the present study was to detect the virulence factor genes in isolated *E. faecalis* strains from rabbits with the aim of contributing to the enterocin spectra (our Laboratory of Animal Microbiology studied and characterised enterocins) and to indicate the possibility of enterocin application in prevention or contaminant elimination in rabbit husbandry. The importance of this study is also related with human health *via* rabbit welfare and the prevention of possible bacterial contamination in the rabbitries themselves (from faeces to skin or other organs) or during slaughter and processing (from fur to meat).

MATERIALS AND METHODS

Isolation and identification of enterococci

Samples from healthy rabbits (n=144, age 35-77 d, Hycole breed, both sexes, faeces, caecum, appendix) were treated using the standard microbiological method (ISO- International Organisation for Standardisation); 1 g of sample was homogenised in a Ringer buffer (Oxoid, United Kingdom) using a Stomacher (IUL Masticator, Spain), diluted and plated on M-Enterococcus Agar (Difco, Maryland, USA, ISO 15214). Plates were incubated at 37°C for 24-48 h. Bacterial counts were expressed in log 10 of colony forming units per gram (log_{10} CFU/g±SD). Presumptive colonies were picked and checked for purity. Isolated strains were identified using protein "fingerprints" measured by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonics MALDI Biotyper, USA) as described by Bessède *et al.* (2011). Taxonomic allocation was evaluated on the basis of highly probable species identification (value score 2300-3000) and to secure probable species identification (2000-2299). Positive controls were those involved in the identification system. Identical colonies evaluated by the MALDI-TOF score value were excluded. Phenotypic properties of the identified strains were tested by the BBL Gram-Positive ID Kit (Becton and Dickinson, Cockeysville, USA).

DNA isolation and virulence factor gene determination

Genomic DNA of each enterococcal strain was isolated according to Baele *et al.* (2010). Screening of the virulence determinant genes *gelE* (gelatinase), *esp* (extracellular surface protein), *agg* (aggregation substance) and *efaAfs* (adhesin *Enterococcus faecalis*) was performed by PCR amplification using specific primers and conditions as previously described by Ribeiro *et al.* (2011). *E. faecalis* P36 (clinical isolate, Eaton and Gasson, 2001) was used as the positive control.

Detection of haemolysis and gelatinase activity

Haemolysis was performed by streaking the strains on Columbia Agar (Oxoid, Basingstoke, United Kingdom) supplemented with 5% defibrinated sheep blood (Semedo-Lemsaddek *et al.,* 2003, 2013). In the gelatinase assay, enterococci were inoculated on Todd-Hewitt agar (Becton and Dickinson) supplemented with gelatine (30 g/L, Becton and Dickinson) and incubated at 37°C for 2 d. Then the plates were flooded with a 15% solution consisting of 15% $HgCl_2$ in 20% HCl. Loss of turbidity halos around colonies was then monitored at 4°C. (Kanemitsu *et al.,* 2001).

Antibiotic testing and susceptibility to enterocins

Antibiotic testing of *E. faecalis* strains (100 µL of an 18-h culture of each strain) was tested on Columbia agar (Becton and Dickinson) enriched with 10% of defibrinated sheep blood following the Clinical and Laboratory Standards Institute agar diffusion method-CLSI (2012). The following antibiotic discs (Oxoid, United Kingdom and Lach-Ner, Czech Republic) were used: ampicillin (AMP10µg), penicillin (P10IU), erythromycin (E15µg), streptomycin (S25µg), chloramphenicol (C30µg), rifampicin (RD30µg), tetracycline (TE30µg), vancomycin (VA30µg) and gentamicin (CN120µg). After incubation at 37°C for 18 h, the strains were evaluated as resistant or sensitive according to the manufacturers' instructions; the inhibitory zones were expressed in mm. Antimicrobial free agar plates were included as a control for obligatory growth. Use of the antimicrobial agents was decided according to the manufacturer's guidance.

Semi-purified enterocins (Ents) produced by the strains listed in Table 3 (isolated in our Laboratory of Animal Microbiology, Institute of Animal Physiology SAS, Košice, Slovakia; except *E. haemoperoxidus* isolated in the Czech Republic (provided by Dr. P. Švec, Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic) and ED 26E/7 (isolated in the Dairy Institute in Žilina but studied in our laboratory). Ents were prepared using the following procedures: a 16-h culture (300 mL) of *E. faecium* CCM7420=EF2019 of rabbit origin strain, CCM7419=EK13, CCM8558=AL41 (environmental strains), EF55 (chicken isolate), CCM4231 (ruminal strain), EF9a (rabbit), EF9296 (silage isolate) and *E. durans* ED26/7 (cheese isolate). Strains in MRS broth (Merck, Germany) were centrifuged for 30 min at 10000 × *g* in order to remove the cells. After adjusting the supernatant to pH 5.0 (5.5 in the case of the AL41 strain), ammonium sulphate was slowly added to the supernatant to obtain 40% (w/v) saturation, and the mixture was stirred at 4°C for 2 h (EK13, EF9296, EF412), for 4 h (EF2019) and for 24 h (EF55, CCM4231) and at 21°C for 1 h (AL41); the ED26/7 strain showed 80% (w/v) saturation. After centrifugation at 10000 × *g for* 30 min, the resulting pellet was resuspended in 10 mmol/L sodium phosphate buffer (pH 6.5). The antimicrobial titre of Ent was defined as the reciprocal of the highest twofold dilution producing a distinct inhibition of the inhibitor lawn, expressed in Arbitrary Units per mL (AU/mL) of culture medium. *E. avium* EA5 (isolated from a piglet from our laboratory) was used as a bacteriocin-sensitive indicator strain (the amount of 200 µL for an 18-h culture of each indicator strain) to determine bacteriocin activity levels; the bacteriocin activity of Ents reached up to 25600 AU/mL.

RESULTS

The total count of enterococci in rabbit faeces varied from 1.33 to 3.74 log10 CFU/g (detected in 30 mixture samples); the bacterial counts in caecum and appendix were lower (from 1.39 to 1.99 log CFU/mL/g; 36 samples). Selected colonies were allotted to the species *Enterococcus faecalis* (25 from faeces, 3 from caecum and 3 from appendix). Their identification score value was in accordance with a high probable species identification (2300-3000) or with a secure probable species identification (2000-2299). Considering the results obtained by phenotyping (the BBL Gram-Positive ID Kit), the strain properties were in accordance with those for *E. faecalis* species in Bergey's Manual (2009); the acid was produced from lactose, sucrose, mannitol, arabinose, glycerol and trehalose. The isolates used were hydrolysed esculin and arginine.

Fifteen strains were absent from the tested virulence factor genes (Table 1). Three strains produced extracellular surface protein (esp; 10%) and 1 had a gene coding for the aggregation substance (*agg*; 3%). In this agg positive isolate the *esp* gene was also detected. The *EfaAfs* gene was detected in 4 isolates (13%). *E. faecalis* strains isolated from rabbits displayed the highest prevalence of the gelE which was present in 13 strains (42%) - in 9 strains only *gel*E gene was identified, in another 4 strains it was in combination with the *efaAfs* gene. Expression of gelatinase *in vitro* (phenotype) did not always correlate with the detection of *gel*E; 8 *gel*E-positive strains were unable to degrade gelatin (Table 1). On the other hand, in 9 isolates gelatinase activity was detected without *gel*E gene presence. Furthermore, all tested *E. faecalis* strains did not produce β-haemolysis on blood agar (Table 1).

All isolates were resistant to streptomycin, tetracycline and vancomycin (Table 2). Most of the strains were resistant to erythromycin, penicillin, gentamicin and chloramphenicol (97%). The isolates were sensitive to rifampicin and ampicillin (48%). Because of virulence factor gene occurrence and dominant resistance to antibiotics, *E. faecalis* strains were treated by Ents.

Table 1: Virulence factor gene occurence, gelatinase phenotype in *E. faecalis* from rabbits.

Isolates	Source	Gelatinase activity	*Esp*	*agg*	efa_{Afs}	*gelE*
P36		+	+	+	+	++
A1b/3	Appendix	+	-	-	-	+
A5b/1	Appendix	+	-	-	-	+
K6/1	Faeces	-	+	-	-	-
T/P/4a	Faeces	-	-	-	-	-
3/3a	Faeces	-	-	-	-	-
K/6a	Faeces	-	-	-	-	+
SŠ 2S	Faeces	+	-	-	-	-
HU 11b	Faeces	-	-	-	+	+
C5b/1	Caecum	+	-	-	+	+
5/2	Faeces	-	-	-	-	-
GMO/5/1	Faeces	+	-	-	-	-
6/3-B	Faeces	-	-	-	-	+
T1b/2	Faeces	-	+	+	-	-
C5b/3	Caecum	+	+	-	-	-
7/2	Faeces	+	-	-	-	-
2/2	Faeces	+	-	-	-	-
8/1	Faeces	+	-	-	-	-
KE K/2	Faeces	+	-	-	-	+
ŠAL 2/B	Faeces	-	-	-	-	+
K8	Faeces	-	-	-	-	+
T/P/1	Faeces	-	-	-	-	-
T/P/5	Faeces	+	-	-	-	-
C6a/2	Caecum	-	-	-	-	-
K5/3b	Faeces	-	-	-	+	+
T1/5	faeces	-	-	-	-	-
A9b/1	Appendix	+	-	-	-	-
1/1	Faeces	-	-	-	-	+
T4b/2	Faeces	-	-	-	-	-
1/6	Faeces	-	-	-	-	+
BIS 2B	Faeces	+	-	-	+	+
VŠ 3S	Faeces	+	-	-	-	-

P36: *E. faecalis* P36, positive control. The strains did not show ß-hemolytic activity.

The strains were sensitive to Ents EK13=(A,P), 2019 and Ent M (activity 6400-102400 AU/mL; Table 3); the most sensitive strain was *E. faecalis* K5/3b (possessing *Efaafs* and *gelE* genes, 12800-102400 AU/mL). The VŠ 3S strain appeared as the most resistant indicator strain (400-25600 AU/mL). However, it did not possess the virulence factor gene.

DISCUSSION

Enterococci are common inhabitants of animal intestines and form the predominant intestinal microflora during the first 2-3 d of many animals' living (Devriese *et al.,* 1991). In a rabbit's gastrointestinal tract, enterococci are present in sufficient amounts (Linaje *et al.,* 2004; Simonová *et al.,* 2005). *E. faecalis* strains were identified by the MALDI-TOF system and confirmed by physiological and biochemical tests according to the Bergey's Manual (Garrity, 2009). The biochemical (phenotypic) characteristics of our *E. faecalis* strains are in agreement with data previously published by Garrity (2009) and also with the results presented by Manero and Blanch (1999). Strains

Table 2: Antibiotic profile of *E. faecalis* strains from rabbits to antibiotics.

Isolates	Source	VA30µg	P10IU	RD30µg	S25µg	TE30µg	CN120µg	C30µg	AMP10µg	E15µg
A1b/3	appendix	R	R	R	R	R	R	R	R	R
A5b/1	appendix	R	R	S	R	R	R	R	S	R
K6/1	faeces	R	R	R	R	R	R	R	R	R
T/P/4a	faeces	R	S	I	R	R	R	R	S	R
3/3a	faeces	R	R	S	R	R	R	R	S	R
K/6a	faeces	R	R	S	R	R	R	R	S	R
SŠ 2S	faeces	R	R	I	R	R	R	R	S	R
HU 11b	faeces	R	R	I	R	R	R	R	R	R
C5b/1	caecum	R	R	S	R	R	R	R	S	R
5/2	faeces	R	R	S	R	R	R	R	R	R
GMO/5/1	faeces	R	R	S	R	R	R	S	S	R
6/3-B	faeces	R	R	R	R	R	R	R	R	R
T1b/2	faeces	R	R	R	R	R	R	R	R	R
C5b/3	caecum	R	R	I	R	R	S	R	S	R
7/2	faeces	R	R	S	R	R	R	R	S	R
2/2	faeces	R	R	R	R	R	R	R	S	R
8/1	faeces	R	R	R	R	R	R	R	R	R
KE K/2	faeces	R	R	S	R	R	R	R	S	R
ŠAL 2/B	faeces	R	R	S	R	R	R	R	R	R
K8	faeces	R	R	S	R	R	R	R	R	R
T/P/1	faeces	R	R	I	R	R	R	R	R	R
T/P/5	faeces	R	R	S	R	R	R	R	S	R
C6a/2	caecum	R	R	I	R	R	R	R	R	R
K5/3b	faeces	R	R	S	R	R	R	R	R	R
T1/5	faeces	R	R	S	R	R	R	R	R	S
A9b/1	appendix	R	R	S	R	R	R	R	R	R
1/1	faeces	R	R	I	R	R	R	R	R	R
T4b/2	faeces	R	R	R	R	R	R	R	S	R
1/6	faeces	R	R	S	R	R	R	R	R	R
BIS 2B	faeces	R	R	R	R	R	R	R	S	R
VŠ 3S	faeces	R	R	I	R	R	R	R	S	R

R: resistant, S: sensitive, I: intermittent reaction, VA: vancomycin (30 µg), P: penicillin (10 IU), TE: tetracycline (30 µg), AMP: ampicillin (10 µg), E: erythromycin (15 µg), RD: rifampicin (30 µg), C: chloramphenicol (30 µg), S: streptomycine (25 µg), CM: gentamicin (120 µg).

of the genus *Enterococcus* are well studied and described in many reviews and studies; however, new permanent species have been discovered and the known features have been diffused (Franz *et al.,* 2011). Although many of the rabbit enterococci properties have been described in previous studies (*e.g.* occurrence, colonisation, counts, sensitivity to ATBs and other antimicrobials, probiotic features and antimicrobial activities), there are still some new attributes/information which are important not only from the basic research point of view but also from the protection possibility aspect and those attributes are also virulence determinants. While the virulence traits in free living animals (Eurasian otters and beavers) have already been described (Semedo-Lemsaddek *et al.,* 2013; Lauková *et al.,* 2015), the detection of virulence factor genes in enterococci from rabbits as food-producing animals had never been tested before. Virulence factor genes were also tested in dogs and cats (Iseppi *et al.,* 2015). In general, animals are not affected by enterococcal infections, but they can be a reservoir of pathogenic strains, for example, which could represent a problem for humans during the manipulation and processing of animals or *via* the food chain (rabbit meat). That is why the detection of virulence factors and/or antibiotic resistance of enterococci in rabbits is important.

Table 3: Sensitivity of *E. faecalis* strains from rabbits to semi-purified enterocins (SPEs) expressed in Arbitrary units per mL (AU/mL) (SPEs are described in Material and Methods section). EA5: *Enterococcus avium* EA5 (positive control-principal indicator).

Isolates	Source	EK13	AL41	EF55	EF2019	CCM4231	EF412	ED26/7	EF9296
EA5		51200	25600	25600	51200	12800	25600	25600	25600
A1b/3	appendix	51200	25600	6400	25600	6400	6400	3200	12800
A5b/1	appendix	25600	6400	12800	25600	6400	12800	3200	6400
K6/1	faeces	51200	25600	3200	25600	12800	12800	3200	12800
T/P/4a	faeces	102400	25600	6400	12800	6400	25600	12800	6400
3/3a	faeces	51200	12800	6400	51200	12800	12800	3200	12800
K/6a	faeces	25600	25600	3200	12800	12800	6400	3200	12800
SŠ 2S	faeces	25600	6400	400	12800	3200	6400	3200	12800
HU 11b	faeces	102400	51200	25600	102400	12800	12800	3200	25600
C5b/1	caecum	25600	25600	6400	12800	6400	6400	3200	6400
5/2	faeces	51200	12800	800	12800	12800	25600	6400	6400
GMO/5/1	faeces	51200	25600	6400	25600	12800	25600	12800	12800
6/3-B	faeces	51200	51200	6400	51200	12800	12800	3200	12800
T1b/2	faeces	25600	6400	3200	12800	1600	12800	3200	6400
C5b/3	caecum	25600	6400	6400	25600	1600	6400	1600	12800
7/2	faeces	51200	25600	6400	51200	12800	25600	25600	12800
2/2	faeces	25600	51200	12800	25600	12800	25600	25600	12800
8/1	faeces	51200	25600	3200	25600	12800	25600	25600	12800
KE K/2	faeces	12800	12800	3200	12800	400	25600	25600	6400
ŠAL 2/B	faeces	102400	51200	6400	102400	12800	25600	25600	25600
K8	faeces	102400	25600	3200	51200	12800	25600	12800	12800
T/P/1	faeces	51200	12800	3200	12800	3200	25600	12800	12800
T/P/5	faeces	25600	6400	3200	12800	1600	25600	12800	12800
C6a/2	caecum	51200	51200	6400	102400	6400	12800	12800	25600
K5/3b	faeces	102400	102400	12800	102400	12800	25600	25600	25600
T1/5	faeces	51200	12800	6400	25600	3200	12800	3200	12800
A9b/1	appendix	25600	25600	12800	25600	3200	25600	12800	6400
1/1	faeces	51200	25600	3200	51200	12800	12800	12800	12800
T4b/2	faeces	51200	12800	6400	25600	6400	25600	25600	25600
1/6	faeces	25600	6400	6400	25600	12800	12800	3200	12800
BIS 2B	faeces	51200	25600	3200	25600	12800	25600	25600	12800
VŠ 3S	faeces	25600	12800	3200	12800	400	6400	6400	12800

The aggregation substance (*agg*) gene coding was identified only in one strain, which is similar to the findings in pet enterococci (Gülhan *et al.,* 2006). Iseppi *et al.* (2015) reported *agg* gene absence in enterococci isolated from dogs and cats. In free-living animals this gene was also absent (Semedo-Lemsaddek *et al.,* 2013; Lauková *et al.,* 2015). There was a low prevalence of the *esp* gene in rabbit isolates (10%); in beavers this gene was detected only in one strain (Lauková *et al.,* 2015) and strains from otter were *esp* gene absent (Semedo-Lemsaddek *et al.,* 2013), similar to those from dogs and cats (Iseppi *et al.,* 2015). The *esp* gene occurs mainly in clinical isolates (Medeiros *et al.,* 2014). While the *efaAfs* was not very prevalent (13%) in our isolates, in beavers this gene was the most detected (Lauková *et al.,* 2015) and it was also detected in *E. faecalis* strains from dogs and cats (Isepp *et al.,* 2015). In general, gelE is a most common factor in *E. faecalis* strains (Eaton and Gasson, 2001). Our results showed a high prevalence of *gel*E gene in rabbit isolates (42%); Lauková *et al.* (2015) and Semedo-Lemsaddek *et al.* (2013) described similar results in free-living animals. However, Iseppi *et al.* (2015) detected the *gelE* gene in *E. faecium, E. faecalis, E. casseliflavus, E. durans* and *E. avium* strains isolated from dogs and cats, but not in representatives of the species *E. gallinarum* and *E. hirae*. Expression of gelatinase phenotype did not always correlate with the detection of the *gel*E gene and 8 gelE-positive strains were unable to degrade gelatin. Similar results were also described by Medeiros *et al.* (2014). The gelatinase coding gene is frequently silent, resulting in the absence of

a gelatinase-positive phenotype. Silent genes may become activated by environmental factors and by changes in the gastrointestinal microflora (Eaton and Gasson 2001). Our enterococci did not produce ß-haemolysis, in accordance with other reports (Eaton and Gasson 2001; Poeta *et al.,* 2007; Iseppi *et al.,* 2015). Despite the fact that the *E. faecalis* isolated from rabbits showed a low frequency of virulence genes, they may act as a reservoir of virulence factors for the environment and also for humans through the food chain. Therefore, the study and detection of the virulence factors, including information concerning the antibiotic profile are important. Enterococci have become a focus of attention because of their increasing resistance to antibiotics. Our strains were multi-resistant and showed high resistance to most of the tested antibiotics; the lowest resistance was detected against rifampicin and ampicillin (48%). However, Iseppi *et al.* (2015) showed high resistance to rifampicin. Although Poeta *et al.* (2007) also detected high percentages of resistance to erythromycin and tetracycline (48, 45%), those % were lower compared to ours (97, 100%). On the other hand, these authors observed low % for streptomycin, chloramphenicol and ampicillin resistance (6.7, 4.5, 3.7%), contrary to our results (100, 97, 48%). A low level of antibiotic resistance for gentamicin, tetracycline and chloramphenicol was expected in free-living otters and in food enterococcal isolates (Özmen Toğay *et al.,* 2010; Semedo-Lemsaddek *et al.,* 2013) and among the antibiotics, attention is focused on the vancomycin-resistant enterococci. All tested strains were resistant to vancomycin, which contradicts other studies (Poeta *et al.,* 2007; Özmen Toğay *et al.,* 2010; Semedo-Lemsaddek *et al.,* 2013). In spite of the fact that vancomycin is not used in rabbit breeding, it is difficult to assess why most of the enterococci strains presented here were resistant to vancomycin. It could probably be caused by an extensive distribution of transposons among enterococci isolated from rabbit faeces and enterococci distributed in environments in which these animals live. As vancomycin and gentamicin are commonly used for human enterococcal infection treatment, the emergence and dissemination of this resistance could have implications for human health. The dramatic increase of antibiotic resistant bacteria and residue in products has evoked and supported the research on new antimicrobials (plant extracts, bacteriocins, etc.) of natural origin. The potential use of bacteriocin-producing strains as well as their bacteriocins as bioprotective agents has recently received increased attention. The antimicrobial activity of bacteriocins/enterocins against spoilage and pathogenic microflora is well documented, although the range of inhibition depends on the sensitivity and cell wall structure of indicator strains, *i.e.* Gram-positive bacteria are more inhibited mainly by Gram-positive producers of bacteriocins (Nes and Holo, 2000; Lauková *et al.,* 2003; Simonová and Lauková, 2007; Franz *et al.,* 2011). Similar to our previous findings concerning the *Pseudomonas* spp. from rabbits, *E. faecalis* strains were more sensitive to natural antimicrobials-enterocins than to ATBs; so "replacing" ATBs with enterocins for the prevention/elimination of potential pathogens seems to be highly effective (Pogány Simonová *et al.,* 2010) and it could be very useful in the case of multidrug resistant isolates. In addition, bacteriocins/enterocins leave no residue in organisms and products, unlike ATBs, so the use of enterocins in the prevention of several infections, diseases and pathological lesions is promising, mainly in the case of food-producing animals.

CONCLUSIONS

In conclusion, our results indicated that *E. faecalis* strains isolated from rabbits display antibiotic resistant traits and the presence of expressed and silent virulence genes. The occurrence of virulence and antibiotic resistant traits in rabbit enterococci underlined the importance of these isolates as reservoirs of virulence factors and possible dissemination of these genes to the human microbiota through the food chain. Nevertheless, these isolates also showed a high level of sensitivity to natural antimicrobials - enterocins. This could be very promising in the avoidance of the possible transference of antibiotic resistance, virulence gene dissemination and in prevention/therapy of multidrug and virulent enterococcal infections.

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