Erythromycin resistance and virulence genes in *Enterococcus faecalis* from swine in China

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SUMMARY

This study aims to describe the erythromycin resistance phenotypes and genotypes, and the prevalence of virulence genes of *Enterococcus faecalis* isolated from swine in China. A total of 117 nonreplicate *E. faecalis* isolates, obtained from 502 clinical samples taken from different pig farms between 2007 and 2009 were included in the study. Minimum inhibitory concentrations were determined using the broth microdilution method. All of the isolates were screened for the presence of seven virulence genes (*ace*, *asa*1, *cylA*, *efaA*, *esp*, *gelE*, and *hyl*). In addition, the DNA from erythromycin-resistant isolates were amplified with primers specific for erythromycin resistance *erm*(A), *erm*(B), *erm*(C), *mef*(A/E), and *msr*(C) genes. Results show that erythromycin, tylosin, and ciprofloxacin resistance rates in *E. faecalis* were 66.67% (n=78), 66.67% (n=78), and 64.10% (n=75), respectively. About 69.23% of isolates (n=81) were positive for *gelE*, 48.72% (n=57) for *ace*, 15.38% (n=18) for *efa*, 7.69% (n=9) for *asa*1, and 6.84% (n=8) for *esp*. Among the erythromycin-resistant isolates, *erm*(B) (n=54) was the most prevalent resistance gene, followed by *erm*(A) (n=37). A significant correlation was found between the presence of the *gelE* virulence gene and erythromycin resistance ($P<0.05$). These findings suggest that enterococci from swine should be regarded with caution because they can be reservoirs for antimicrobial resistance and virulence genes.

KEY WORDS: Erythromycin resistance, Virulence genes, *Enterococcus faecalis*, Swine

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INTRODUCTION

Enterococcus spp. are natural inhabitants of the gastrointestinal tract of humans and animals (Creti *et al.,* 2004; De Marques and Suzart 2004), but can be also found in soil, water, and vegetables (Burgos *et al.,* 2009). The two most important species, *Enterococcus faecium* and *E. faecalis,* are most frequently implicated in human and animal infections (Aakra *et al.,* 2005). *E. faecalis* is an opportunistic pathogen known to cause serious infections, such as bacteraemia, septicaemia, urinary tract infections, wound infections, meningitis, and endocarditis (Giacometti *et al.,* 2000; De Marques and Suzart 2004; Hällgren *et al.,* 2009).

Although the virulence determinants of enterococci are largely unknown, the presence of gene encoding virulence factors, including collagen-binding protein (*ace*), aggregation substance (*asa*1), cytolysin (*cylA*), endocarditis antigen (*efaA*), enterococcal surface protein (*esp*), gelatinase (*gelE*), and hyaluronidase (*hyl*), have been analysed (Dupre *et al.,* 2003; Creti *et al.,* 2004; Vankerckhoven *et al.,* 2004; Hebert *et al.,* 2007; Billström *et al.,* 2008; Hällgren *et al.,* 2009).

Enterococci readily acquire antibiotic resistance determinants, which spread rapidly among pop-

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ulations (Aakra *et al.,* 2005). Because of the general use of macrolides, macrolide-resistant *Enterococcus* spp. has been isolated in humans and animals (Portillo *et al.,* 2000; Jackson *et al.*, 2004; Barbosa *et al.,* 2009). Two major resistance mechanisms that cause macrolide resistance in enterococci isolates are target modification due to the ribosomal methylase encoded by *erm* genes (MLS_B phenotype) and an efflux pump system mediated by the membrane-bound efflux protein encoded by *mef*(A/E) and *msr* genes (M phenotype) (Sutcliffe *et al.,* 1996; Portillo *et al.,* 2000; Singh *et al.,* 2001; Schwaiger and Bauer 2008).
Although much has been learned about the epidemiology of nosocomial enterococci infections, the distribution of virulence genes in *Enterococcus* sp. of food animals in China is poorly documented. Macrolides, especially tylosin, are used in swine as therapeutic and prophylactic agents to treat bacterial infections or as growth promotants. Antimicrobial resistance has increased among isolates of animal origin on pig farms in recent years (Chen *et al.,* 2007; Guo-Bao *et al.,* 2009). Therefore, the objective of this study is to describe the erythromycin resistance phenotypes and genotypes, and the prevalence of virulence genes of *E. faecalis* isolated from swine in China.

MATERIALS AND METHODS

Bacteria isolation and identification

Samples, including lungs, lymph nodes, livers, hearts, spleens, kidneys, intestines, and blood from pigs with clinical signs of digestive and respiratory disorders, taken between 2007 and 2009, were used for the isolation of *E. faecalis*. A total of 502 pigs were sampled from 81 different pig farms in China. Bacteria were isolated as previously described (Jackson *et al.,* 2004) and routinely grown in trypticase soy broth or agar at 37°C. They were purified by standard methods and identified to the species level by the conventional biochemical identification scheme of De Marques and Suzart (2004). In addition, the 16S rDNA gene was amplified (Hong-Zhou *et al.,* 2002), and the amplicons were sequenced by Shanghai Sangon Bioengineering Co., Ltd. The nucleotide sequences were analysed using the BLAST algorithm available from the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov). Confirmed isolates were stored in trypticase soy broth containing 20% glycerol at -80°C until further characterisation could be performed.

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of ciprofloxacin, clindamycin, tylosin, kitasamycin, and erythromycin were determined using the broth microdilution method, and susceptibility to vancomycin was performed according to the standard disk diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI 2009). The concentration range used to determine the MICs of all antibiotics was 0.06-128 g/ml. *Staphylococcus aureus* ATCC 25923 and *E. faecalis* ATCC 29212 were used for quality control.

Detection of virulence and resistance genes

Template DNA was prepared, as previously described (Vankerckhoven *et al.* 2004). Five microlitres of templates were used in amplification reactions, with seven oligonucleotide primers for *ace* (Creti *et al.,* 2004), *asa*1 (Billström *et al.* 2008), *cylA* (Creti *et al.,* 2004), *efaA* (Dupre *et al.,* 2003), *esp*, *gelE*, and *hyl* (Billström *et al.,* 2008), as previously described. The amplified PCR products of the virulence genes were analysed on 2% (w/v) agarose gels. To detect the erythromycin resistance genes, the DNA was amplified with primers specific for *erm*(A), *erm*(B), *erm*(C), *mef*(A/E) (Sutcliffe *et al.,* 1996), and *msr*(C) (Sutcliffe *et al.* 1996; Singh *et al.* 2001) genes. The amplified PCR products of these genes were analysed on 1% (w/v) agarose gels.
The positive and negative controls for amplification were *E. faecalis* clinical isolates, which were confirmed by PCR and sequencing. The nucleotide sequences were aligned with ClustalX 2.0 and analysed using the BLAST algorithm. All tests were repeated three times, in parallel with the positive and negative controls. The 12 oligonucleotide primer pairs used to amplify both the virulence and resistance genes are shown in Table 1.

Statistical analysis

The correlation between the occurrence of antibiotic-resistant and virulence genes was calculated using the 2 or Fisher's exact test. The tests

TABLE 1 - *PCR primers used in the amplification for the detection of virulence and resistance genes.*

Gene	*Description*	*Sequence(5'-3')*	*Amplicon size (bp)*	*Reference*
erm(A)	Ribosomal methylase	TCTAAAAAGCATGTAAAAGAA CTTCGATAGTTTATTAATATTAGT	645	Sutcliffe et al., 1996
erm(B)	Ribosomal methylase	GAAAAGGTACTCAACCAAATA AGTAACGGTACTTAAATTGTTTAC	639	Sutcliffe et al., 1996
erm(C)	Ribosomal methylase	TCAAAACATAATATAGATAAA GCTAATATTGTTTAAATCGTCAAT	642	Sutcliffe et al., 1996
mef(A/E)	Efflux protein	AGTATCATTAATCACTAGTGC TTCTTCTGGTACTAAAAGTGG	348	Sutcliffe et al., 1996
msr(C)	Efflux protein	GCAAATGGTGTAGGTAAGACAACT ATCATGTGATGTAAACAAAAT	399	Sutcliffe et al., 1996 Singh et al., 2001
ace	Collagen-binding protein	GGAATGACCGAGAACGATGGC GCTTGATGTTGGCCTGCTTCCG	616	Creti et al., 2004
*asa*1	Aggregation substance	CACGCTATTACGAACTATGA TAAGAAAGAACATCACCACGA	375	Billström et al., 2008
cylA	Cytolysin	ACTCGGGGATTGATAGGC GCTGCTAAAGCTGCGCTT	688	Creti et al., 2004
efaA	Endocarditis antigen	CGTGAGAAAGAAATGGAGGA CTACTAACACGTCACGAATG	499	Dupre et al., 2003
esp	Enterococcal surface protein	AGATTTCATCTTTGATTCTTGG AATTGATTCTTTAGCATCTGG	510	Billström et al., 2008
gelE	Gelatinase	TATGACAATGCTTTTTGGGAT AGATGCACCCGAAATAATATA	213	Billström et al., 2008
hyl	Hyaluronidase	ACAGAAGAGCTGCAGGAAATG GACTGACGTCCAAGTTTCCAA	276	Billström et al., 2008

were performed using the SPSS statistical package. A p-value of <0.05 was considered significant.

RESULTS

Antimicrobial susceptibility

A total of 117 non-duplicate *E. faecalis* isolates, obtained from 502 pigs from different pig farms in China between 2007 and 2009, were studied, providing an isolation frequency of 23.31%. Table 2 shows the MICs of six antimicrobial agents tested against the *E. faecalis* isolates. A high incidence of antibiotic resistance was detected among the *E. faecalis* isolates. The results show that the majority of the *E. faecalis* isolates were resistant to erythromycin (66.67%; n=78), tylosin (66.67%; n=78), and ciprofloxacin (64.10%; n=75) (Table 2). No vancomycin-resistant enterococci were isolated. Unexpectedly, erythromycin-resistant isolates showed high-level resistance to the three macrolides, erythromycin, kitasamycin, and tylosin. Among 78 erythromycin-resistant isolates, 75 were found highly resistant (MIC, ≥126 g/ml) to tylosin, which is only used in animals.

Distribution of virulence genes

The *E. faecalis* isolates were tested for the presence of seven virulence factors. The *cylA* and *hyl* genes were not detected in any of the isolates. The frequency of the other five virulence genes

ranged in prevalence from 6.84% (*esp*) to 69.23% (*gelE*). The *gelE* gene was the most widespread virulence determinant, found in 69.23% of the isolates (n=81). The second most frequently occurring virulence gene, *ace*, was found in 48.72% of the isolates (n=57). Furthermore, multiple virulence genes co-existed in the *E. faecalis* isolates (Table 3).

TABLE 2 - *MICs for 6 antimicrobial agents against 117 clinical isolates of E. faecalis.*

Antimicrobial agent	*No. of isolates with MIC of (μg/ml):*												*Breakpoint MIC (μg/ml)*	*Resistance (%)*
	0.06	*0.125*	*0.25*	*0.5*	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*		
Ciprofloxacin		6	6	8	12	10	22	32	10	1	8		≥4[a]	64.10
ancomycin				117										0
Clindamycin		4	9	9	12	8	2	3		7	5	58	-	/
Tylosin					1	9	9	20		3		75	≥16[b]	66.67
Kitasamycin		3	3	5	10	6	12	3	1	1	8	65	-	/
Erythromycin		3	3	3	7	8	15	2	1		6	69	≥8[a]	66.67

[a] = CLSI breakpoints for resistance; [b] = NARMS (The National Antimicrobial Resistance Monitoring System) breakpoints for resistance; - = no breakpoints were available.

TABLE 3 - *Frequency of different virulence genes in E. faecalis.*

No. of the isolates	*Virulence gene*							*Percentage (%)*
	gelE	*ace*	*efaA*	*asa1*	*esp*	*hyl*	*cylA*	
35	+							29.91
17		+						14.53
10			+					8.55
7					+			5.98
2				+				1.71
31	+	+						26.50
4	+		+					3.42
2	+			+				1.71
4	+	+	+					3.42
4	+	+		+				3.42
1	+	+		+	+			1.71
Total	117	81 69.23%	57 48.72%	18 15.38%	9 7.69%	8 6.84%	0 0	0 0

+ = positive.

TABLE 4 - *Distribution of the resistance genotypes in erythromycin-resistant isolates.*

No. of the isolates	**MIC Range (μg/ml)*	*Erythromycin resistance genotype*					*No. of virulence genes*				
		erm(A)	*erm(B)*	*erm(C)*	*mef (A/E)*	*msr(C)*	*gelE*	*ace*	*efaA*	*esp*	*asa1*
16	≥128	+					16	6	0	0	2
34	64 to ≥128		+				32	11	9	1	2
1	≥128			+			1	0	0	0	0
3	8 to 16					+	3	3	0	0	1
20	≥128	+	+				19	14	0	1	2
2	≥128			+	+		2	2	0	0	0
1	≥128		+		+		1	1	0	0	0
1	≥128	+	+			+	1	1	0	0	0
Total	78	37	58	3	1	4	75	37	9	2	7

* = erythromycin; + = positive.

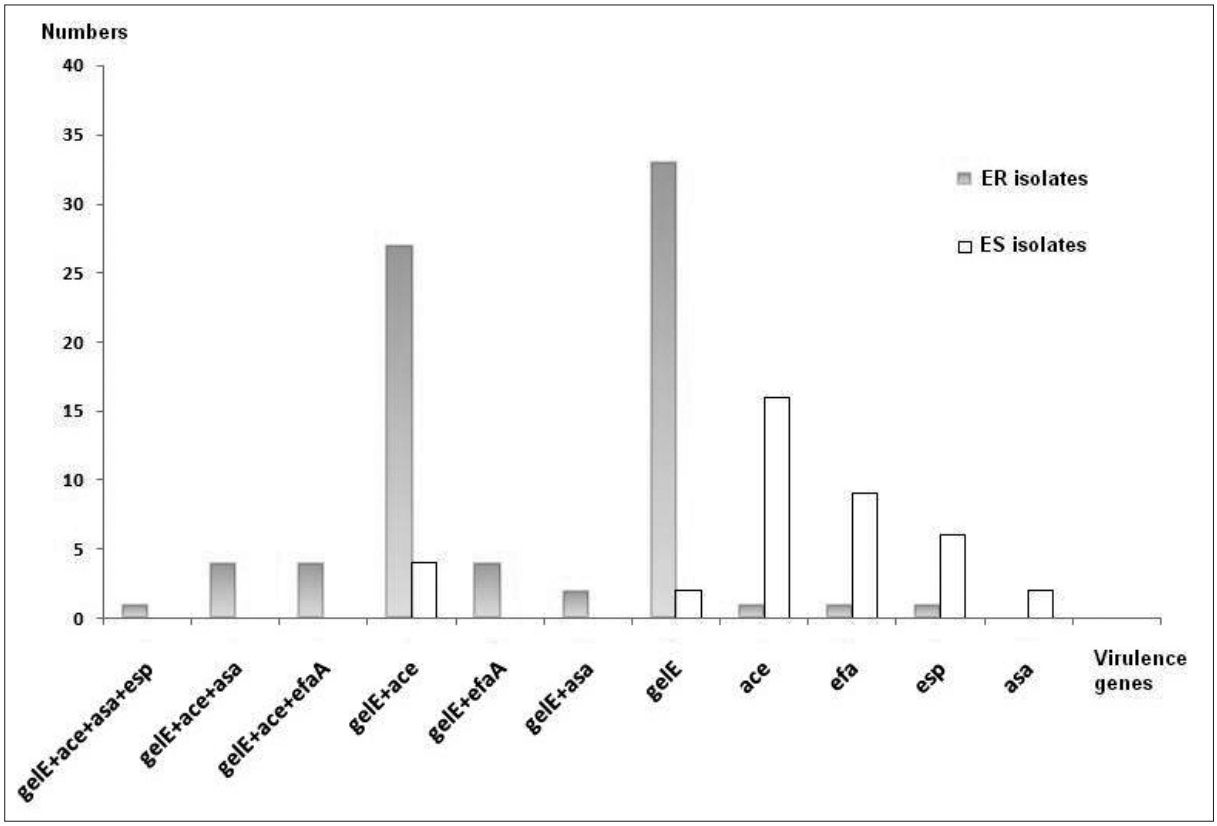

FIGURE 1 - *Incidence of virulence genes in 78 erythromycin-resistant (ER) and 39 erythromycin-susceptible (ES) E. faecalis islolates.*

Distribution of erythromycin resistance genes

All the 78 erythromycin-resistant isolates were tested for the presence of *erm*(A), *erm*(B), *erm*(C), *mef*(A/E), and *msr*(C) genes. The distribution of erythromycin resistance genes according to the phenotypes of macrolide resistance is presented in Table 4. Among the erythromycin-resistant isolates, *erm*(B) (n=54) was the most prevalent resistance gene, followed by *erm*(A) (n=37). Overall, 34 isolates had *erm*(B), 20 carried both *erm*(A) and *erm*(B), and 16 had the *erm*(A) genes. Only three isolates had *msr*(C), two had both *erm*(B) and *erm*(C), one carried *erm*(C), one had both *erm*(B) and *mef*(A/E), and one carried the *erm*(A), *erm*(B), and *msr*(C) genes.

Correlation between erythromycin and virulence resistance genes

Of all the 81 *gelE*-positive isolates, the majority (n=75) were resistant to erythromycin (Table 4). However, only three isolates were resistant to erythromycin among the 36 *gelE*-negative isolates.

Of all the 78 erythromycin-resistant isolates, 42.32% (n=33) harboured the *gelE* gene separately, 34.62% (n=27) carried both *gelE* and *ace* genes, 3.42% (n=4) had the *gelE*, *ace*, and *asa*1 genes, 3.42% (n=4) harboured *gelE*, *ace*, and *efaA*, 3.42% (n=4) carried *gelE* and *efaA*, 1.72% (n=2) had *gelE* and *asa*1, and 0.85% (n=1) harboured the *gelE*, *ace*, *asa*1, and *esp* genes. The three other erythromycin-resistant isolates did not carry the *gelE* gene. Nevertheless, in the 39 erythromycin-susceptible isolates, only six isolates harboured the *gelE* gene. The correlation between the presence of the *gelE* virulence gene and erythromycin resistance was statistically significant. Details of detected virulence determinants in erythromycin-resistant and susceptible isolates are shown in Figure 1.

DISCUSSION

Enterococci infections have become increasingly common because of their intrinsic resistance to several antimicrobial agents and their propensity to acquire resistance from the environment (Laevis *et al.,* 2006). Approximately 80% to 90% of all enterococcal infections are attributed to *E. faecalis*, whereas *E. faecium* is responsible for about 5%-10% of these infections (Simjee *et al.,* 2002; Dupre *et al.,* 2003). The zoonotic dissemination of antibiotic-resistant and virulent strains of enterococci has become a significant public health concern. Jensen *et al.* (1999) reported on *E. faecium* strains isolated from pigs and a hospitalised patient in Denmark, suggestive of a foodborne zoonotic route of transmission of VRE. Hong-Zhou *et al.* (2002) also reported an outbreak of *E. faecium* in China, in which thousands of pigs died and 40 farmers were hospitalised due to severe illness after contact with sick pigs, strongly suggesting the spread of a virulent enterococcal strain from pigs to humans.

As shown in Table 2, a high frequency of resistance to ciprofloxacin (64.10%) and erythromycin (66.7%) was observed. The clinical *E. faecalis* isolates were also resistant to ciprofloxacin as reported by Hershberger *et al.* (2005) and Barbosa *et al.* (2009). Our study shows that *E. faecalis* clinical isolates exhibited high resistance to macrolides. The high level of resistance to erythromycin in these isolates is likely related to the wide use of these classes of antibiotics in husbandry activities, especially the widespread use of tylosin for growth promotion and treatment of disease. The same results were obtained by other authors (Jackson *et al.,* 2004; Poeta *et al.,* 2006; Barbosa *et al.,* 2009; Valenzuela *et al.,* 2009).

Of the 78 erythromycin-resistant *E. faecalis* clinical isolates, *erm*(B) was the most common resistance gene (n=58) detected. This gene plays a predominant role in the development of high-level resistance to macrolides, lincosamides, and streptogramin B (MLS_B phenotype) in *Enterococcus* spp. (Del Grosso *et al.,* 2007). Out of the 78, 37 erythromycin-resistant *E. faecalis* clinical isolates possessed *erm*(A) and displayed higher MICs to erythromycin (≥128 g/ml). However, there are few reports on the presence of *erm*(A) in *E. faecalis* (Schwaiger and Bauer 2008). The *msr*(C) gene encodes a putative efflux pump of the ABC transporter for macrolide and streptogramin B antibiotics (Portillo *et al.* 2000; Graham *et al.,* 2009). The level of resistance resulting from the expression of the efflux pump is generally lower than that produced by target modification due to the ribosomal methylase encoded by *erm* genes. Accordingly, the strain carrying *msr*(C) showed a low level of resistance

(Sutcliffe *et al.,* 1996; Portillo *et al.,* 2000; Singh *et al.,* 2001).

The *E. faecalis* isolates were further screened for potential virulence genes. The virulence genes *ace*, *asa*1, *cylA*, *efaA*, *esp*, *gelE*, and *hyl* were detected at different levels in *E. faecalis*. According to previous studies (Creti *et al.,* 2004; Billström *et al.,* 2008), the prevalence of virulence genes in *E. faecalis* isolated from swine is lower than that reported for human strains. The *gelE* gene was present in 69.2% of the 117 isolates and was the most widespread virulence determinant. The *gelE* positive isolates were significantly more frequent among clinical and food animal isolates - a result also in accord with the reports by Dupre *et al.* (2003), Creti *et al.* (2004), Martín-Platero *et al.* (2009), and Belgacem *et al.* (2010). A relatively high number of isolates carried *ace* (n=57; 48.72%), also distributed with high frequency in isolates from meat (Cariolato *et al.* 2008; Belgacem , 2010). However, the low prevalence of other virulence genes *efa* (n=18; 15.38%), *esp* (n=9; 7.64%), and *asa*1 (n=8; 6.84%) is in contrast with the data reported by other researchers on clinical and food animal isolates (Creti *et al.,* 2004; De Marques and Suzart 2004; Martin *et al.,* 2005; Hällgren *et al.,* 2009; Martín-Platero *et al.,* 2009; Valenzuela *et al.,* 2009). The *cylA* and *hyl* genes were not detected in all isolates as reported in a previous study on food animal isolates (Martin *et al.,* 2005). In addition, the clustering of virulence genes in *E. faecalis* was more frequent in erythromycin-resistant than in erythromycin-susceptible isolates. Therefore, our results confirm a different distribution of virulence genes in clinical *E. faecalis* isolates from swine. In this study, the correlation between erythromycin resistance and the *gelE* virulence gene was statistically significant - a result that has not been observed in previous studies (Billström *et al.,* 2008; Cariolato *et al.,* 2008). Virulent genes and antimicrobial resistance may be transferred together to food strains, although few foodborne enterococcal infections have been reported (Valenzuela *et al.,* 2009).

In conclusion, erythromycin resistance in *E. faecalis* clinical isolates from swine is associated primarily with the presence of *erm*(B) and *erm*(A) genes. Our results also confirm a distribution of virulence genes in clinical *E. faecalis* isolates from swine in China, *gelE* gene being the most common virulence determinant. A strong correlation between the presence of the *gelE* virulence gene and erythromycin resistance was observed. The results from the present study suggest that the studied enterococci from swine should be regarded with caution because they may constitute a reservoir for antimicrobial resistance and virulence genes, and that effective measures should be taken to control antibiotic use in pig farms.

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REFERENCES

Aakra A., Vebø H., Snipen L., Hirt H., Aastveit A., Kapur V., Dunny G., Murray B., Nes. I.F. (2005). Transcriptional response of *Enterococcus faecalis* V583 to erythromycin. *Antimicrob. Agents Ch.* **49**, 2246-2259.

Barbosa J., Ferreira V., Teixeira P. (2009). Antibiotic susceptibility of enterococci isolated from traditional fermented meat products. *Food Microbiol.* **26**, 527-532.

Belgacem Z.B., Abriouel H., Ben Omar N., Lucas R., Martínez-Canamero M., Gálvez A., Manai M. (2010). Antimicrobial activity, safety aspects, and some technological properties of bacteriocinogenic *Enterococcus faecium* from artisanal Tunisian fermented meat. *Food Control.* **21**, 462-470.

Billström H., Lunda B., Sullivana A., Nord C.E. (2008). Virulence and antimicrobial resistance in clinical *Enterococcus faecium*. *Int. J. Antimicrob. Agents.* **32**, 374-377.

Burgos M.J.G., Lopez R.L., Abriouel H, Omar N.B., Galvez A. (2009). Multilocus sequence typing of *Enterococcus faecalis* from vegetable foods reveals two new sequence types. *Foodborne Pathog. Dis.* **6**, 321-327.

Cariolato D., Andrighetto C., Lombardi A. (2008). Occurrence of virulence factors and antibiotic resistances in *Enterococcus faecalis* and *Enterococcus faecium* collected from dairy and human samples in North Italy. *Food Control.* **19**, 886-892

Chen L., Zhang-Liu C., Jian-Hua L., Zhen-Ling Z., Jun-Ying M., Hong-Xia J. (2007). Emergence of RmtB methylase- producing *Escherichia coli* and *Enterobacter cloacae* isolates from pigs in China. *J. Antimicrob. Chemoth.* **59**, 880-885

Clinical and Laboratory Standards Institute.

(2009).Performance standards for antimicrobial susceptibility testing: 19th informational supplement M100-S19. CLSI, Wayne, PA, USA.

Creti R., Imperi M., Bertuccini L., Fabretti F., Orefici G., Rosa D.R., Baldassarri L. (2004). Survey for virulence determinants among *Enterococcus faecalis* isolated from different sources. *J. Med. Microbiol.* **53**, 13-20.

De Marques E.B., Suzart S. (2004). Occurrence of virulence-associated genes in clinical *Enterococcus faecalis* strains isolated in Londrina, Brazil. *J. Med. Microb.* **53**, 1069-1073.

Del Grosso M., Northwood J.G.E., Farrell D.J., Pantosti A. (2007). The macrolide resistance genes *erm*(B) and *mef*(*E*) are carried by Tn2010 in dual-gene *Streptococcus pneumoniae* isolates belonging to clonal complex CC271. *Antimicrob. Agents. Ch.* **50**, 4184-4186.

Dupre I., Zanetti S., Schito A.M., Fadda G., Sechi L.A. (2003). Incidence of virulence determinants in clinical *Enterococcus faecium* and *Enterococcus faecalis* isolates collected in Sardinia (Italy). *J. Med. Microbiol.* **52**, 491-498.

Giacometti A., Cirioni O., Schimizzi A.M., Del Prete, M.S., Barchiesi F., D'Errico M.M., Petrelli E., Scalise G. (2000). Epidemiology and microbiology of surgical wound infections. *J. Clin. Microbiol.* **38**, 918-922.

Graham J.P., Price L.B., Evans S.L., Graczyk T.K., Silbergeld E.K. (2009). Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations. *Sci. Total Environ.* **407**, 2701-2710.

Guo-Bao T., Hong-Ning W., Li-Kou Z., Jun-Ni T., Ying-Wang Z., Man-Yu Ye, Jing-Yuan T., Yi Z., An-Yun Z., Xin Y., Chang-Wen X,, Yue-Jun F. (2009). Detection of CTX-M-15, CTX-M-22, and SHV-2 Extended-Spectrum -Lactamases (ESBLs) in *Escherichia coli* Fecal-Sample Isolates from Pig Farms in China. *Foodborne Pathog. Dis.* **6**, 297-304.

Hällgren A., Claesson C., Saeedi B., Hans-Jürg Isaksson, Hanberger H., Nilsson L.E. (2009). Molecular detection of aggregation substance, enterococcal surface protein, and cytolysin genes and in vitro adhesion to urinary catheters of *Enterococcus faecalis* and *E. faecium* of clinical origin. *Int. J. Med. Microbiol.* **299**, 323-332.

Hebert L., Courtin P., Torelli R., Sanguinetti M., Chapot-Chartier M.P., Auffray Y., Benachour A. (2007). *Enterococcus faecalis* constitutes an unusual bacterial model in lysozyme resistance. *Infect. Immun.* **75**, 5390-5398.

Hershberger E., Oprea S.F., Susan M., Perri D.M. Bozigar P., Bartlett P., Zervos M.J. (2005). Epidemiology of antimicrobial resistance in enterococci of animal origin. *J. Antimicrob. Chemoth.* **55**, 127-130.

Hong-Zhou, L., Xin-Hua W., Haijing L., You-Kuan Y., Mao-Yin P., Yi-Wei T. (2002). *Enterococcus faecium*-related outbreak with molecular evidence of transmission from pigs to humans. *J. Clin. Microbiol.* **40**, 913- 917.

Jackson C.R., Fedorka-Cray P.J., Barrett J.B., Ladely S.R. (2004). Effects of tylosin use on erythromycin resistance in enterococci isolated from swine. *Appl. Environ. Microb.* **70**, 4205-4210.

Martin B., Garriga M,, Hugas M,, Aymerich T. (2005). Genetic diversity and safety aspects of enterococci from slightly fermented sausages. *J. Appl. Microbiol.* **98**, 1177-1190.

Martín-Platero A.M., Valdivia E., Maqueda M., Martínez-Bueno M. (2009). Characterization and safety evaluation of enterococci isolated from Spanish goats'milk cheeses. *Int. J. Food Microbiol.* **132**, 24-32.

Poeta P., Costa D., Rodrigues J., Torres C. (2006). Antimicrobial resistance and the mechanisms implicated in faecal enterococci from healthy humans, poultry and pets in Portugal. *Int. J. Food Microbiol.* **27**, 131-137.

Portillo A., Ruiz-Larrea F., Zarazaga M., Alonso A., Martinez J.L., Torres C. (2000). Macrolide resistance genes in *Enterococcus* spp. *Antimicrob. Agents Ch.* **44**, 967-971.

Schwaiger K., Bauer J. (2008) Detection of the erythromycin rRNA methylase gene *erm*(A) in *Enterococcus faecalis*. *Antimicrob. Agents Ch.* **52**, 2994-2995.

Simjee S., White D.G., Wagner D.D., Meng J., Qaiyumi S., Zhao S., McDermott P.F. (2002). Identification of vat(E) in *Enterococcus faecalis* isolates from retail poultry and its transferability to *Enterococcus faecium*. *Antimicrob. Agents Ch.* **46**, 3823-3828.

Singh K.V., Malathum K., Murray B.E. (2001). Disruption of an *Enterococcus faecium* species-specific gene, a homologue of acquired macrolide resistance genes of *Staphylococci*, is associated with an increase in macrolide susceptibility. *Antimicrob. Agents Ch.* **45**, 263-266.

Sutcliffe J., Grebe T., Tait-Kamradt A., Wondrack L. (1996). Detection of erythromycin-resistant determinants by PCR. *Antimicrob. Agents Ch.* **40**, 2562-2566.

Valenzuela A.S., Ben Omar N., Abriouel H., López R.L., Veljovic K., Cañamero M.M., Topisirovic M.K.L., Gálvez A. (2009) .Virulence factors, antibiotic resistance, and bacteriocins in enterococci from artisan foods of animal origin. *Food Control.* **20**, 381-385.

Vankerckhoven V., Van Autgaerden T., Vael C., Lammens C., Chapelle S., Rossi R., Jabes D., Goossens H. (2004). Development of a multiplex PCR for the detection of *asa*1, *gelE*, *cylA*, *esp*, and *hyl* genes in *Enterococci* and survey for virulence determinants among European hospital isolates of *Enterococcus faecium*. *J. Clin. Microbiol.* **42**, 4473-4479.