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Species distribution, antibiotic resistance and virulence traits in enterococci from meat in Tunisia

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ABSTRACT

Antimicrobial resistance and the mechanisms implicated were studied in 119 enterococci from 105 meat samples from Tunisian markets. Almost 24.5% of recovered enterococci showed resistance against four or more antimicrobial agents and these isolates were identified to the species level. *Enterococcus faecalis* was the most prevalent species (41%). High percentages of erythromycin and tetracycline resistances were found among our isolates, and lower percentages were identified to aminoglycosides, ciprofloxacin and chloramphenicol. All tetracycline-resistant isolates carried the *tet*(M) and/or *tet*(L) genes. The *erm*(B) gene was detected in 78.5% of erythromycin-resistant isolates, *ant*(6)-Ia gene in 58.8% of streptomycin-resistant isolates, and *cat*(A) gene in one chloramphenicol-resistant isolate. Forty-eight isolates carried the *gel*E gene and exhibited gelatinase activity. The *hyl* and *esp* genes were detected in one and three *Enterococcus faecium* isolates, respectively. Streptomycin-resistant isolates showed a high genetic diversity by PFGE and MLST. Meat might play a role in the spread through the food chain of enterococci with these virulence and resistance characteristics to humans.

1. Introduction

Enterococci are commensal microorganisms that colonize the gastrointestinal tract of humans and animals (Günter, 2003). They are also found in different food sources such as meat, milk, cheese, vegetables and olives, among others, where they can play a beneficial role during food maturation processes by contributing to the organoleptic properties of fermented food products. Conversely, enterococci can be also used as an enteric contamination indicator (Foulquié Moreno, Sarantinopoulos, Tsakalidou, & De Vuyst, 2006; Franz, Stiles, Schleifer, & Holzapfel, 2003; Hugas, Garriga, & Aymerich, 2003). In addition, enterococci have a notable resistance to adverse environmental conditions (low and high temperature, extreme pH and salinity) that explain their ability to colonize different ecological niches and they present a high capacity of spreading within the food chain through contaminated animals and foods (Bradley & Fraise, 1996; Foulquié Moreno et al., 2006). Enterococci can be implicated in human infections, have an intrinsic resistance to many antibiotics and can acquire multiple mechanisms of resistance to other agents, due to the possibility of DNA exchange mechanisms (Aarestrup, Agerso, Gerner-Smidt, Madsen, & Jensen, 2000; Ben Belgacem et al., 2010; Del Campo et al., 2000; Hammerum, Lester, & Heuer, 2010; Novais et al., 2005; Sánchez Valenzuela et al., 2009). The high prevalence of resistance and virulence factors makes the safety assessment of enterococcal strains a difficult task, therefore, enterococci are not recommended in the qualified presumption of safety approaches proposed by the European Food Safety Authority (EFSA, 2008). In 2012, the EFSA have developed a guidance document on the safety of the use of *Enterococcus faecium* in animal nutrition which should allow the discrimination between safe strains and those which are more likely to cause human infections (EFSA, 2012). In fact, the consumption of meat carrying antibiotic resistant bacterial populations is a possible route of transfer from animals to humans and could result in either colonization or transfer of antibiotic resistance determinants to host adapted strains (Lester, Frimodt-Møller, Lund Sørensen, Monnet, & Hammerum, 2006; Smith, Harris, Johnson, Silbergeld, & Morris, 2002; Sparo et al., 2012; Vignaroli, Zandri, Aquilanti, Pasquaroli, & Biavasco, 2011). The presence of enterococci in the gastrointestinal tract of animals leads to a high potential for the contamination of meat at the time of slaughter (Franz et al., 2003). The present study was designed to determine the frequency, antibiotic resistance, virulence factors and genetic diversity of enterococci isolated from food animal products mainly derived from chicken, sheep and beef in Tunisia.

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2. Methods

2.1. Bacterial strains

One-hundred-and-five fresh meat samples were obtained from October 2010 to February 2011 and of these, 51 were of poultry origin (turkey or chickens) and 54 were of red meat origin (beef or sheep). All these samples were collected from different supermarkets of Tunisia.

2.2. Bacterial identification

Enterococci were isolated from the food samples using the following protocol: approximately 3 g of sample was mixed with 27 ml of sterile saline solution in a stomacher, and 1 ml of the stomacher solution was added to 5 ml of BHI broth, being incubated at 37 °C for 24 h. After that, several dilutions of the enrichment broth were seeded on Slanetz–Bartley agar plates either supplemented or not with vancomycin (8 μg/ml), that were later incubated 48 h at 37 °C. One or two colonies per sample, with typical enterococcal morphology, were selected and identified to the genus and species level by their cultural characteristics, Gram's stain, catalase test, bile esculin reaction and capacity to grow in hypersaline medium. The species identification was confirmed by polymerase chain reaction (PCR), using primers and conditions for the different enterococcal species (Torres et al., 2003, Table 1), and by 16S rDNA sequencing for some isolates. Positive controls were used in all reactions.

2.3. Antibiotic susceptibility testing

Susceptibility was tested for 11 antibiotics (vancomycin, teicoplanin, ampicillin, streptomycin, gentamicin, trimethoprim–sulfamethoxazole, chloramphenicol, tetracycline, erythromycin, pristinamycin and ciprofloxacin) by the CLSI disk diffusion method and, for vancomycin and teicoplanin, also by the CLSI agar dilution method (CLSI. 2010). High-level resistance was considered for aminoglycosides. *E. faecalis* strain ATCC 29212 was used for quality control.

2.4. Detection of antibiotic resistance genes by PCR

The resistance genes for macrolides [*erm*(A), *erm*(B), *erm*(C)], tetracycline [*tet*(M), *tet*(K), *tet*(L), *tet*(S), *tet*(O)], aminoglycosides [*aph*(3)-IIIa, *aac*(6′)-*aph*(2″), *ant*(6)-Ia], pristinamycin [*vat*(D) *and vat*(E)], and chloramphenicol [*cat*(A)], were tested by PCR in all the enterococcal isolates that showed resistance or reduced susceptibility for these antibiotics, using the previously reported primers and conditions (Table 1).

2.5. Production of gelatinase and hemolysin

For the detection of gelatinase activity, enterococci were inoculated on blood agar plates containing 3% gelatin (Difco), which were then incubated at 37 °C for 24 h, as described elsewhere (Su et al., 1991). Gelatinase activity was observed as a transparent halo around the colonies after the plate was flooded with Frazier solution (Clarke, 1953).

Table 1
Primers used in PCR reactions for the enterococcal identification and for the detection of genes implicated in antibiotic resistance and virulence.

Primer	Sequence	Ta (°C)	Reference
$ddl_{E.\ faecalis}$	F: ATCAAGTACAGTTAGTCT R: ACGATTCAAAGCTAACTG	54	Dutka-Malen, Evers, and Courvalin (1995)
aac(6′)-Ii *E. faecium*	F: GCGGTAGCAGCGGTAGACCAAG R: GCATTTGGTAAGACACCTACG	55	Costa, Galimand, Leclercq, Duval, and Courvalin (1993)
*van*C1 (*E. gallinarum*)	F: GCTGAAATATGAAGTAATGACC R: CGGCATGGTGTTGATTTCGTT	58	Miele, Bandera, and Goldstein (1995)
*van*C2/C3 (*E. casseliflavus*)	F: CTCCTACGATTCTCTTG R: CGAGCAAGACCTTTAAG	58	Dutka-Malen et al. (1995)
$mur\text{-}2_{ed}$ (*E. durans*)	F: AACAGCTTACTTGACTGGACGC R: GTATTGGCGCTACTACCCGTATC	60	Robredo, Singh, Baquero, Murray, and Torres (1999)
mur-2 (*E. hirae*)	F: CGTCAGTACCCTTCTTTTGCAGAGTC R: GCATTATTACCAGTGTTAGTGGTTG	61	Chu, Kariyama, Daneo-Moore, and Shockman (1992)
erm(A)	F: TCTAAAAAGCATGTAAAAGAA R: CTTCGATAGTTTATTAATATTAG	52	Sutcliffe, Grebe, Tait-Kamradt, and Wondrack (1996)
erm(B)	F: GAAAAGTACTCAACCAAATA R: AGTAACGGTACTTAAATTGTTTA	52	Sutcliffe et al. (1996)
erm(C)	F: TCAAAACATAATATAGATAAA R: GCAAATATTGTTTAAATCGTCAAT	52	Sutcliffe et al. (1996)
tet(M)	F: GTTAAATAGTGTTCTTGGAG R: CTAAGATATGGCTCTAACAA	55	Aarestrup et al. (2000)
tet(L)	F: CATTTGGTCTTATTGGATCG R: ATTACACTTCCGATTTCGG	50	Aarestrup et al. (2000)
tet(K)	F: TTAGGTGAAGGGTTAGGTCC R: GCAAACTCATTCCAGAAGCA	55	Aarestrup et al. (2000)
tet(O)	F: F-GATGGCATACAGGCACAGAC R: R-CAATATCACCAGAGCAGGCT	50	Aarestrup et al. (2000)
aac(6′)-Ie-*aph*(2″)-Ia	F: CCAAGAGCAATAAGGGCATA R: CACTATCATAACCACTACCG	60	van de Klundert and Vliegenthart (1993)
aph(3′)-IIIa	F: GCCGATGTGGATTGCGAAAA R: GCTTGATCCCCAGTAAGTCA	60	van de Klundert and Vliegenthart (1993)
ant(6)-Ia	F: ACTGGCTTAATCAATTTGGG R: GCCTTTCCGCCACCTCACCG	58	Clark, Olsvik, Swenson, Spiegel, and Tenover (1999)
*cat*A (PIP501)	F: GGATATGAAATTTATCCCTC R: CAATCATCTACCCTATGAAT	50	Aarestrup et al. (2000)
vat(E)	F: F-ACGTTACCCATCACTATG R: R-GCTCCGATAATGGCACCGAC	55	Robredo, Singh, Torres, and Murray (2000)
esp	F: TTGCTAATGCTAGTCCACGACC R: GCGTCAACACTTGCATTGCCGAA	63	Eaton and Gasson (2001)
hyl	F: GAGTAGAGGAATATCTTAGC R: AGGCTCCAATTCTGT	50	Klare et al. (2005)