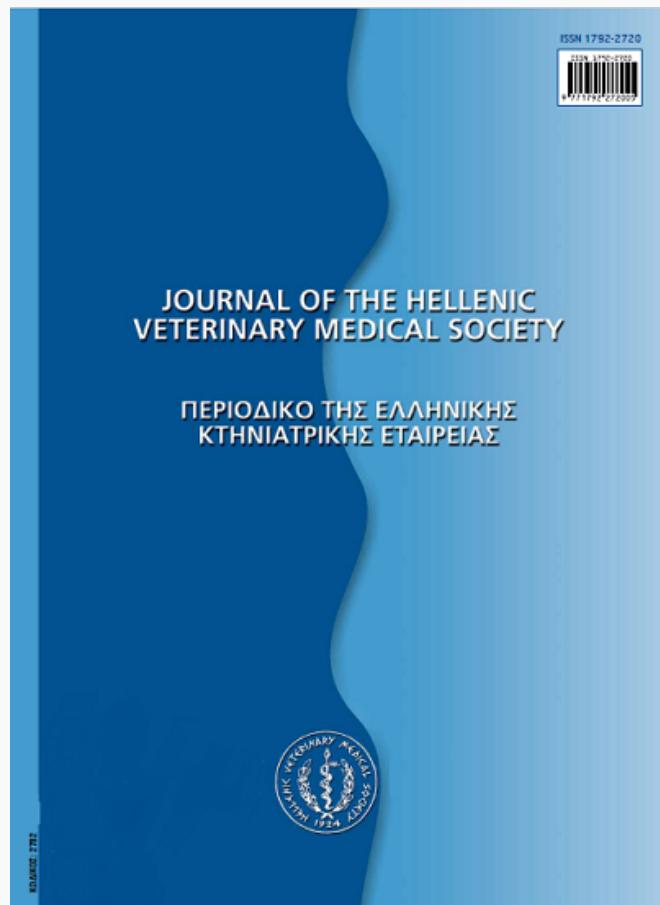


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■ Antimicrobial susceptibility of *Enterococcus* spp. isolated from freshwater fish and personnel and equipment of fish markets in northern Greece

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■ Ευαισθησία έναντι αντιμικροβιακών φαρμάκων στελεχών *Enterococcus* spp. από ψάρια του γλυκού νερού και προσωπικό και εξοπλισμό υχθυοπωλείων στη Βόρεια Ελλάδα

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ABSTRACT. In total, 270 samples from freshwater fish and personnel and equipment from retail fish markets in three cities in northern Greece, were examined for presence of antimicrobial resistance and biogenic amine production of *Enterococcus* spp. strains. Enterococci were isolated from 9.6% of the samples; from 7.4% and 2.2%, respectively, *Enterococcus faecium* and *Enterococcus casseliflavus* were recovered. Isolates were tested for antibacterial susceptibility to 20 antibiotics used regularly in Greek hospitals. All isolates except one were multi drug resistant, to 7-15 antibiotics. Increased rates of resistance were recorded to penicillin, cephalosporins and erythromycin. Relatively increased rates were recorded to quinupristin/

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dalfopristin and linezolid, drugs commonly used as treatment options of infections by vancomycin-resistant enterococci. One *E. faecium* and one *E. casseliflavus* isolate showed intermediate resistance to vancomycin. Multiplex PCR assay for presence of *van* genes in *E. faecium* was negative. All *E. faecium* isolates and one-third of *E. casseliflavus* isolates were able to decarboxylate tyrosine, but not histidine, ornithine or lysine. The results indicate that freshwater fish and fish markets are potential reservoirs of multi-drug resistant enterococci.

Keywords: antimicrobial susceptibility, biogenic amines, *Enterococcus* spp., freshwater fish.

ΠΕΡΙΛΗΨΗ. Διερευνήθηκε η εναισθησία στα αντιμικροβιακά φάρμακα και η ικανότητα παραγωγής βιογενών αμινών από στελέχη *Enterococcus* spp. από ψάρια του γλυκού νερού και το προσωπικό και τον εξοπλισμό ιχθυοπωλείων στη Βόρειο Ελλάδα. Εξετάσθηκαν 270 συνολικά δείγματα και απομονώθηκαν εντεροκόκκοι από 9,6% των δειγμάτων, συγκεκριμένα από 7,4% των δειγμάτων απομονώθηκε *Enterococcus faecium* και από 2,2% των δειγμάτων απομονώθηκε *Enterococcus casseliflavus*. Τα απομονωθέντα στελέχη εξετάσθηκαν για την εναισθησία τους έναντι 20 αντιμικροβιακών ουσιών που χρησιμοποιούνται συνήθως σε ελληνικά νοσοκομεία. Όλα τα στελέχη, εκτός ενός, ήταν πολυανθεκτικά εμφανίζοντας αντοχή σε 7-15 αντιμικροβιακά φάρμακα. Αυξημένη συχνότητα ανθεκτικότητας παρατηρήθηκε στην πενικιλίνη, στις κεφαλοποροίνες και στην ερυθρομυκίνη. Σχετικά αυξημένη συχνότητα παρατηρήθηκε στην κινουπριστίνη/δαλφοπριστίνη και τη λινεζολίδη, φάρμακα χρησιμοποιούμενα στη θεραπευτική αγωγή λοιμώξεων από ανθεκτικούς στη βανκομυκίνη εντεροκόκκους. Σε ένα στέλεχος *E. faecium* και ένα *E. casseliflavus* παρατηρήθηκε μέτρια ανθεκτικότητα έναντι της βανκομυκίνης. Με εξέταση με πολλαπλή αλυσιδωτή αντίδραση πολυμεράσης (multiplex PCR) των στελεχών *E. faecium* δεν ανιχνέυτηκαν γονίδια αντοχής στη βανκομυκίνη. Όλα τα στελέχη *E. faecium* και ένα τρίτο των στελεχών *E. casseliflavus* είχαν την ικανότητα αποκαρβοξυλώσης της τυροσίνης, όχι όμως της ιστιδίνης, ορνιθίνης και λυσίνης. Τα αποτελέσματα υποδεικνύουν ότι τα ψάρια του γλυκού νερού και το περιβάλλον των ιχθυοπωλείων αποτελούν πιθανές πηγές διασποράς πολυανθεκτικών στελεχών εντεροκόκκων σε ανθρώπους.

Λέξεις ευρετηρίασης: αντιμικροβιακή εναισθησία, βιογενείς αμίνες, *Enterococcus* spp., ψάρια γλυκού νερού.

INTRODUCTION

Enterococcus spp. includes over twenty species widely distributed in nature, with a few of them involved in clinical infections of humans (de Perio et al., 2006; Fisher and Phillips, 2009; Al Bulushi et al., 2010). *Enterococcus faecium* and *Enterococcus faecalis* are the most frequently encountered species and constitute part of the normal intestinal flora of humans and animals (Fernández et al., 2007). Enterococci are frequently isolated from various foods of animal origin (Chingwaru et al., 2003; Çitak et al., 2004; Jaffrès et al., 2009; Sergelidis et al., 2010). They have also been isolated from aquatic habitats, fish and seafood (Rice et al., 1995; Wilson and McAfee, 2002; Petersen and Dalgaard, 2003; Quigg et al., 2009; Al Bulushi et al., 2010; Valenzuela et al., 2010). The organisms are also part of the spoilage flora of processed fish products (Dalgaard et al., 2003; Mejlholm et al., 2008; Tomé et al., 2008; Jaffrès et al., 2009).

Enterococci exhibit interesting technological and probiotic properties and their beneficial role in

food fermentations, ripening and biopreservation has been recognised (Giraffa, 2003; Hugas et al., 2003; Tomé et al., 2008). Despite their beneficial properties, they are considered to be faecal contamination indicators and responsible for food spoilage (Franz et al., 1999; Dalgaard et al., 2003; Jaffrès et al., 2009). Moreover members of this genus often develop antimicrobial resistance (Chingwaru et al., 2003; Çitak et al., 2004; Fisher and Phillips, 2009) and produce biogenic amines (BA) in foods through decarboxylation of amino acids (Giraffa et al., 1997; Gardini et al., 2001; Sarantinopoulos et al., 2001).

In the past, these organisms were generally believed to be of decreased pathogenicity for humans. However, in recent years they have become important pathogens of nosocomial infections (Chatterjee et al., 2007). It is estimated that 80% to 90% of enterococcal infections in humans are caused by *E. faecalis*, 10% to 15% are caused by *E. faecium* and <5% are caused by other species of lesser importance, e.g., *E. raffinosus*, *E. cas-*

seliflavus, *E. durans* or *E. avium* (Reid et al., 2001; Karmakar et al., 2004). The bacteria are intrinsically resistant to a wide range of antibiotics, including the semi-synthetic penicillins (e.g., oxacillin), aminoglycosides, vancomycin (*E. gallinarum*, *E. casseliflavus*, *E. flavescentis*), lincosamides, polymyxines, streptogramin A (*E. faecalis*) and monobactams (Giraffa et al., 2000; Pavia et al., 2000; Chingwaru et al., 2003; Koluman et al., 2009). During the last decades, vancomycin-resistant enterococci (VRE) have been recognized as emerging pathogens and have been incriminated as causative agents of a variety of severe infections (Chatterjee et al., 2007; Fisher and Phillips, 2009). According to CDC's National Nosocomial Infections Surveillance in the USA, over a period of 15 years there has been a 20-fold increase in VRE-associated nosocomial infections (National Nosocomial Infections Surveillance, 2004). This dramatic increase highlights the need for a better understanding of these bacteria: their ecology, epidemiology and virulence. Isolates from humans show the highest virulence, followed by isolates from food and isolates from starter cultures (Busani et al., 2004; Omar et al., 2004). However, it is difficult to separate safe and unsafe enterococcal strains, since virulence and antibiotic resistance genes can be easily exchanged between strains (Eaton and Gasson, 2001; Hummel et al., 2007).

Decarboxylation of amino acids and production of biogenic amines is another feature of enterococci. Enterococci have been found to be the most abundant tyramine producers (Chong et al., 2011). Ingestion of food containing increased concentration of biogenic amines may cause various problems in humans. Many biogenic amines have been found in fish and fish products, with histamine, cadaverine and putrescine being the ones most frequently detected (Al Bulushi et al., 2009). Decarboxylation of amino acids by bacteria in seafood has been considered an important factor for seafood poisoning of humans (Fernández et al.s 2007).

Presence of multi-drug resistant bacteria as well as VRE in foods is a matter of concern, because these bacteria may contribute in transmission of resistance determinants through the food chain. Freshwater fish have the potential to harbour enterococci from multiple sources, e.g. water from aquaculture, rivers and lakes, which frequently accept

treated or untreated urban wastewater. Additionally, fish may be contaminated due to multiple transfer, improper handling and storage, temperature abuse or contamination through colonized food handlers and contaminated boxes and tools. Consequently fish may serve as reservoir of resistant bacteria.

Objective of the present study was to investigate antibiotic resistance and decarboxylase activity of enterococci isolated from freshwater fish, as well as from personnel and equipment in fish markets in northern Greece.

MATERIALS AND METHODS

Sampling

In total, 150 samples of freshwater fish, specifically 75 samples from rainbow trout (*Oncorhynchus mykiss*) and 75 samples from gibel carp (*Carassius gibelio*), were collected from 16 fish markets and 4 open air markets located in three towns of northern Greece (Florina, Komotini, Ptolemaida). Samplings were performed during a 6-month period, from January to June 2011.

Gibel carp originated from three nearby lakes; rainbow trouts from fish farms located in streams near these lakes. Body weight of each fish sampled was ~300g. Usually, fish were brought into the markets by shop owners or fishermen in ice within the same day after fishing. Moreover, 100 swab samples were collected from the equipment in each fish market shop (20 from workers' knives, 20 from work surfaces, 20 from wooden boxes, 20 from plastic boxes, 16 from floor surfaces, 2 from drainage lids and 2 from refrigerator knobs) and another 20 from the hands of staff (in each fish market shop, one person was sampled). Fish were aseptically put into sterile bags. About 100 cm² of flat surfaces were swabbed by means of the wet-dry double swab technique, using sterile cotton swabs moistened with 0.1% sterile peptone water containing 0.85% sodium chloride. The swabs were immersed into tubes containing 10 mL of tryptone soy broth containing 7.5% NaCl (LAB M, Lancashire, UK). Fish samples and swabbed samples were transported to the laboratory under refrigerated storage and processed within 2 h of collection.

Isolation, enumeration and identification of enterococci

Tubes with swabs were directly incubated at 37 °C for 24 h. A 10 cm² sample, consisting of skin and flesh, was aseptically excised from the anterior dorsal region of each fish using sterile template, scalpel and forceps and suspended in 100 mL of buffered peptone water (Oxoid, Basingstoke, UK). The sample was homogenized for 2 min in a stomacher (Lab Blender 400; A. J. Seward and Co. Ltd., London, UK) and serial 10-fold dilutions were prepared in buffered peptone water; 1 mL from each dilution was plated, using pour plating technique, onto Slanetz Bartley agar (LAB M, Lancashire, UK). For the detection of <10 cfu g⁻¹, the first dilution was incubated for enrichment at 37 °C for 16 h. One loopful of the enriched culture was spread onto Slanetz Bartley agar and incubated at 37 °C for 24 to 48 h. All raised colonies with a red, maroon or pink colour, either in the centre of the colony or throughout it, were tentatively considered to be enterococci (Domig et al., 2003). Three colonies were transferred onto trypticase soy agar (LAB M, Lancashire, UK) supplemented with 0.6% yeast extract for identification and further studies. Initial identification of the isolates was based on tests for Gram staining, catalase and oxidase production, growth at 10 °C and 45 °C, growth in the presence of 6.5% NaCl and at pH 9.6 in tryptone soy broth. The lower detection limit of the technique was <1 log cfu g⁻¹ or cm⁻².

Isolates were identified based on biochemical characterization by the semi-automated system WIDER (Francisco Soria Melguizo, Madrid, Spain) using the Gram positive minimal inhibitory concentration/identification (MIC/ID) panels. The following biochemical tests were included in these panels: acidification of arabinose, cellobiose, lactose, mannitol, ribose and saccharose, use of aesculin, arginine and urea, production of phosphate, α -glucosidase and β -glucuronidase, transformation of pyruvate to acetoin, growth in the presence of optochin, bacitracin and novobiocin, growth in the presence of 6.5% sodium chloride and haemolysis.

E. faecium ATCC 19434, *E. faecalis* ATCC 19433, *E. durans* ATCC 19432 and *E. hirae* LMG 6399 were used as control strains.

Antimicrobial susceptibility tests

Isolates were tested for antimicrobial susceptibility to 20 antimicrobials commonly used in Greek hospitals. MIC was evaluated according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2008) in the semi-automated system WIDER (Francisco Soria Melguizo, Madrid, Spain) using the Gram Positive MIC/ID Panels. The antibiotics were: beta-lactams (penicillin, ampicillin, oxacillin and amoxicillin/clavulanic acid), cephalosporins (cefazolin and cefotaxime), aminoglycosides (streptomycin 1000, gentamicin, gentamicin 500 and amikacin), glycopeptides (vancomycin and teicoplanin), fluoroquinolones (levofloxacin), macrolides (erythromycin), lincosamides (clindamycin), streptogramins (quinupristin/dalfopristin), oxazolidinones (linezolid), rifamycins (rifampin), chloramphenicol, fosfomycin and trimethoprim/sulfamethoxazole.

E. faecalis ATCC 29212 and *E. faecium* BM 4147 (vanA+) were used as reference strains.

Detection of van genes

Total DNA from tested isolates was extracted by proteinase K and phenol-chloroform treatment and a multiplex PCR assay was performed on DNA for detection of *vanA*, *vanB*, *vanC1* and *vanC2/3* genes according to Christidou et al. (2004) using the same sets of primers.

Screening for decarboxylase activity

Enterococci were screened for the ability to produce biogenic amines by decarboxylation of amino acids according to the screening method on decarboxylase medium proposed by Bover-Cid and Holzapfel (1999). All isolates were cultured, in duplicate, on plates with 1% of each precursor amino acid (tyrosine, histidine, ornithine or lysine) or without them (as control) and incubated aerobically at 37°C for 4 days. Development of a purple color around the colonies and a clear zone in the case of tyrosine was recorded as a positive reaction.

RESULTS

Enterococcus spp. were isolated from 9.6% (26/270) of the samples. *E. faecium* (from 7.4% of samples) and *E. casseliflavus* (from 2.2% of samples) were the two species identified (Table 1). *E. faecium* was isolated from 5.3% and 6.7% of samples

Table 1. Incidence of *Enterococcus* spp. in freshwater fish, handlers and environment of fish markets in northern Greece.

Origin of sample	n	n samples that yielded enterococci	
		<i>E. faecium</i>	<i>E. casseliflavus</i>
Fish			
Rainbow trout	75	4 (5.3%)	3 (4%)
Gibel carp	75	5 (6.7%)	-
Equipment			
Knives	20	2 (10%)	-
Refrigerator knobs	2	-	-
Wooden containers (boxes)	20	2 (10%)	2 (10%)
Plastic containers (boxes)	20	2 (10%)	1 (5%)
Cutting boards	20	1 (5%)	-
Floors	16	-	-
Drainage lids	2	-	-
Personnel			

of rainbow trout and gibel carp, respectively, and *E. casseliflavus* was isolated from 4% of samples of rainbow trout. Population of enterococci on fish skin samples did not exceed 2 log CFU cm⁻².

From equipment samples, *E. faecium* was isolated from 10%, 10% and 5% of samples from wooden box, plastic box and cutting board, respectively, and *E. casseliflavus* from 10% and 5% of samples from wooden box and plastic box, respectively. *E. faecium* was the only species isolated from 20% of samples from personnel (Table 1).

Enterococcal isolates with distinct antibiotic resistance patterns were resistant to 2-15 antimicrobial agents (Table 2). One *E. faecium* and one *E. casseliflavus* isolate were found to have intermediate resistance to vancomycin (Table 3). Multiplex PCR for detection of *van* genes did not reveal that *E. faecium* and *E. casseliflavus* carried *vanC2/3* gene, which is common in motile enterococci. Resistance to penicillin was evident in 33% and 75% of *E. faecium* strains from equipment and personnel, respectively (Table 3). All isolates from fish and from equipment, as well as 75% of strains isolated from personnel were found to be resistant to cefazolin and

cefotaxime. Moreover, 44% and 57% of *E. faecium* isolates from fish and equipment, respectively, were found to be resistant to linezolid. Both *Enterococcus* species isolated showed remarkable rates of resistance to erythromycin, reaching 75% among *E. faecium* isolates from personnel.

All *E. faecium* isolates and one-third (2/6) of *E. casseliflavus* isolates were able to decarboxylate tyrosine, but none of the other amino acids tested (histidine, ornithine and lysine).

DISCUSSION

Data regarding isolation of enterococci from fish and fish markets are limited. *E. faecium* was found to be the predominant species in the present study, isolated from such samples. Similar results have been obtained from other studies that included seafood (Fisher and Phillips, 2009; Barros et al., 2011) and retail meats (Hayes et al., 2003; Poeta et al., 2006; 2007).

Barros et al. (2011) isolated 73 enterococci from 118 faecal samples of gilthead seabream; 92% and 8% of these were found to be *E. faecium* and *E. fae-*

Table 2. Detailed antimicrobial resistance pattern and tyramine decarboxylation activity of *Enterococcus* spp. strains isolated from freshwater fish and personnel and equipment in fish markets in northern Greece.

Species (n=1)	Antimicrobial resistance pattern	TDA	Source
<i>E. faecium</i>	AK, CTX, CZ, DA, CN, CN500, L, OX, RA (I), SXT	Yes	Rainbow trout
<i>E. faecium</i>	AK, CTX, CZ, CN, LEV, OX, RA, S1000, SXT	Yes	Rainbow trout
<i>E. faecium</i>	AK, CTX, CZ, DA, CN, OX, RA (I), SXT	Yes	Rainbow trout
<i>E. faecium</i>	AK, CTX, CZ, DA, FF, CN, OX, SXT	Yes	Rainbow trout
<i>E. faecium</i>	AK, CTX, CZ, DA (I), CN, CN500, LEV, L, OX, P, RA, S1000, SXT	Yes	Gibel carp
<i>E. faecium</i>	AK, CTX, CZ, DA, E, CN, CN500, LEV, L, OX, P, RA, S1000, SXT	Yes	Gibel carp
<i>E. faecium</i>	AK, CTX, CZ, DA, CN, LEV, L, OX, SXT	Yes	Gibel carp
<i>E. faecium</i>	AK, CTX, CZ, E, CN, LEV, OX, P, RA (I), SXT	Yes	Gibel carp
<i>E. faecium</i>	AK, CTX, CZ, DA, FF, CN, OX, RA, SXT	Yes	Gibel carp
<i>E. faecium</i>	AM, CTX, CZ, DA, E, CN, P	Yes	Personnel
<i>E. faecium</i>	AM, P	Yes	Personnel
<i>E. faecium</i>	CTX, CZ, DA, E, OX, P, RA	Yes	Personnel
<i>E. faecium</i>	CTX, CZ, DA, E, CN, LEV, OX, SXT	Yes	Personnel
<i>E. faecium</i>	AK, CTX, CZ, DA, E, CN, LEV, L, OX, Q/D, SXT	Yes	Equipment
<i>E. faecium</i>	AK, CTX, CZ, DA, CN, OX, Q/D (I), SXT, VA (I)	Yes	Equipment
<i>E. faecium</i>	AK, CTX, CZ, CL (I), E (I), FF, CN, LEV, L, OX, SXT	Yes	Equipment
<i>E. faecium</i>	AK, CZ, CFT, DA, E, CN, OX, Q/D (I), RA, SXT	Yes	Equipment
<i>E. faecium</i>	AK, CTX, CZ, E (I), FF, CN, LEV, OX, RA (I), SXT	Yes	Equipment
<i>E. faecium</i>	AK, CTX, CZ, DA, CN, LEV, L, OX, Q/D (I), SXT	Yes	Equipment
<i>E. faecium</i>	AK, CTX, CZ, E, CN, LEV, LIN, OX, SXT	Yes	Equipment
<i>E. casseliflavus</i>	AK, CTX, CZ, DA, E, FF, CN, LEV, OX, Q/D, RA, S1000, SXT	No	Rainbow trout
<i>E. casseliflavus</i>	AK, CTX, CZ, DA, FF, CN, OX, Q/D, S1000, SXT	Yes	Rainbow trout
<i>E. casseliflavus</i>	AK, CTX, CZ, DA, CN, OX, RA (I), S1000, SXT	No	Rainbow trout
<i>E. casseliflavus</i>	AK, CTX, CZ, DA, CN, OX, Q/D, RA (I), SXT	Yes	Equipment
<i>E. casseliflavus</i>	AK, CTX, CZ, DA, E (I), CN, LEV (I), OX, Q/D (I), RA, SXT, VA (I)	No	Equipment
<i>E. casseliflavus</i>	AK, CTX, CZ, DA, E, FF, CN, CN500, LEV, L, OX, Q/D, RA, S1000, SXT	No	Equipment

TDA: tyramine decarboxylation activity

AK: amikacin, AM: ampicillin, CTX: cefotaxime, CZ: cefazolin, C: chloramphenicol, CL: clindamycin, E: erythromycin, FF: fosfomycin, CN: gentamicin, CN500: gentamicin 500, LEV: levofloxacin, L: linezolid, OX: oxacillin, P: penicillin, Q/D: quinupristin/dalfopristin, RA: rifampin, S1000: streptomycin 1000, SXT: trimethoprim/sulfamethoxazole, VA: vancomycin - (I) = Intermediate resistance.

Table 3. Cumulative results of antimicrobial resistance of *Enterococcus* spp. isolated from freshwater fish and personnel and equipment of fish markets in northern Greece.

Antimicrobial drug	Identity and origin of <i>Enterococcus</i> spp.				
	<i>E. faecium</i>		<i>E. casseliflavus</i>		
	Fish (n=9)	Equipment (n=7)	Personnel (n=4)	Fish (n=3)	Equipment (n=3)
Amikacin	9	6	0	3	3
Amoxicillin/Clavulanic acid	0	0	0	0	0
Ampicillin	0	0	2	0	0
Cefazolin	9	7	3	3	3
Cefotaxime	9	7	3	3	3
Chloramphenicol	0	0	0	0	1
Clindamycin	7	4	3	3	3
Erythromycin	2	5	3	1	2
Fosfomycin	2	2	0	2	1
Gentamicin	9	7	2	3	3
Gentamicin 500	3	0	0	0	0
Levofloxacin	5	5	1	1	2
Linezolid	4	4	0	0	1
Oxacillin	8	7	2	3	3
Penicillin	3	0	3	0	0
Quinupristin/Dalfopristin	0	3 (I)	0	2	3
Rifampin	7	2	1	2	3
Trimethoprim/Sulfamethoxazole	0	7	1	3	3
Streptomycin 1000	3	0	0	2	1
Teicoplanin	0	0	0	0	0
Vancomycin	0	1 (I)	0	0	1 (I)

(I) = Intermediate resistance.

calis, respectively. Vancomycin- and/or teicoplanin-resistance was not detected in any of them; the strains were resistant to erythromycin (59%) and tetracycline (18%), while decreased resistance (<13%) to quinupristin/dalfopristin, ampicillin, gentamicin, streptomycin, kanamycin, ciprofloxacin and chloramphenicol was observed.

Although enterococci are considered intrinsically resistant to β -lactams, the results of the present study are not in agreement with this concept, since all isolates were sensitive to ampicillin and a high proportion of them to penicillin. Similar results have

been reported in other studies (Peters et al., 2003; Omar et al., 2004). David et al. (2010) reported that enterococci isolated from water and the intestine of tilapia fish showed the highest resistance rate to penicillin and the lowest to gentamicin. The majority of enterococci (74%) were resistant to overone antibiotic tested. In southeastern Asia, Petersen and Dalgaard (2003) reported that *Enterococcus* spp., isolated from fish intestinal samples from integrated broiler-fish farms, showed significant levels of resistance to chloramphenicol (8%), erythromycin (91%), oxytetracycline (75%) and streptomycin (72%), these

levels being higher to those in isolates from control farms culturing only fish (0%, 23%, 16% and 31%, respectively).

Intermediate resistance to vancomycin recorded in one *E. casseliflavus* isolate is a naturally occurring characteristic for this species (Toye et al., 1997), who, in general, have decreased resistance to vancomycin and sensitivity to teicoplanin, and has not been shown to be transferrable (Leclercq and Courvalin, 1997; Reid et al., 2001). The fact that no vancomycin resistance genes were detected in the *E. faecium* isolate with the intermediate resistance may be the result of a decreased, undetectable level of expression.

Isolation of enterococci from personnel is not considered to be a potential risk, at least no greater than that potentially caused by enterococci present in their intestinal flora. Nevertheless, application of good hygiene practices is necessary, in order to eliminate risk of spread of these bacteria in foods.

With regard to decarboxylation activity of enterococci, the results of the study are in accord with those of others, who have reported that many strains of enterococci can produce tyramine (Masson et al.,

1996; Silla Santos, 1996; Giraffa et al., 1997; Bover-Cid et al., 2001; Gardini et al., 2001; Mejhlholm et al., 2008; Tuncer, 2009), but not significant amounts of putrescine and/or cadaverine (Bover-Cid and Holzapfel, 1999; Hayes et al., 2003).

CONCLUDING REMARKS

The results of the present study indicate that freshwater fish sold in fish markets may represent a potential source of multi-drug resistant enterococci, of possible concern to public health. The role of fish in the spread of antimicrobial resistance traits is not clear and deserves further investigation. Compliance with codes of good practice in the processing and transport of fish is important, in order to ensure health of workers and consumers. Prevalence and antibiotic resistance of enterococci should be continuously monitored.

CONFLICT OF INTEREST STATEMENT

None of the authors of this article has any conflict of interest. 

REFERENCES

Al Bulushi IM, Poole S, Deeth HC, Dykes GA (2009) Biogenic amines in fish: roles in intoxication, spoilage, and nitrosamine formation - A review. *Crit Rev Food Sci Nutr* 49:369-377.

Al Bulushi IM, Poole SE, Barlow R, Deeh HC, Dykes GA (2010) Speciation of Gram-positive bacteria in fresh and ambient-stored sub-tropical marine fish. *Int J Food Microbiol* 138:32-38.

Barros J, Igrejas G, Andrade M, Radhouani H, López M, Torres C, Poeta P (2011) Gilthead seabream (*Sparus aurata*) carrying antibiotic resistant enterococci. A potential bioindicator of marine contamination? *Mar Pollut Bull* 62:1245-1248.

Bover-Cid S, Holzapfel WH (1999) Improved screening procedure for biogenic amine production by lactic acid bacteria. *Int J Food Microbiol* 53:33-41.

Bover-Cid S, Hugas M, Izquierdo-Pulido M, Vidal-Carou MC (2001) Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages. *Int J Food Microbiol* 66:185-189.

Busani L, Del Grosso M, Paladini C, Graziani C, Pantosti A, Biavasco F, Caprioli A (2004) Antimicrobial susceptibility of vancomycin-susceptible and -resistant enterococci isolated in Italy from raw meat products, farm animals, and human infections. *Int J Food Microbiol* 97:17-22.

Chatterjee I, Iredell JR, Woods M, Lipman J (2007) The implications of enterococci for the intensive care unit. Critical care and resuscitation: J Australasian Academy of Critical Care Medicine 9:69-75.

Chingwaru W, Mpuchane SF, Gashe BA (2003) Enterococcus faecalis and Enterococcus faecium isolates from milk, beef, and chicken and their antibiotic resistance. *J Food Prot* 66:931-936.

Chong CY, Bakar FA, Russly AR, Jamilah B, Mahyudin NA (2011) The effects of food processing on biogenic amines formation. *Int Food Research J* 18:867-876.

Christidou A, Gikas A, Scoulica E, Pediaditis J, Roumbelaki M, Georgilidakis A, Tselenitis Y (2004) Emergence of vancomycin-resistant enterococci in a tertiary hospital in Crete, Greece: a cluster of cases and prevalence study on intestinal colonization. *Clinical Microbiology and Infection* 10:999-1005.

Çitak S, Yucel N, Orhan S (2004) Antibiotic resistance and incidence of Enterococcus species in Turkish white cheese. *Int J Dairy Technol* 57:27-31.

CLSI (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals Approved Standard CLSI Document M31-A3. Wayne, PA, USA.: Clinical and Laboratory Standards Institute.

Dalgaard P, Vancanneyt M, Euras Vilalta N, Swings J, Fruekilde P, Leisner JJ (2003) Identification of lactic acid bacteria from spoilage associations of cooked and brined shrimps stored under modified atmosphere between 0°C and 25°C. *J Appl Microbiol* 94:80-89.

David OM, Falegan CR, Ogunlade JT (2010) Levels of heavy and other metals in tilapia species raised in different ponds and resistant pattern of associated Enterococcus species. *Advances in Environ Biology* 4:47-52.

De Perio MA, Yarnold PR, Warren J, Noskin GA (2006) Risk Factors and Outcomes Associated with Non-Enterococcus faecalis, Non-Enterococcus faecium Enterococcal Bacteremia. *Infect Control Hosp Epidemiol* 27:28-33.

Domig KJ, Mayer HK, Kneifel W (2003) Methods used for the isolation, enumeration, characterisation and identification of Enterococcus spp.: 1. Media for isolation and enumeration. *Int J Food Microbiol* 88:147-164.

Eaton TJ, Gasson MJ (2001) Molecular Screening of Enterococcus Virulence Determinants and Potential for Genetic Exchange between Food and Medical Isolates. *Appl Environ Microbiol* 67:1628-1635.

Fernández M, Linares D, Rodríguez A, Alvarez M (2007) Factors affecting tyramine production in *Enterococcus durans* IPLA 655. *Appl Microbiol Biotechnol* 73:1400-1406.

Fisher K, Phillips C (2009) The ecology, epidemiology and virulence of *Enterococcus*. *Microbiol* 155:1749-1757.

Franz CMAP, Holzapfel WH, Stiles ME (1999) Enterococci at the crossroads of food safety? *Int J Food Microbiol* 47:1-24.

Gardini F, Martuscelli M, Caruso MC, Galgano F, Crudele MA, Favati F, Guerzoni ME, Suzzi G (2001) Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of *Enterococcus faecalis*. *Int J Food Microbiol* 64:105-117.

Giraffa G (2003) Functionality of enterococci in dairy products. *Int J Food Microbiol* 88:215-222.

Giraffa G, Carminati D, Neviani E (1997) Enterococci Isolated from Dairy Products: A Review of Risks and Potential Technological Use. *J Food Prot* 60:732-737.

Giraffa G, Olivari AM, Neviani E (2000) Isolation of vancomycin-resistant *Enterococcus faecium* from Italian cheeses. *Food Microbiol* 17:671-677.

Hayes JR, English LL, Carter PJ, Proescholdt T, Lee KY, Wagner DD, White DG (2003) Prevalence and Antimicrobial Resistance of *Enterococcus* Species Isolated from Retail Meats. *Appl Environ Microbiol* 69:7153-7160.

Hugas M, Garriga M, Aymerich MT (2003) Functionality of enterococci in meat products. *Int J Food Microbiol* 88:223-233.

Hummel A, Holzapfel WH, Franz CMAP (2007) Characterisation and transfer of antibiotic resistance genes from enterococci isolated from food. *Systematic Appl Microbiol* 30:1-7.

Jaffrès E, Sohier D, Leroi F, Pilet MF, Prévost H, Joffraud JJ, Dousset X (2009) Study of the bacterial ecosystem in tropical cooked and peeled shrimps using a polyphasic approach. *Int J Food Microbiol* 131:20-29.

Karmakar MG, Gershon ES, Mehta PR (2004) Enterococcal infections with special reference to phenotypic characterization and drug resistance. *Indian J Med Res* 119 (Suppl.):22-25.

Kolumn A, Akan LS, Çakiroğlu FP (2009) Occurrence and antimicrobial resistance of enterococci in retail foods. *Food Control* 20:281-283.

Leclercq R, Courvalin P (1997) Resistance to glycopeptides in enterococci. *Clin Infect Dis* 24:545-554.

Marine-Font A, Vidal-Carou C, Izquierdo-Pulido M, Veciana-Nogues T, Hernandez-Jover T (1995) Les amines biogènes dans les aliments: leur signification, leur analyse. Paris, France: Soci des experts-chimistes de France.

Masson F, Talon R, Montel MC (1996) Histamine and tyramine production by bacteria from meat products. *Int J Food Microbiol* 32:199-207.

Mejlholm O, Kjeldgaard J, Modberg A, Vest MB, Bøknæs N, Koort J, Björkroth J, Dalgaard P (2008) Microbial changes and growth of *Listeria monocytogenes* during chilled storage of brined shrimp (*Pandalus borealis*). *Int J Food Microbiol* 124:250-259.

National Nosocomial Infections Surveillance (2004) System Report, data summary from January 1992 through June 2004, issued October 2004. A report from the NNIS System. *Am J Infect Control* 32:470-485.

Omar NB, Castro A, Lucas R, Abriouel H, Yousif NMK, Franz CMAP, Holzapfel WH, Rubén PP, Martínez-Canámero M, Gálvez A (2004) Functional and Safety Aspects of Enterococci Isolated from Different Spanish Foods. *Systematic and Appl Microbiol* 27:118-130.

Pavia M, Nobile CGA, Salpietro L, Angelillo IF (2000) Vancomycin Resistance and Antibiotic Susceptibility of Enterococci in Raw Meat. *J Food Prot* 63:912-915.

Peters J, Mac K, Wichmann-Schauer H, Klein G, Ellerbroek L (2003) Species distribution and antibiotic resistance patterns of enterococci isolated from food of animal origin in Germany. *Int J Food Microbiol* 88:311-314.

Petersen A, Dalsgaard A (2003) Antimicrobial resistance of intestinal *Aeromonas* spp. and *Enterococcus* spp. in fish cultured in integrated broiler-fish farms in Thailand. *Aquaculture* 219:71-82.

Poeta P, Costa D, Igrejas G, Rodrigues J, Torres C (2007) Phenotypic and genotypic characterization of antimicrobial resistance in faecal enterococci from wild boars (*Sus scrofa*). *Veterinary Microbiol* 125:368-374.

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 Antimicrobial Agents* 27:131-137.

Quigg A, Broach L, Denton W, Miranda R (2009) Water quality in the Dickinson Bayou watershed (Texas, Gulf of Mexico) and health issues. *Marine Pollution Bulletin* 58:896-904.

Reid K C, Cockerill III FR, Patel R (2001) Clinical and epidemiological features of *Enterococcus casseliflavus/flavescens* and *Enterococcus gallinarum* bacteremia: a report of 20 cases. *Clin Infect Dis* 32:1540-1546.

Rice E, Messer J, Johnson C, Reasoner D (1995) Occurrence of high-level aminoglycoside resistance in environmental isolates of enterococci. *Appl Environ Microbiol* 61:374-376.

Sarantinopoulos P, Andriguetto C, Georgalaki MD, Rea MC, Lombardi A, Cogan TM, Kalantzopoulos G, Tsakalidou E (2001) Biochemical properties of enterococci relevant to their technological performance. *Int Dairy J* 11:621-647.

Sergelidis D, Ibrahim A, Anagnostou V, Papa A, Papadopoulos T (2010) Prevalence and antimicrobial susceptibility of *Enterococcus* spp. in ready-to-eat salads (dips), the environment and the personnel of a salad processing plant in Northern Greece. *J. Hellenic Vet Med Soc* 61:308-315.

Silla Santos MH (1996) Biogenic amines: Their importance in foods. *Int J Food Microbiol* 29:213-231.

Tomé E, Pereira VL, Lopes CI, Gibbs PA, Teixeira PC (2008) In vitro tests of suitability of bacteriocin-producing lactic acid bacteria, as potential biopreservation cultures in vacuum-packaged cold-smoked salmon. *Food Control* 19:535-543.

Toye B, Shymanski J, Bobrowska M, Woods W, Ramotar K (1997) Clinical and epidemiological significance of enterococci intrinsically resistant to vancomycin (possessing the vanC genotype). *J Clin Microbiol* 35:3166-3170.

Tuncer Y (2009) Some technological properties of phenotypically identified enterococci strains isolated from Turkish tulum cheese. *African J Biotechnol* 8:7008-7016.

Valenzuela AS, Benomar N, Abriouel H, Cañamero MM, Gálvez A (2010) Isolation and identification of *Enterococcus faecium* from seafoods: Antimicrobial resistance and production of bacteriocin-like substances. *Food Microbiology* 27:955-961.

Wilson IG, McAfee GG (2002) Vancomycin-resistant enterococci in shellfish, unchlorinated waters, and chicken. *Int J Food Microbiol* 79:143-151.