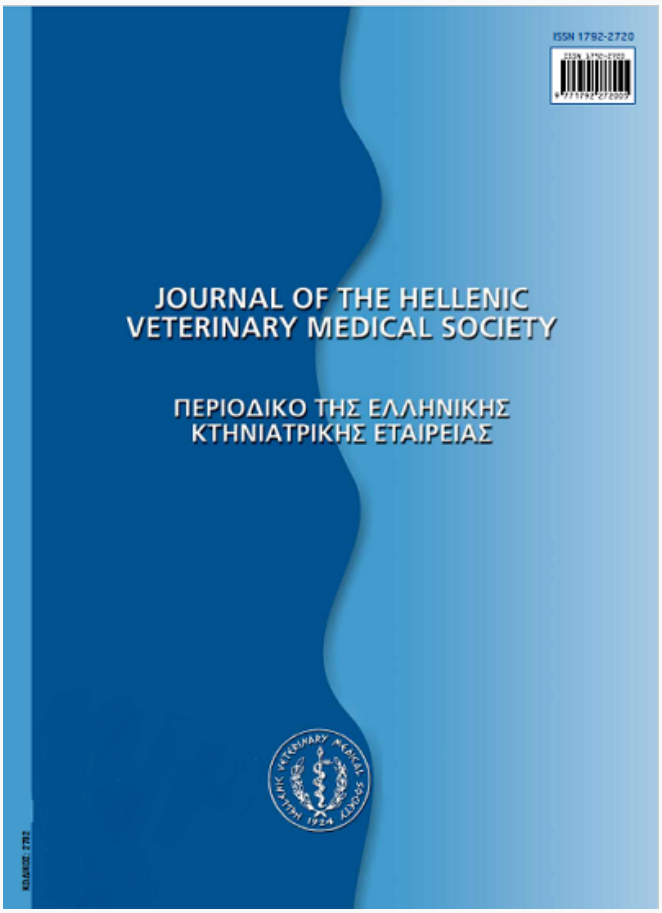
Journal of the Hellenic Veterinary Medical Society

Vol 64, No 4 (2013)



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

D. SERGELIDIS (Δ. ΣΕΡΓΚΕΛΙΔΗΣ), A. ABRAHIM (Α. ΑΜΠΡΑΧΙΜ), T. PAPADOPOULOS (Θ. ΠΑΠΑΔΟΠΟΥΛΟΣ), J. KIRKOUDIS (Ι. ΚΥΡΚΟΥΔΗΣ), V. ANAGNOSTOU (Β. ΑΝΑΓΝΩΣΤΟΥ), A. PAPAVERGOU (Α. ΠΑΠΑΒΕΡΓΟΥ), A. PAPA (Α. ΠΑΠΑ)

doi: 10.12681/jhvms.15503

J HELLENIC VET MED SOC 2013, 64(4): 239-248
ΠΕΚΕ 2013, 64(4): 239-248

Research article
Ερευνητικό άρθρο

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

Sergelidis D.[1], Abrahim A.[1], Papadopoulos T.[2], Kirkoudis J.[3], Anagnostou V.[4], Papavergou A.[1], Papa A.[4]

[1]Department of Hygiene and Technology of Foods of Animal Origin, School of Veterinary Medicine, Aristotle University of Thessaloniki. 54124 Thessaloniki, Greece

[2]National Agricultural Research Foundation, Veterinary Research Institute of Thessaloniki, NAGREF Campus of Thermi, 57001 Thermi, Greece

[3]Laboratory of Ecology-Wildlife Management, Depart ment of Forestry and Environmental Management and Natural Resources, Democritus University of Thrace, 68200 Orestiada, Greece

[4]Laboratory of Microbiology, Medical School, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.

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

Σεργκελίδης Δ.[1], Αμπραχίμ Α.[1], Παπαδόπουλος Θ.[2], Κιρκούδης Ι.[3], Αναγνώστου Β.[4], Παπαβέργου Α.[1], Παπά Α.[4]

[1]Τομέας Υγιεινής και Τεχνολογίας Τροφίμων Ζωικής Προέλευσης, Τμήμα Κτηνιατρικής, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, 54124 Θεσσαλονίκη

[2]Εθνικό Ίδρυμα Αγροτικής Έρευνας, Ινστιτούτο Κτηνιατρικών Ερευνών Θεσσαλονίκης, 57001 Θέρμη

[3]Εργαστήριο Οικολογίας-Διαχείρισης Άγριας Ζωής, Τμήμα Δασολογίας και Διαχείρισης Περιβάλλοντος και Φυσικών Πόρων, Δημοκρίτειο Πανεπιστήμιο Θράκης, 68200 Ορεστιάδα

[4]Ά Εργαστήριο Μικροβιολογίας, Τμήμα Ιατρικής, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, 54124 Θεσσαλονίκη

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 recoreded to quinupristin/

Correspondence: D. Sergelidis,
Department of Hygiene and Technology of Foods of Animal Origin, School of Veterinary Medicine, Aristotle University of Thessaloniki. 54124 Thessaloniki, Greece. E-mail: dsergkel@vet.auth.gr

Αλληλογραφία: Δ. Σεργκελίδης,
Τομέας Υγιεινής και Τεχνολογίας Τροφίμων Ζωικής Προέλευσης, Κτηνιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης. 54124 Θεσσαλονίκη, Ελλάδα. E-mail:dsergkel@vet.auth.gr

Date of initial submission: 03 July 2013
Date of revised submission: 23 August 2013
Date of acceptance: 28 August 2013

Ημερομηνία αρχικής υποβολής: 03 Ιουλίου 2013
Ημερομηνία αναθεωρημένης υποβολής: 23 Αυγούστου 2013
Ημερομηνία αποδοχής: 28 Αυγούστου 2013

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 ευαισθησία 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 εντερόκοκκοι 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 ευαισθησία 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 πενικιλλίνη, στις κεφαλοσπορίνες και στην ερυθρομυκίνη. Σχετικά αυξημένη συχνότητα παρατηρήθηκε στην κινουπριστίνη/δαλφοπριστίνη και τη λινεζολίδη, φάρμακα χρησιμοποιούμενα στη θεραπευτική αγωγή λοιμώξεων από ανθεκτικούς στη βανκομυκίνη εντερόκοκκους. 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 ανιχνεύτηκαν γονίδια αντοχής στη βανκομυκίνη. Όλα τα στελέχη *E. faecium* και ένα τρίτο των στελεχών *E. casseliflavus* είχαν την ικανότητα αποκαρβοξυλίωσης 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 εντερόκοκκων σε ανθρώπους.

Λέξεις ευρετηρίασης: αντιμικροβιακή ευαισθησία, 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 Dalsgaard, 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. flavescens*), lincosamides, polymycines, 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 cm^2 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 cm^2 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	
		E. faecium	*E. casseliflavus*
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
E. faecium	AK, CTX, CZ, DA, CN, CN500, L, OX, RA (I), SXT	Yes	Rainbow trout
E. faecium	AK, CTX, CZ, CN, LEV, OX, RA, S1000, SXT	Yes	Rainbow trout
E. faecium	AK, CTX,CZ, DA, CN, OX, RA (I), SXT	Yes	Rainbow trout
E. faecium	AK, CTX, CZ, DA, FF, CN, OX, SXT	Yes	Rainbow trout
E. faecium	AK, CTX, CZ, DA (I), CN, CN500, LEV, L, OX, P, RA, S1000, SXT	Yes	Gibel carp
E. faecium	AK, CTX, CZ, DA, E, CN, CN500, LEV, L, OX, P, RA, S1000, SXT	Yes	Gibel carp
E. faecium	AK, CTX, CZ, DA, CN, LEV, L, OX, SXT	Yes	Gibel carp
E. faecium	AK, CTX, CZ, E, CN, LEV, OX, P, RA (I), SXT	Yes	Gibel carp
E. faecium	AK, CTX, CZ, DA, FF, CN, OX, RA, SXT	Yes	Gibel carp
E. faecium	AM, CTX, CZ, DA, E, CN, P	Yes	Personnel
E. faecium	AM, P	Yes	Personnel
E. faecium	CTX, CZ, DA, E, OX, P, RA	Yes	Personnel
E. faecium	CTX, CZ, DA, E, CN, LEV, OX, SXT	Yes	Personnel
E. faecium	AK, CTX, CZ, DA, E, CN, LEV, L, OX, Q/D, SXT	Yes	Equipment
E. faecium	AK, CTX, CZ, DA, CN, OX, Q/D (I), SXT, VA (I)	Yes	Equipment
E. faecium	AK, CTX, CZ, CL (I), E (I), FF, CN, LEV, L, OX, SXT	Yes	Equipment
E. faecium	AK, CZ, CFT, DA, E, CN, OX, Q/D (I), RA, SXT	Yes	Equipment
E. faecium	AK, CTX, CZ, E (I), FF, CN, LEV, OX, RA (I), SXT	Yes	Equipment
E. faecium	AK, CTX, CZ, DA, CN, LEV, L, OX, Q/D (I), SXT	Yes	Equipment
E. faecium	AK, CTX, CZ, E, CN, LEV, LIN, OX, SXT	Yes	Equipment
E. casseliflavus	AK, CTX, CZ, DA, E, FF, CN, LEV, OX, Q/D, RA, S1000, SXT	No	Rainbow trout
E. casseliflavus	AK, CTX, CZ, DA, FF, CN, OX, Q/D, S1000, SXT	Yes	Rainbow trout
E. casseliflavus	AK, CTX, CZ, DA, CN, OX, RA (I), S1000, SXT	No	Rainbow trout
E. casseliflavus	AK, CTX, CZ, DA, CN, OX, Q/D, RA (I), SXT	Yes	Equipment
E. casseliflavus	AK, CTX, CZ, DA, E (I), CN, LEV (I), OX, Q/D (I), RA, SXT, VA (I)	No	Equipment
E. casseliflavus	AK, CTX, CZ, C, 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 *Enterococcus* spp.				
	E. faecium			*E. casseliflavus*	
	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; Mejlholm 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.

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