ANTIBIOTIC RESISTANCE OF ENTEROCOCCI ISOLATED FROM FROZEN FOODS AND ENVIRONMENTAL WATER

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Abstract. We evaluated 239 isolates of enterococci (113 from frozen foods and 126 from environmental water) for their resistance to 8 antibiotics by agar disk diffusion method. Most isolates from both sources were resistant to tetracycline (64.1% food strains; 46.8% water strains) and ciprofloxacin (53.4% food strains; 48.4% water strains). A relatively high prevalence of chloramphenicol, trimethoprim-sulfamethoxazole and vancomycin resistance was present, ranging from 9.7 to 27.2% for food strains and 10.3 to 15.9% for water strains; while other drug resistance (ampicillin, gentamicin and teicoplanin) was minimal (≤ 0.9% for food strains; ≤ 1.6% for water strains). No significant differences in resistant rates between the two sources were found for any of the drugs ($p>0.05$) except tetracycline ($p<0.05$). The majority of isolates from both sources were multi-resistant strains (50% for food strains and 42% for water strains). Most of them showed resistance to two drugs. There was no significant difference in the non-resistance patterns and the multidrug resistance patterns ($p>0.05$) between the frozen food and environmental water strains, but a significant difference was seen in the single drug resistance pattern ($p<0.05$). Vancomycin resistant enterococci (VRE) were isolated from nearly all sources studied, 9.7% food isolates and 10.3% water isolates, with no significant difference between the two sources ($p>0.05$). This study shows a high prevalence of multidrug resistance among enterococci isolated from foods of animal origin and environmental water. This may serve as a potential transfer route of antibiotic-resistant bacteria and resistant genes into the human food-chain and environment which could potentially pose a health threat to humans in the future. The use of antibiotics for purposes other than human health, *ie* in animal feeds and in the treatment of infection in animals, should be reduced and eventually eliminated. Improved hygiene practices and controlled use of antibiotics in agriculture, animal husbandry, and fisheries are desirable for environmental management and public health protection.

INTRODUCTION

During the past decade, enterococci have emerged as important human pathogens in both nosocomial- and community-acquired infections (Murray, 1997; Edmond *et al*, 1999; Vergis *et al*, 2001). Of increasing concern is the rising antibiotic resistance of enterococci throughout the world, creating a serious problem concerning the effective therapy of enterococcal infection. Enterococci belong to the normal flora of humans and animals and are intrinsically resistant to many antibiotics, including beta-lactams and clinically available levels of aminoglycosides. In addition, they demonstrate a remarkable ability to acquire resistance to antibiotics through exchange of resistance-encoding genes carried on transposons and plasmids (Rice *et al*, 1995).

The acquisition of vancomycin resistance by enterococci has had serious implications for treatment and infection control of these organisms because vancomycin-resistant enterococci (VRE) are often resistant to all antibiotics effective for treating infections caused by more susceptible enterococci (Murray, 1997). The potential for transferring of VRE genes from enterococci to *Staphylococcus aureus* and pneumococci increase the importance of finding ways to limit the spread of VRE.

The emergence of VRE has been encour-

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aged by the increased use of glycopeptides in hospitals for the treatment of infections due to staphylococci and *Clostridium difficile*, and of aminoglycosides, cephalosporins, and quinolones for infections due to gram-negative bacteria. In Western Europe, the prevalence of VRE may be due to the widespread use of avoparcin in animal husbandry at sub-therapeutic levels as antimicrobial growth promoters (Bates *et al*, 1994). This antibiotic is a vancomycin analogue, that is not used in human medicine but in the livestock industry. Avoparcin is a substance which farmers mix in feed meal in order to speed up the maturity of animals. Use of avoparcin in food animals provides selective pressure that contributes to the increasing prevalence of transferable resistance genes to glycopeptides in enterococci in animals. These organisms are spread via the food chain as indicated by some data that raw poultry and raw minced meat harbored VRE (Bates *et al*, 1994; Bager *et al*, 1997; Klein *et al*, 1998). More recently, an investigation in Japan of imported chicken revealed a high level (MIC ≥ 128 μg/ml) of VRE isolated from 3 of 14 samples and 9 of 43 samples from Thailand in 1998 and 1999, respectively (Ike *et al*, 1999). In order to ensure the export quality of frozen chickens, the Thai Agriculture and Cooperative Ministry had banned avoparcin as an ingredient in animal feed meal. However, several other powerful antibiotics, generally used for human medicine, are still mixed in these animal feed meals.

Thus the aim of this study was to investigate the enterococci contamination of environmental water and food of animal origin, and to determine the prevalence of antibiotic resistance among these enterococci isolates in order to provide some information on the emergence of resistant enterococci in environmental water and food of animal origin.

MATERIALS AND METHODS

Samples and collection

A total of 228 samples for isolation of enterococci were obtained from two main sources: foods of animal origin (n = 103) and water samples (n = 125). A total of 103 frozen food samples comprised of frozen chicken (n =50) and frozen shrimp (n = 53, 23 cooked shrimp and 30 uncooked shrimp) were collected from frozen food factories in Bangkok and Samut Prakan Provinces. All food samples were separately collected in a gas sterilized plastic bag, kept in a dry ice box and then transferred to the laboratory as soon as possible.

A total of 125 water samples were obtained from various sources, including water from agricultural wells on animal farms (n = 50), rivers and canals (n = 25), piped water (n = 25) and bottled drinking water (n = 25). The water samples were collected using aseptic technique avoiding sample contamination. Each water sample consisted of 100 ml collected in a sterilized glass bottle, placed on ice and transported to the laboratory within 2 hours of collection. The analyses were completed within 6 hours of collection.

Isolation of enterococci

Frozen foods. To isolate the enterococci, a 50-g portion of food sample was placed in 50 ml of buffered peptone water (Merck) and homogenized with a stomacher for 1 minute. Then 0.1 ml of the diluted sample was spread onto Enterococcus (EC) agar (Merck). The agar plates were incubated aerobically at 37°C for 24 hours. Typical pink or maroon colonies were counted and the densities in CFU (colony forming unit) were recorded per gram of food sample. Two typical colonies were randomly selected for further verification at the species level.

Environmental water. A membrane filter (MF) technique was used for analysis of enterococci in bottled drinking water and piped water according to standard methods (APHA, 1999). A volume of 100 ml of each water sample was filtered through a sterile membrane filter (pore size of 0.45 μm) on which bacteria were trapped, then the filter was placed on a plate of membrane enterococcus agar (Merck). After incubation, typical pink or maroon colonies were counted and the densities of enterococci in CFU were recorded per 1 ml of water. Two typical colonies were randomly selected for further verification at the species level.

The isolation of enterococci from canals, rivers and agricutural wells was accomplished by using a spread plate technique. The water

sample was first diluted in suitable dilutions and 0.1 ml of each dilution was spread onto EC agar. The method described for the frozen food samples was then followed and the densities of enterococci in CFU were recorded per 1 ml of water. A MF technique was used for analysis of water samples from shrimp farms which usually contain few enterococci. The water sample was first diluted in suitable dilutions and 10 ml of each dilution was filtered, then the method described for drinking water was followed.

Species identification

A single well isolated colony from secondary subculture on blood agar plate was evaluated by Gram stain to confirm the presence of gram-positive cocci. The following tests were then performed: the catalase test, the bile esculin test and growth at 10°C and 45°C in the presence of 6.5% NaCl. The colonies that were catalase negative and able to hydrolyze esculin in the presence of 40% bile salt and able to grow at 10°C and 45°C in 6.5% NaCl at 37°C were classified as group D enterococci. Strains were identified to the species level by biochemical tests following the recommendations of Facklam *et al* (1999). Two to three identical colonies were kept in 1 ml of trypticase soy broth with 20% glycerol and stored at -70°C until use for further study.

Antibiotic susceptibility test

A single disk diffusion method as described by NCCLS (1999) was used for susceptibility testing of all enterococci isolates. The following commercial disks (Oxoid) were employed: ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (30 μg), gentamicin (120 μg), teicoplanin (30 μg), tetracycline (30 μg) trimethoprim-sulfamethoxazole (1.25/23.75 μg) and vancomycin (30 μg). The organism was adjusted to a viable count of $1X10^8$ cfu ml^{-1}. This inoculum was spread uniformly over the entire surface of the Mueller-Hinton agar plate (Merck). After 24 hours of incubation at 37°C, the diameters of inhibition zone produced around the disks were measured, and compared to those in an interpretive table (NCCLS, 1999). In addition, vancomycin susceptibility testing was performed using an E-test (AB Biodisk, Solna, Sweden) as described by the manufacturer.

Entercoccus faecalis ATCC 29212, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 were used as control organisms. The tests with these control organisms followed the same procedures as the test strains. If the zone diameters obtained for each antibiotic were within the acceptable range, the control was satisfied.

RESULTS

Of the 228 samples examined, a total of 239 enterococcal isolates were obtained, 113 isolates (47.3%) were from frozen foods, while 126 isolates (52.7%) were from environmental water. The number of enterococcal isolates obtained from these samples is shown in Table 1. Of the 239 isolates of enterococci obtained, 60 isolates (25.1%) were recovered from frozen chicken, 53 isolates (22.2%) from frozen shrimp, 45 isolates (18.8%) from the water of shrimp farms, 41 isolates (17.2%) from the water of chicken farms, and 40 isolates (16.7%) from the water of rivers and canals. Enterococci were not recovered from any of the 50 samples of bottled drinking water and piped water analyzed. Of 53 isolates from frozen shrimp, most were recovered from uncooked samples. Forty-one isolates (17.2%) were from uncooked samples and 12 isolates (5.0%) were from pasteurized samples.

Of the 239 isolates of enterococci investigated, 114 (47.7%) were identified as *E. faecalis*, 61 (25.5%) were *E. faecium,* 14 (5.9%) were *E. durans* and *E. raffinosus*, and 36 (15.0%) were other species (comprised of 7 *E. avium,* 3 *E. malladoratus*, 1 *E. classeliflavus*, 6 *E. hirae*, 4 *E. gallinarum,* and 15 unidentified *Enterococcus* spp) (Table 2). *E. faecalis* was the most common species isolated from both frozen foods and environmental water samples, comprising 69 (28.9%) frozen foods and 45 (18.8%) environmental water samples. *E. faecium* was ranked the second most recovered, 21 (8.8%) from frozen foods and 40 (16.7%) from environmental water samples. The majority of *E. faecalis* was from frozen foods, especially frozen chicken (18.4%), while *E. faecium* was from environmental water. Other species were recovered much less often than *E. faecalis* and *E. faecium,* ac-

Table 1
Number and percentage of samples and enterococci isolated from frozen foods and environmental water.

Sources of samples	No. of sample (%)	No. of isolate (%)
Frozen foods	103 (45.2)	113 (47.3)
Chicken	50 (22.0)	60 (25.1)
Shrimp	53 (23.2)	53 (22.2)
Cooked	23 (10.0)	12 (5.0)
Uncooked	30 (13.2)	41 (17.2)
Environmental water	125 (54.8)	126 (52.7)
Piped	25 (10.9)	0 (0.0)
Bottled drinking	25 (10.9)	0 (0.0)
Chicken farms	25 (10.9)	41 (17.2)
Shrimp farms	25 (10.9)	45 (18.8)
Rivers and canals	25 (10.9)	40 (16.7)
Total	228 (100.0)	239 (100.0)

Table 2
Species identification of enterococci isolated from frozen foods and various environmental water sources.

Species	No. of isolates (%) obtained from:		Total
	Frozen foods	Environmental water	
E. faecalis	69 (28.9)	45 (18.8)	114 (47.7)
E. faecium	21 (8.8)	40 (16.7)	61 (25.5)
E. durans	10 (4.2)	4 (1.7)	14 (5.9)
E. raffinosus	2 (0.8)	12 (5.1)	14 (5.9)
Other species[a]	11 (4.6)	25 (10.5)	36 (15.0)
Total	113 (47.3)	126 (52.8)	239 (100.0)

[a] Other species including *E. avium, E. mallodoratus, E. hirae, E. gallinarum* and unknown species.

Table 3
Prevalence of antibiotic-resistant enterococci among 113 isolates from frozen foods and 126 isolates from environmental water samples.

Antibiotic	Number (%) of isolates showing resistance			p-value
	Total(n=239)	Frozen foods(n=113)	Water(n=126)	
Ampicillin	2 (0.8)	0 (0.0)	2 (1.6)	0.277[a]
Chloramphenicol	48 (20.0)	28 (27.2)	20 (15.9)	0.495
Ciprofloxacin	117 (48.5)	55 (53.4)	61 (48.4)	0.430
Gentamicin (high-level)	3 (1.3)	1 (0.9)	2 (1.6)	0.541[a]
Tetracycline	125 (52.3)	66 (64.1)	59 (46.8)	0.036
Teicoplanin	1 (0.4)	1 (0.9)	0 (0.0)	0.473[a]
Trimethoprim-sulfamethoxazole	35 (14.6)	17 (16.5)	18 (14.3)	0.434
Vancomycin	23 (9.6)	10 (9.7)	13 (10.3)	0.350

[a] Fisher exact test

Table 4
Frequency distribution of the antibiotic resistance patterns of enterococci isolated from frozen food and environmental water.

Antibiotic resistance pattern	% of isolates with antibiotic resistance patterns			p-value[a]
	Total n=239	Frozen food isolates n=113	Environmental water isolates n=126	
No resistance	80 (33.5)	41 (36.3)	39 (31.0)	0.191
Single resistance	49 (20.5)	15 (13.3)	34 (27.0)	0.004
Multiple resistance	110 (46.02)	58 (50.4)	52 (42.0)	0.097
2	58 (24.3)	30 (26.5)	28 (22.2)	
3	32 (13.4)	15 (13.3)	17 (13.5)	
4	17 (7.0)	11 (9.7)	6 (4.7)	
5	3 (1.3)	1 (1.1)	2 (1.6)	

n, number of isolates
[a] p-value by χ^2 test, df = 1

counting for less than 6% of all isolates.

Antibiotic susceptibility results

Prevalence of resistance. Antibiotic susceptibility results (Table 3) revealed that most isolates from both frozen foods and environmental water were resistant to tetracycline (52.3%) and ciprofloxacin (48.5%). In addition, the rates of resistant to other antibiotics were in the range of 0.4-20%, with the rate of resistant to teicoplanin being the lowest (0.4%).

A high prevalence of resistance in the samples for all frozen foods and environmental water was observed for ciprofloxacin (53.4% food strains; 48.4% aquatic strains) and tetracycline (64.1% food strains; 46.8% aquatic strains). A relatively high prevalence of resistance was seen for chloramphenicol, trimethoprim-sulfamethoxazole and vancomycin (ranged from 9.7 to 27.2% for food strains; 10.3 to 15.9% for aquatic strains). For other compounds the prevalence was less than 0.9%. Teicoplanin resistance was present in only one sample from frozen foods (0.9%). No teicoplanin-resistant enterococci were found in the environmental water samples. Ampicillin-resistance was present in only two (1.6%) samples from water samples, but no ampicillin-resistant enterococci were found in the food samples.

The prevalence of high-level gentamicin resistance in food strains and aquatic strains ranged from 0.9 to 1.6%; while the prevalence of resistance to vancomycin ranged from 9.7 to 10.3%. No significant differences in rates of resistance between the food and aquatic strains were found for any of the drugs ($p > 0.05$) except tetracycline ($p < 0.05$).

For all the tested antibiotics combined, the proportion of resistant enterococci in both the food and water samples with a high degree of resistance was approximately 50% (range 48-52%) and for a moderate degree of resistance was 15% (range 9.6-20%), and a low degree of resistance was 0.8% (range 0.4 -1.3%).

The prevalence of antibiotic-resistant enterococci was also subdivided according to food and water sources (data not shown). In frozen chicken: 81.7% were resistant to tetracycline, followed by 63.3% to ciprofloxacin, and 23.3, 18.3, 6.7 and 1.7%, to chloramphenicol, trimethoprim-sulfamethoxazole, vancomycin, and teicoplanin, respectively. No ampicillin- or high level gentamicin-resistant enterococci were found. In frozen shrimp, most were resistant to ciprofloxacin and tetracycline equally (32.1%), followed by resistance to cotrimoxazole, vancomycin, and chloramphenicol (range 7.5-11.3%). High level gentamicin-resistant enterococci were found in 1.9%. No ampicillin- or teicoplanin-re-

sistant enterococci were found in the frozen shrimp.

In water from chicken farms, most were resistant to tetracycline and ciprofloxacin (75.6% and 63.4%), 34.1% were resistant to trimethoprim-sulfamethoxazole, followed by chloramphenicol, vancomycin, and high level resistance to gentamicin (26.8, 7.3 and 4.9%, respectively). No ampicillin- or teicoplanin-resistant enterococci were found. In water from shrimp farms, most (46.7%) were resistant to ciprofloxacin, 31.1% to tetracycline, 17.8% to vancomycin, 15.6% to chloramphenicol, and 8.9% to trimethoprim-sulfamethoxazole. No resistance to ampicillin, teicoplanin or high level resistance to gentamicin were observed. In rivers and canals, the majority of isolates (35%) were resistant to both ciprofloxacin and tetracycline, but only 5% to ampicillin, chloramphenicol and vancomycin. No trimethoprim-sulfamethoxazole, teicoplanin or high level resistance to gentamicin were found.

Distribution of resistance determinants

Most enterococci (66.5%) were resistant to one or more drugs tested, while 33.5% of isolates had no resistance. Of the 66.5% of antibiotic resistant strains, 46.0% were multi-resistant (resistant to two or more antibiotics), and 20.5% were single drug resistant. The frequency of drug resistance in these strains is shown in Table 4, which ranged from one to five drugs. Most (24.3%) showed resistance to two drugs, 20.5% showed resistance to one drug, 13.4% to 3 drugs, 7.0% to four drugs, and 1.3% to five drugs. When the distribution of resistance was compared, there was a significant difference ($p<0.05$) in the isolates with one drug resistance and no significantly difference ($p>0.05$) in the isolates with no resistance and multidrug resistance between the two sources isolates.

Resistance patterns

One hundred fifty-nine of 239 isolates of enterococci demonstrated 29 different kinds of resistance patterns, 72 of 113 frozen food isolates showed 15 patterns and 87 of 126 environmental water isolates showed 20 patterns. While 41 of 113 frozen food isolates and 39 of 126 environmental water isolates had no resistance patterns (data not shown).

DISCUSSION

As normal gastrointestinal flora of humans and animals, enterococci have been traditionally considered as indicators of fecal contamination in water (Godfree *et al*, 1997). They are now well-recognized nosocomial pathogens that can cause variety of infections in hospitalized patients. Recent attention has focused on enterococci because of their remarkable and increasing resistance to many antibiotics. The organisms have been frequently recovered from environmental sources, both in and out of the hospital. Release of antibiotic-resistant enterococci in the community is therefore of particular concern since they might proliferate in soil and surface water (Iversen *et al*, 2004; Vilanova *et al*, 2004), transferring antibiotic resistance genes to different species (Harwood *et al*, 2001). The increased use of antibiotics in farming, together with the practice of raw sewage discharge into surface water, has resulted in a significant increase in the number of antibiotic resistant bacteria present in aquatic environments (Young, 1993).

In this study, a cross-sectional design was utilized to investigate the species prevalence of enterococci isolated from frozen foods and environmental water; it assessed the occurrence of antibiotic resistance in these enterococcal isolates. The focus was on frozen shrimp and chicken, various brands of bottled drinking water, piped water, water from chicken and shrimp farms, from canals and rivers and agricultural runoff wells. Antibiotic resistance among these isolates was compared in order to evaluate the penetration of antibiotic resistant enterococcal strains in farms, food animal populations and the subsequent risk of transfer to humans.

In this study, enterococci was isolated from a wide variety of sources, even foods of animal origin kept frozen for a long time. This may due to their ability to grow and survive under harsh conditions. There is little data on antibiotic resistance among environmental isolates of enterococci in Thailand, since most reports on antibiotic susceptibility are for bacteria isolated from

patients or from sick and dying animals with few from bacteria isolated from healthy humans, animals or foods of animal origin.

Large numbers of bacteria, usually found in the large intestine, may be exposed to antibiotics, exchange genetic material with other bacteria, and on excretion contaminate the environment or colonize other animals and humans. The intestinal flora of animals and humans are considered to be the most important reservoir of resistant bacteria and resistance genes. As contamination of carcasses with fecal flora during slaughtering inevitably occurs, foods of animal origin may serve as a vehicle to transport resistant bacteria and genes between animals and humans (Khachatourians, 1998).

There has been considerable interest in the role of antibiotics for growth promotion and therapy of infections in food-producing animals, especially as a potential transfer route of antibiotic-resistant pathogens, or the genes encoding antibiotic resistance, into the human food chain. In this study we sought to examine the susceptibility of enterococci isolated from foods of animal origin and their water environment, to antibiotics commonly used in human medicine.

The present study showed that the strains of enterococci isolated from environmental water and food had similar susceptibilities to antibiotics ($p>0.05$). The proportion of enterococci in both food and water samples with a high level of resistance was approximately 50%, with a moderate degree of resistance was 15%, and a low degree of resistance was 0.8%. No significant differences in prevalences and rates of resistance between the food and aquatic strains were found for any of the drugs ($p > 0.05$) except tetracycline. A study by van den Bogaard *et al* (2000) showed the enterococci in pig fecal samples with a high level of resistance was approximately 80% (range 73 - 86%); the samples with a low level of resistance varied from 2% to 28% (mean 17%). A study by Knudtson and Hartman (1993) found water isolates were more resistant than either pork or clinical isolates to all cephalosporins, amikacin, gentamicin, imipenam and rifampin. The possibility exists that some of the water samples contain animal or human sewage runoff, which may explain some of the resistance patterns seen in these samples.

In the present study, enterococci were undetected in piped water and bottled drinking water, indicating an effective treatment process for drinking water, particularly disinfection with chlorine or filtration, which can be used to destroy or remove these organisms. Pasteurization was also able to reduce the frequency and concentration of enterococci (data not shown).

Enterococci were least detected in water samples of shrimp farms. This may be explained by the widespread use of several broad spectrum antibiotics (ciprofloxacin, norfloxacin, sulfamethoxazole-trimethoprim, tetracycline) in shrimp feed by shrimp farms found during our collection of these water samples.

The species prevalence of enterococci among food isolates and environmental water isolates in this study was similar to those found in most clinical isolates of enterococci, where *E. faecalis* and *E. faecium* were the two most common species, comprising 80-90% and 5-10% of the species, respectively (Murray *et al*, 1998). There are increasing numbers of reports of infections due to other species of enterococci (Moellering, 1992). In this study, *E. durans* and *E. raffinosus* were also detected among food isolates and environmental water isolates but much less often than *E. faecalis* and *E. faecium.*

Antibiotic susceptibility testing showed most enterococcal isolates from both frozen foods and environmental water were generally resistant to two antibiotics commonly used in humans, tetracycline and ciprofloxacin, while resistance to other drugs tested was minimal. Tetracycline and ciprofloxacin are common drugs used to treat and prevent animal diseases. We found a significantly greater incidence of resistance to these antibiotics, which is probably related to prolonged exposure. Van den Bogaard *et al* (2000) also found enterococcal isolates from pig feces had a high degree of resistance to erythromycin and oxytetracycline: 70% and 46%, respectively.

The prevalence of vancomycin resistant enterococci (VRE) was 9.6% (23 of 239 strains tested). These VRE strains were isolated from nearly all sources studied, 4 and 6 strains from

frozen chicken and frozen shrimp, respectively, 3 and 8 strains from water of chicken and shrimp farms, respectively, and 2 strains from river and canals. The rates of vancomycin resistance for strains isolated from foods and aquatic sources (9.7% food strains; 10.3% aquatic strains) were not statistically significant different ($p>0.05$).

In a study by Klein *et al* (1998) regarding the occurrence of VRE in raw minced beef and pork, a total of 34 VRE strains were isolated; 38% VRE isolates were identified as *E. faecium*, 35% were *E. faecalis.* Other species were members of the *E. faecium* group. All VRE isolated from beef and pork exhibited intermediate resistance or were resistant to methicillin, cephalothin, vancomycin, erythromycin, clindamycin, avoparcin, virginiamycin and tylosin. The present study also is in agreement with Klein *et al* (1998), of VRE from food isolates, 4 of 10 strains (40%) were also resistant to all 3 drugs tested, ciprofloxacin, cotrimoxazole and tetracycline, while other strains were resistant to at least to 1-4 drugs.

VRE in imported chicken investigated in 1998 and 1999 (lke *et al*, 1999) were commonly found in 2 species, *E. faecalis*, and *E. durans.* In 1998, high level VRE (MIC > 128 $\mu g\ ml^{-1}$) was isolated from 3 of 14 (21%) samples (2 samples were identified as *E. faecalis*, and 1 sample as *E. durans*) and in 1999 VRE was isolated from 9 of 43 (21%) samples (3 were identified as *E. faecalis*, and 6 as *E. durans*). In our study, VRE was isolated from 4 of 50 (8%) exported frozen chicken samples and were identified as *E. faecalis.*

At present, there is little data regarding VRE resistance among environmental isolates of enterococci, since most reports of antibiotic susceptibilities are for bacteria isolated from humans. This study shows that extensive agricultural use of glycopeptides or other antibiotics (tetracycline, cotrimoxazole, ciprofloxacin) in chicken or shrimp feed has created an animal reservoir of resistant enterococci which may lead to more enterococci species resistant to glycopeptides and other antibiotics in animals and complicates the control of such infections.

High-level aminoglycoside resistance is of great clinical importance because it eliminates the synergism between the affected aminoglycosides and β-lactams or glycopeptide (Murray, 1990). In this study, two water isolates and one frozen food isolate were also found to exhibit high level resistance to gentamicin. High-level resistance to aminoglycosides (HLAR) results when enterococci acquire plasmids coding for aminoglycoside-modifying enzymes or develop ribosomal mutations (Patterson and Zervos, 1990). This study indicates that enterococci which exhibit HLAR are not limited to the clinical setting, but may be recovered from food animals and variety of aquatic environmental sources. The increasing prevalence of HLAR enterococci associated with nosocomial disease and the current finding of these bacteria in environmental samples should be a matter of concern for both clinician and public health authorities.

In conclusion, this study indicates a high prevalence of multidrug resistance among enterococci isolated from food of animal origin and water environments that may serve as a vehicle to transport these resistant bacteria and genes from animals to humans and become a serious threat to public health in the future. The prevalence and level of antibiotic resistance found in the fecal flora of humans and animals are considered to be good indicators of selective pressure caused by antibiotic usage. In food animals, a low prevalence and level of antibiotic resistance in the intestinal flora should be considered a distinguishing quality and safety mark. The use of antibiotics for purposes other than human use, in animal feed and in the treatment of infection in animals, should be reduced and eventually eliminated. Improvement in hygiene practices and controlled use of antibiotics in agriculture, animal husbandry, and fisheries is desirable for environmental management and public health protection.

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