

## First isolation of *Enterococcus* strains in pig faeces in Turkey and determination of antibiotic susceptibilities

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### Abstract

In this trial, *Enterococcus* strains were isolated from a total of 69 faecal samples obtained from 238 pigs (< 6 months old and 133 > 6 months old) on three pig farms located in Istanbul and Tekirdag Provinces in the Marmara Region of Turkey in the summer season of 2003. Forty-seven of the isolates were determined as *Enterococcus faecium* (68%), 15 isolates as *Enterococcus faecalis* (21.7%), three isolates as *Enterococcus gallinarum* (4.3%) and one of each as *Enterococcus hirae* (1.4%), *Enterococcus casseliflavus* (1.4%), *Enterococcus cecorum* (1.4%) and *Enterococcus sulfurens* (1.4%). In addition, antimicrobial susceptibilities of isolates were assessed through the disk diffusion method. Among 47 *E. faecium* isolates, 44 were determined to be resistant to erythromycin, 38 to ciprofloxacin, and three isolates were resistant to vancomycin. All *E. faecalis* isolates were resistant to erythromycin (100%) and four were resistant to vancomycin (27%). Five *E. faecium* (11%) and five *E. faecalis* isolates (33%) were found to exhibit intermediate resistance to vancomycin. In this study, isolates obtained from pig faeces were determined to exhibit a high rate of antimicrobial resistance. This study is the first report on isolation and determination of antimicrobial resistance of Enterococci in Turkey.

*Pig, stool samples, Enterococcus spp., antibiotic sensitivity*

Enterococci have emerged as important pathogens associated with serious nosocomial infections (Silverman et al. 1998; Hayes et al. 2003; Hershberger et al. 2005). They are the second most common cause of nosocomial infections and the third most common cause of nosocomial bacteraemia (Silverman et al. 1998; Peters et al. 2003). Although the role of non-human sources and reservoirs in the spread of *Enterococcus* strains is controversial and not clearly understood (Thal et al. 1995; Hershberger et al. 2005), animals, human foods and inanimate environments have been suspected to be sources for several resistant clinical isolates (Thal et al. 1995). It has been suggested that food animals may be a reservoir of enterococci and resistance genes capable of transferring to humans through the food chain (Arestrup et al. 2002; Hayes et al. 2003; Hershberger et al. 2005). Today, we know that antibiotic resistant bacteria are selected in the intestinal flora of animals, contaminate the foods of animal origin and transfer their resistance to other bacteria in the human gut, no matter if they are pathogenic or non-pathogenic (van den Bogaard et al. 2000). Thus, the most important reservoir for resistant bacteria and resistance genes is the intestinal flora of healthy animals and humans (van den Bogaard et al. 2000). The prevalence of antibiotic resistance found in indicator bacteria isolated from the faecal samples of humans and animals are thought to be a good indicator of the selective pressure of antibiotic usage (Knudtson et al. 1992; van den Bogaard et al. 1997; van den Bogaard et al. 2000). It has also been suggested that to determine of carriage of low level antibiotic resistance in microorganisms (especially in food animals) should be a public health goal (van den Bogaard et al. 1997; van den Bogaard et al. 2000).

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Resistance to antimicrobials may be an inborn characteristic or it may be acquired by new genes (through e.g. conjugation) that are susceptible to antimicrobials or via mutations. The virulence of *E. faecium* species gradually increases due to resistance against commonly used antimicrobials (Lukasova et al. 2003). *E. faecalis* has been reported to transfer plasmids harbouring antibiotic-resistance traits to other enterococci (Marcinek et al. 1998).

Microbial resistance to antimicrobials is a serious issue worldwide, both in humans and veterinary medicine. In general, the wide use of antimicrobials is regarded as the main risk factor for the increase in antimicrobial resistance. The wide use of antimicrobials has led to development and spread of resistance genes and bacterial resistance in humans and among animals (Lukasova et al. 2003).

In Turkey, there are many studies on the epidemiology of nosocomial *Enterococcus* strains in humans; however, we have almost no information on the situation in animals. The aim of this study was to examine the antibiotic susceptibilities of the *Enterococcus* strains isolated from the faecal samples of pigs.

### Materials and Methods

#### Stool samples

Faecal samples were collected in the 2003 summer season on three different pig farms located in Çorlu (Tekirdağ), Ayazma (İstanbul) and Arnavutköy (İstanbul). The samples were obtained from a total of 238 pigs, 105 of which were under six months of age and 133 of which were older than six months. The samples (approximately 20 g in a plastic vial) were stored at 4 °C and delivered to the Istanbul Medical Faculty's bacteriological laboratory on the same day.

#### Isolation and identification of the strains

On the day of arrival at the laboratory, the samples were diluted ( $10^{-1}$ ) in saline. Each of the samples was cultured through the enrichment method. Bile esculin azide (BEA) broth and BEA agar (Merck, Germany) were used for the isolation of strains. Gram staining, growth in 6.5% NaCl, aeskulin hydrolysis, absence of oxidase and catalase and Streptococcal group antigens were also detected using group D antiserum (Streptococcal grouping kit, Oxoid, Basingstoke, Hampshire, England), Presumptive colonies were identified using the conventional biochemical methods (Faclam et al. 1989; Faclam et al. 1998; Quinn et al. 1994; Manero et al. 1999; Winn et al. 2006).

Susceptibilities of the isolated bacterial strains to antibiotics ampicillin (10 µg), vancomycin (30 µg), teicoplanin (30 µg), gentamicin (120 µg), erythromycin (15 µg), ciprofloxacin (5 µg) and nitrofurantoin (300 µg) were investigated using the Kirby-Bauer disc diffusion method according to the CLSI criteria (CLSI document M100-S18, 2008).

### Results

In total 69 *Enterococcus* strains were isolated from 69 faecal samples; the others (169 samples) were negative. Forty-seven (68.1%) of the strains were identified as *E. faecium*, 15 strains (21.7%) as *E. faecalis*, one strain (1.4%) as *E. hirae*, one strain (1.4%) as *E. casseliflavus*, three strains (4.3%) as *E. gallinarum*, one strain (1.4%) as *E. cecorum*, and one strain (1.4%) as *E. sulfurens*.

When testing antibiotic susceptibilities, 44 (93.6%) *E. faecium* and 15 (100 %) *E. faecalis* strains were resistant to erythromycin and 38 (80.9%) *E. faecium* strains were found to be resistant to ciprofloxacin. Three (6.4%) *E. faecium* and four (26.6%) *E. faecalis* strains were resistant and five (10.6%) *E. faecium* and five (33.3%) *E. faecalis* strains were moderately resistant to vancomycin (Tables 1, 2).

### Discussion

Sensible use of antibiotics in humans but also in animals is very important to minimize the infections caused by antibiotic resistant bacteria. Surveillance is an essential part of the antibiotic policy and should consist of regular monitoring not only of antibiotic resistance

Table 1. Resistance patterns of the enterococci isolated from pigs

Enterococcus species	n = 69	Antibiotics - No. (%) of resistant strains						
		AMP	VAN	TE	GN	E	CIP	NIT
<i>E. faecium</i>	47	-	3 R (6.4%) 5 I (10.6%)	-	-	44 R (93.6%)	38 R (80.9%)	-
<i>E. faecalis</i>	15	-	4 R (26.6%) 5 I (33.3%)	-	-	15 R (100%)	-	-
<i>E. hirae</i>	1	-	-	-	-	-	-	-
<i>E. casseliflavus</i>	1	-	-	-	-	-	-	-
<i>E. gallinarum</i>	3	-	-	-	-	-	-	-
<i>E. cecorum</i>	1	-	-	-	-	-	-	-
<i>E. sulfurens</i>	1	-	-	-	-	-	-	-

R - resistant, I - intermediate resistant, AMP - ampicillin (10 µg), VAN - vancomycin (30 µg), TE - teicoplanin (30 µg), GN - gentamicin (10 µg), E - erythromycin (15 µg), CIP - ciprofloxacin (5 µg), NIT - nitrofurantoin (300 µg)

Table 2. Antibiotic susceptibilities of *Enterococcus* isolated from pigs

Enterococcus species	Antibiotics						
	AMP	VAN	TE	GN	E	CIP	NIT
<i>E. faecium</i> (n = 47)	47 S	3R, 5 I, 39 S	47 S	47 S	44 R, 3 S	38R, 9 S	47 S
<i>E. faecalis</i> (n = 15)	15 S	4R, 5 I, 6 S	15 S	15 S	15R	15 S	15 S
<i>E. hirae</i> (n = 1)	1 S	1 S	1 S	1 S	1 S	1 S	1 S
<i>E. casseliflavus</i> (n = 1)	1 S	1 S	1 S	1 S	1 S	1 S	1 S
<i>E. gallinarum</i> (n = 3)	3 S	3 S	3 S	3 S	3 S	3 S	3 S
<i>E. cecorum</i> (n = 1)	1 S	1 S	1 S	1 S	1 S	1 S	1 S
<i>E. sulfurens</i> (n = 1)	1 S	1 S	1 S	1 S	1 S	1 S	1 S

R – resistant, I – intermediate resistant, S – sensitive, AMP - ampicillin (10 µg), VAN - vancomycin (30 µg), TE - teicoplanin (30 µg), GN - gentamicin (10 µg), E - erythromycin (15 µg), CIP - ciprofloxacin (5 µg), NIT - nitrofurantoin (300 µg)

but also of antibiotic use in both animal and human populations (van den Bogaard et al. 2000). Previous studies have indicated that frequent usage of a particular antibiotic in an animal population seemed to correlate not only with high prevalence of resistance to that antibiotic, but also with higher prevalence of animals with a high degree of resistance in the population (van den Bogaard et al. 2000; Arestrup et al. 2002). Therefore, numerous surveillance studies have been carried out worldwide, investigating both meat products and animal colonization.

In the study of Hayes et al. (2003) a total of 981 retail raw meat samples (chicken, turkey, pork, beef) were cultured and a total of 1 357 *Enterococcus* strains were isolated, and *E. faecium* was found to be the predominant species. Hernshberger et al. (2005) investigated the antimicrobial resistance in enterococci from animal farms. They evaluated 1 256 *E. faecium* strains and 656 *E. faecalis* strains from the faecal samples of cattle, swine, chickens and turkeys. None of them was vancomycin-resistant, but resistance to quinupristin/dalfopristin, gentamicin and fluroquinolones was common in various food animals (Hernshberger et al. 2005). Van den Bogaard AEJM et al. (2000) investigated the antibiotic resistance of faecal indicator bacteria in 1 321 faecal samples collected from pigs at five abattoirs in the Netherlands and 100 samples from Swedish pigs. All the enterococci were found to be susceptible to amoxicillin and 4–6% of them were found to be

resistant to high doses of gentamicin. Vancomycin resistance ranged from 24% to 46% (van den Bogaard et al. 2000). In the study of Peters et al. (2003), a total of 416 *Enterococcus* strains were isolated from 155 different animal origin food samples (sausage, ham, minced meat, cheese). The most frequently isolated species were *E. faecalis* (299 strains) and *E. faecium* (54 strains). Only one *E. faecium* strain was resistant to penicillin, whereas all strains were sensitive to glycopeptides. Similarly to our results, 74% of *E. faecalis* strains and all *E. faecium* strains were moderately resistant or resistant to erythromycin (Peters et al. 2003).

The results of a trial conducted in Denmark, Spain and Sweden to determine antimicrobial susceptibilities of samples isolated from pigs were as follows: In Denmark, 85% of 102 *E. faecalis* isolates were resistant to erythromycin and 1% were resistant to gentamicin, while no vancomycin-resistant samples were isolated; 81% of 88 *E. faecium* isolates were resistant to erythromycin and 17% to vancomycin, while the isolates were determined to be susceptible to gentamicin; in Spain, 86% of 124 *E. faecium* were resistant to erythromycin, 5% to gentamicin and 2% to vancomycin; 17% of 48 *E. faecalis* samples isolated in Sweden were resistant to erythromycin, while they were regarded as susceptible to gentamicin and vancomycin; 6% of 18 *E. faecium* isolates were resistant to erythromycin and 6% to vancomycin, while all the isolates were reported to be susceptible to gentamicin (Aarestrup et al. 2002).

The antimicrobial resistance rates found in Enterococci isolates in our study are in agreement with the results of the trial conducted by Aarestrup et al. (2002). On the other hand, erythromycin resistance was detected in 100% of 15 *E. faecalis* isolates and in 93.6% of 44 *E. faecium* isolates. In contrast to gentamicin resistance in 1% of 102 *E. faecalis* isolates in Denmark and 5% of 124 *E. faecium* isolates in Spain, all the isolates in the current trial were susceptible to gentamicin.

Aarestrup et al. (2002) determined 100% susceptibility to vancomycin in all *E. faecalis* isolates; however, 26.6% of *E. faecalis* isolates in our trial were determined to be resistant to vancomycin. The rate of vancomycin resistance among *E. faecium* isolates determined in the current trial (6.4%) was strikingly similar to the vancomycin resistance rates of *E. faecium* isolated in Sweden (Aarestrup et al. 2002). Utilization of similar antimicrobials for therapeutic purposes in Europe and in Turkey will lead to similar values in antimicrobial resistance rates.

Türkyılmaz et al. (2010) isolated 91 Enterococci from cats and dogs; among these isolates, only 5 *E. faecium* isolates exhibited intermediate resistance to vancomycin, and the authors did not determine any *vanA*, *vanB* and *vanC* genes; in the same trial; 30.8% of enterococci were reported to be resistant to erythromycin and 84.6% were found to be resistant to gentamicin. In the current trial, 10 of 69 isolates (14.49%) showed intermediate resistance to vancomycin, whereas 7 isolates (10.14%) were determined as resistant. While no gentamicin-resistant isolates were determined, 59 of 69 enterococci isolates (85.50%) were found to be resistant to erythromycin. The close correspondence between the erythromycin-resistance rates in our trial and in enterococci isolated from cats and dogs in our country indicate that similar antimicrobials are used in animals, and this is regarded as a major potential factor in the transmission of the agent pathogen among animals.

In a trial conducted on humans in 2005 by Kaçmaz and Aksoy (2005), 109 isolates obtained from a total of 264 urine, wound, blood, secretions and catheter samples were reported to be susceptible to vancomycin. In the current trial, 10 of 69 isolates (14.49%) of 238 faecal samples isolated from pigs demonstrated intermediate resistance to vancomycin, while 7 isolates (10.14%) were regarded as resistant.

Although a number of human and food-borne isolates have been determined in various animal species in our country, we found no specific study on enterococci isolation in pig

species. This trial is important as it is the first study conducted on isolation of enterococci in pigs.

We determined a high frequency of erythromycin and ciprofloxacin resistance among enterococci in our study. In view of our study and others, we may suggest that food animals can serve as a potential reservoir for resistant *Enterococcus* strains.

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