



RESEARCH ARTICLE

Antibiotic resistances of enterococci isolated from bovine subclinical mastitis

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Özet

Kuyucuoğlu Y. Subklinik mastitisli ineklerden izole edilen enterokok suşlarının antibiyotik dirençlilikleri. **Eurasian J Vet Sci, 2011, 27, 4, 231-234**

Amaç: Bu çalışma ile subklinik mastitisli süt ineklerinden izole edilen enterokok suşlarında antibiyotik dirençliliğinin araştırılması amaçlandı.

Gereç ve Yöntem: Afyonkarahisar'da bulunan çiftliklerden toplam 392 CMT pozitif süt örneği toplandı. *Enterococcus* türlerinin tiplendirilmesinde API 20 Strep kiti kullanıldı. Antibiyotik duyarlılıkları National Committee for Clinical Laboratory Standards'ın önerdiği disk difüzyon metoduna göre yapıldı.

Bulgular: Sığır mastitislerinden toplam 43 enterococci izole edildi. İzole edilen suşların çoğunluğunu *E. faecalis* (%53.4) ve *E. faecium* (%18.6) oluşturdu. Diğer 12 (%27.9) suş sınıflandırılmadı. Bu çalışmada izole edilen *E. faecalis* suşları, tetrasiklin (%91.3), eritromisin (%82.6) ve penisilin (%78.2)'e dirençli bulundu.

Öneri: Bu çalışma ile CMT pozitif süt örneklerinden izole edilen *E. faecalis*, *E. faecium* ve diğer *Enterococci* suşlarında yüksek oranlarda antibiyotik dirençliliği belirlenmiştir. Enterokok suşlarındaki antibiyotik dirençliliği halk sağlığı ve çevre açısından önemsenmelidir.

Abstract

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Aim: The aim of this study was to investigate the antibiotic resistances of enterococci species isolated from bovine subclinical mastitis in dairy cows.

Materials and Methods: A total of 392 CMT positive milk samples were collected from dairy farms in Afyonkarahisar. Typing of enterococcus species were performed using API 20 Strep, subsequently, antibiotic sensitivities were determined by disk diffusion method according to the National Committee for Clinical Laboratory Standards.

Results: A total of 43 isolated enterococci, involved in bovine mastitis. The majority of isolates were *E. faecalis* (53.4%), followed by *E. faecium* (18.6%). The remaining 12 (27.9%) strains could not be classified. This study observed that the highest percentage of resistance by *E. faecalis* were to tetracycline (91.3%), erythromycin (82.6%) and penicillin (78.2%).

Conclusion: This study showed a high prevalence of antimicrobial resistant *E. faecalis*, *E. faecium* and other *Enterococci* species isolated from CMT positive milk samples. Resistance to antibiotics in enterococci is becoming a concern for human and environmental health.

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Keywords: Enterococci, antimicrobial susceptibility, bovine mastitis

► Introduction

Enterococci have been found in a number of environments including the intestinal tract of mammals, soil, water, plants and insects (Witte et al 1999, Aarestrup et al 2002). Enterococci, one of the leading causes of nosocomial infections, pose a significant threat to public health with their intrinsic resistance to many broad-spectrum antimicrobials (Hayes et al 2004) and the prevalence of vancomycin-resistant enterococci has been on the rise in the last decades. Enterococci host a wide variety of mobile genetic elements and are considered a reservoir for acquisition and distribution of antibiotic resistance genes among gram-positive bacteria (Courvalin 2006).

In cattle, enterococci have been associated with diarrhoea in calves and bovine mastitis in dairy cattle (Madsen et al 1974, Rogers et al 1992). Enterococci can also cause many economically important diseases including bovine mastitis (Devriese et al 1992) considered environmental pathogens as they are transmitted between the environment and the animal rather than from animal to animal (Rossitto et al 2002). In addition, enterococci are also important because of their ability to antimicrobial resistance genes (Klare et al 2001). Particularly species of *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*) carry some genes that encode resistance to antimicrobial agents. The contribution of dairy cattle as a source of antimicrobial-resistant enterococci that can be transmitted to humans remains unclear (Jackson et al 20011). *Enterococcus* spp. are used as indicator bacteria for the development of antimicrobial resistance and provide accurate information on previous antibiotic treatment of the animals (CDC 2002).

Transmission of resistant bacteria to humans may still occur via raw milk products contaminated with resistant bacteria from subclinical mastitis infections (Tenhagen et al 2006). Studies on the prevalence of enterococci in dairy cattle have been reported, but have mainly focused on mastitis and contamination of raw milk products; few studies have reported the prevalence of enterococci in the milk samples of dairy cattle (Aarestrup et al 1995, Pitkala et al 2004, Nam et al 2010).

The aim of this study was to determine the antimicrobial resistance of various species of enterococci strains isolated from bovine milk samples taken from dairy cattle in Afyonkarahisar, Turkey.

► Materials and Methods

A total of 392 California Mastitis Test (CMT) positive milk samples were collected from 274 lactating cows in different dairy farms from Afyonkarahisar, Turkey. Milk samples were aseptically collected from individual mammary quarters. CMT was used to make a diagnosis of subclinical mastitis. The identification of *Enterococcus* strains were determined by using conventional methods (Quinn 2002, Aydın 2006) and API 20 Strep (bioMerieux, France) system as described by the manufacturer. *E. faecalis* ATCC 29212 was used as the control strain.

In vitro antimicrobial susceptibility testing was examined using the disk diffusion method on Mueller Hinton Agar, according to the National Committee for Clinical Laboratory Standards (NCCLS). The antimicrobial susceptibility of enterococcus species was tested with penicillin G, ampicillin, gentamycin, erythromycin, cephalothin, tetracycline, vancomycin and ciprofloxacin (Oxoid Ltd, Basingstoke, UK).

Chi-square tests were used to determine the difference of antibiotic resistance between *E. faecalis* and *E. faecium*. The significance level was defined at $p < 0.05$.

► Results

A total of 43 isolated enterococci, involved in bovine mastitis, were analyzed. The majority of isolates were *E. faecalis* (53.4%), followed by *E. faecium* (18.6%). The remaining 12 (27.9%) strains could not be classified. Of the 392 CMT positive milk samples from the 274 dairy cows tested for the presence of enterococci, 10.9 % were positive.

Resistance rates of the strains to antibiotics are shown in Table 1. There were statistically significant differences between the antimicrobial resistance of *E. faecalis* and *E. faecium* strains ($p < 0.05$).

Table 1. Antimicrobial resistance profiles of the enterococci species isolated from bovine milk samples.

Antimicrobials	<i>E. faecalis</i> (n:23)		<i>E. faecium</i> (n:8)		Other <i>Enterococci</i> spp.(n:12)	
	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Penicillin G	5 (21.7)	18 (78.2)	2 (25.0)	6 (75.0)	3 (25.0)	9 (75.0)
Ampicillin	23 (100)	--	8 (100)	--	10 (83.3)	2 (16.6)
Gentamycin	17 (73.9)	6 (26.6)	4 (50.0)	4 (50.0)	8 (66.6)	4 (33.3)
Erythromycin	4 (17.3)	19 (82.6)	2 (25.0)	6 (75.0)	3 (25.0)	9 (75.0)
Cephalothin	13 (56.5)	10 (43.4)	5 (62.5)	3 (37.5)	9 (75.0)	3 (25.0)
Tetracycline	2 (8.6)	21 (91.3)	3 (37.5)	5 (62.5)	1 (8.3)	11 (91.6)
Vancomycin	22 (95.6)	1 (4.3)	8 (100)	--	10 (83.3)	2 (16.6)
Ciprofloxacin	16 (69.5)	7 (30.4)	4 (50.0)	4 (50.0)	8 (66.6)	4 (33.3)

S; sensitive, R; resistant.

► Discussion

Studies on the prevalence of enterococci in dairy cattle have been reported, but have mainly focused on mastitis and contamination of raw milk products (Aarestrup et al 1995, Rossitto et al 2002, Makovec and Ruegg 2003). In this study, both the prevalence and antimicrobial resistance of enterococci from bovine milk samples were examined. This study will be useful of antimicrobial employ practices and public health. Because commensally bacteria such as enterococci have natural gene transfer mechanisms and can cause multiple resistances (Nam et al 2010, Jackson et al 2011).

In previous studies on the prevalence of enterococci from dairy cattle faecal samples, few enterococcal species were isolated (Devriese et al 1992, Edrington et al 2009). Overall, *E. faecalis* and *E. faecium* were the most common enterococci species found in cattle. These species were shown to be found in different rates by previous studies (Jayarao and Oliver 1992, Chingwaru et al 2003, Nam et al 2010, Cengiz et al 2011). Cengiz et al (2011) reported that a total of 75 *Enterococcus* spp. isolated from dairy farms in districts of Ankara and Balıkesir. Sixty-one *E. faecium* and 14 *Enterococcus avium* strains were identified according to Restriction Fragment Length Polymorphism-Polymerase Chain Reaction results. In the present study, two enterococcal species, *E. faecalis* and *E. faecium* were isolated from CMT positive milk samples in dairy cows. In addition other 12 species belonging to enterococci were not identified in this study (Table 1).

Generally, the prevalence of antimicrobial resistance showed in this study (Table 1) was much higher than those of the previous studies on mastitis (Pitkala et al 2004, Nam et al 2010, Jackson et al 2011). It was observed (Table 1) that the highest percentage of resistant *E. faecalis* were to tetracycline (91.3%), erythromycin (82.6%) and penicillin G (78.2%). Four antibiotics were sensitive against *E. faecalis* in this study: ampicillin, vancomycin, gentamycin and ciprofloxacin. While vancomycin and ampicillin also showed the most effective against *E. faecium*. Our findings were similar to results of a previous study (Nam et al 2010). Gentamycin resistant enterococci were previously reported (Lopes et al 2003) and possibility of gene transfer, probably from clinical or commensally bacteria to dairy enterococci, has been suggested (Lopes et al 2005), although gentamycin resistance was observed 26.6% and 50.0% in this study. *Fluoroquinolones* can be used in enterococci infections. In some studies, an increase in the resistance of *Enterococcus* spp. strains to quinolones is observed. Quinones et al (2005) found that the resistances of *E. faecium* strains to ciprofloxacin were 30%. In our study, corresponding rate were 50.0% for ciprofloxacin. Tetracyclines are also used in *Enterococcus* spp. infections. Quinones et al (2005) reported that tetracycline resistance rates in *E. faecalis* and *E. faecium* were 78.6 and

90%, respectively, which can be evaluated to close to our findings. The resistance rates of *E. faecalis* strains to vancomycin 4.3% in this study. A similar ratio was reported by Daza et al (2001). However, we also found that all the *E. faecium* strains were susceptible to vancomycin.

► Conclusions

This study showed a high prevalence of antimicrobial resistant *E. faecalis*, *E. faecium* and other *Enterococci* species isolated from CMT positive milk samples in lactating cows. Although all *E. faecium* strains were susceptible to ampicillin and vancomycin, only one *E. faecalis* strains were resistant to vancomycin. Since *Enterococci* could have a role in environmental contamination, antibiotic resistance in enterococci is pointing out a possible concern of human and environmental health.

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