

Full Length Research Paper

Antibacterial drug resistance patterns in poultry isolated enterococci

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The increasing use of antibiotics in both human medicine and animal agriculture leads to the decline in the effectiveness of antibiotics. Enterococci are natural intestinal flora which are important reservoir of antibiotic resistance genes and have the ability to transfer their resistance traits to other bacteria. In this report, antibacterial drug resistance of broiler chickens isolated *Enterococcus faecalis* and *Enterococcus faecium* were examined using Kirby-Bauer disk diffusion method. 93% of *E. faecalis* and 98% of *E. faecium* isolates were resistant to one or more antibiotics. The most frequent resistance properties were resistance to erythromycin (80% in *E. faecalis* and 82% in *E. faecium*) and tetracycline (73% in *E. faecalis* and 75% in *E. faecium*). No vancomycin-resistant *E. faecalis* were isolated but 9% of *E. faecium* isolates were resistant to vancomycin (VRE). These high amounts of drug resistant enterococci can be transfer from animal to human and suggest the need for restriction on the use of antibiotics as food additives and rational use of antimicrobial therapy of infections.

Key words: Antibacterial drug resistance, enterococci, poultry.

INTRODUCTION

Recently, there is a growing concern about the decline in the effectiveness of antibiotics in veterinary and human medicine (Catry et al., 2003; Mateu and Martin, 2000). The main risk factor for this problem is the increasing use of antibiotics in both human medicine and animal agriculture (Antony et al., 2000). In modern poultry industry, antibiotics are used in high quantities for therapy and prevention of bacterial disease and also to promote growth, increase feed efficacy and decrease waste production (Antony, 2000; van den Bogaard and Stobberingh, 1999). Poultry litter has been found to contain large amounts of antibiotic resistant bacteria and resistance genes (Nandi et al., 2004). Antibiotic-resistant bacteria that have been identified in animals can enter meat during slaughtering or contaminate raw vegetable when the soil is fertilized with animal excrement (Teuber, 2001; Phillips et al., 2004). Enterococci are natural intestinal flora and a wide range of *Enterococcus* species such as *Enterococcus avium*, *Enterococcus casseliflavus*, *Enterococcus cecorum*, *Enterococcus durans*, *Enterococcus gallinarum*, *Enterococcus hirae*, *E. malodoratus* and the predominant species *Enterococcus faecalis* and *Enterococcus faecium* have been isolated from poultry (Debnam et al., 2005; Devriese et al., 2008).

Enterococci are not important pathogens for animals but can cause life-threatening opportunistic infections in immune-compromised patients (Murray, 1990). Their role in such infections has increased due to their intrinsic and acquired resistance to various antimicrobial agents which renders them difficult to treat (Linden and Miller, 1999; Iversen et al., 2002). These important reservoir of antibiotic resistance genes have the ability to transfer their resistance traits to other species of enterococci or to other serious human pathogens through gene transfer (Devriese, 1992). Enterococci usually contaminate raw meats and are obviously resistant to temperature, pH and salinity effects (Teuber et al., 1996) and can be transfer from animal to man. According to Mateu and Martin (2000) foodstuffs contaminated with enterococci after consumption become source of resistance genes for bacteria present in the intestinal contents of humans. Especially, the spread of vancomycin resistance in enterococci which is one of the considerable concern. Vancomycin – resistant enterococci (VRE) cause a health risk, in patients with severe underlying disease or immune-suppression (Chadwick et al., 1996; van den Braak et al., 1998). Nobel et al. (1992) reported *in vitro* conjugative transfer of high level vancomycin resistance

Table 1. Number and percentage of antibiotic resistance in *Enterococcus faecalis* and *Enterococcus faecium* isolated from broiler chickens.

Antibiotic	<i>E. faecalis</i>		<i>E. faecium</i>	
	No.	%	No.	%
V (30 µg)	0	-	4	9
M (5 µg)	18	60	33	73
Amx (25 µg)	0	-	5	11
Amc (30 µg)	0	-	7	15
G (30 µg)	18	60	22	48
E (15 µg)	24	80	37	82
SXT (25 µg)	16	53	29	64
TE (30 µg)	22	73	34	75
NFX (5 µg)	17	56	24	53

V = Vancomycin; M = Methicillin; Amx = Amoxicillin; Amc = Amoxicillin-clavulanic acid; G = Gentamycin; E = Erythromycin; SXT = Trimethoprim-sulfamethoxazole; TE = Tetracycline; NFX = Enrofloxacin.

gene from *E. faecalis* to *Staphylococcus aureus*. The present study was designed to evaluate the level of antibacterial drug resistance in poultry intestinal isolated enterococci and determine their resistance patterns.

MATERIALS AND METHODS

Test microorganism isolation

One gram of broiler chicken caecal digesta was added to 9 ml buffered peptone water and homogenized. Then 0.1 ml of the homogenate was plated on Bile Esculin Azide Agar and incubate aerobically at 37°C for 24 h. Enterococci colonies were identified on the basis of esculin hydrolysis, gram stain and catalase reaction (Pavia et al., 2000) and identified to the species using different biochemical tests (Facklam and Collin, 1989; Bauer et al., 1966; CLSI, 2006).

Antimicrobial susceptibility test of isolates

Totally 30 *E. faecalis* and 45 *E. faecium* isolated from the poultry were tested against 9 commonly used anti-microbial drugs. The antimicrobial susceptibility test of the isolates were performed according to the National Committee for Clinical Laboratory Standards (NCCLS) method using Kirby-Bauer disk diffusion test on Muller-Hinton agar. Standard strain of *S. aureus* ATCC 6538 was used as a quality control organism. The susceptibility pattern of the isolates to the selected antimicrobial agents including: vancomycin (30 µg), methicillin (5 µg), amoxicillin (25 µg), amoxicillin-clavulanic acid (30 µg), gentamycin (30 µg), erythromycin (15 µg), tetracycline (30 µg), enrofloxacin (5 µg) and trimethoprim-sulfamethoxazole (25 µg) provided from Padtan Teb Company (Iran), was determined.

RESULTS

Of 30 *E. faecalis* isolates, 28 (93%) and of 45 *E. faecium* isolates, 44 (98%) were resistant to one or more antibiotics. Table 1 shows the frequency of drug

resistance in *E. faecalis* and *E. faecium* isolates. Majority of the isolates were resistant to erythromycin also a high frequency of antibiotic resistance to tetracycline, methicillin, gentamycin, trimethoprim-sulfamethoxazole and enrofloxacin was found. All of the *E. faecalis* isolates were sensitive to vancomycin, amoxicillin, and amoxicillin-clavulanic acid, but 9% of *E. faecium* isolates were resistant to vancomycin (VRE), 11 and 15% were resistance to amoxicillin and amoxicillin-clavulanic acid. Twelve different resistant patterns in *E. faecalis* and 17 different patterns in *E. faecium* were found among 28 and 44 resistant strains (Tables 2 and 3).

DISCUSSION

Clearly the use of antibiotics leads to increased antibiotic resistance in human, animals and the environment. Enterococci often acquire antibiotic resistance through exchange of resistance encoding genes carried on conjugative transposons, pheromone-responsive plasmids and other broad-host range plasmids (Rice et al., 1995). The results of van den Bogaarda et al. (2002) study provided evidence for dissemination of resistant enterococci from animals to man and, probably the exchange of resistance genes between poultry and human enterococci. According to these authors the transposon encoding vancomycin resistance was similar between VRE isolated from farmers and chickens (van den Bogaard et al., 2002). Also in enterococcal isolates from pigs, vancomycin resistance were found to be transferable *in vitro* (Aarestrup, 2000) and rifampin resistant *E. faecium* fed to broiler chicks transferred resistance to indigenous flora at a higher rate than that observed *in vitro* (Nether wood et al., 1999). Enterococci have always been considered low pathogenic bacteria, but in the last two decades *E. faecium* and *E. faecalis* have emerged as important pathogens responsible for

Table 2. Antibiotic resistance patterns in *E. faecalis* strains isolated from broiler chickens.

No. of resistance markers	No. of resistance strains	Resistance patterns
1	1	E
3	3	E, SXT, TE
3	2	G, NFX, TE
3	4	E, TE, G
3	1	G, M, NFX
4	2	E, G, M, TE
4	1	SXT, M, TE, V
4	1	SXT, E, M, NFX, TE
5	4	E, M, NFX, SXT, TE
5	2	E, G, M, NFX, TE
5	4	E, G, M, NFX, SXT
6	3	E, G, M, NFX, SXT, TE

V = Vancomycin, M = Methicillin, Amx = Amoxicillin, Amc = Amoxicillin-clavulanic acid, G = Gentamycin, E = Erythromycin, SXT = Trimethoprim-sulfamethoxazole, TE = Tetracycline, NFX = Enrofloxacin.

Table 3. Antibiotic resistance patterns in *E. faecium* strains isolated from broiler chickens.

No. of resistance markers	No. of resistance strains	Resistance patterns
2	4	E, TE
2	3	E, SXT
3	1	E, NFX, TE
3	2	AMC, M, SXT
4	1	AMC, G, SXT, TE
4	6	E, G, M, TE
4	3	E, M, SXT, TE
4	2	E, G, SXT, TE
4	1	G, M, SXT, V
4	1	G, M, NFX, TE
5	6	E, G, M, SXT, TE
5	4	E, M, NFX, SXT, TE
5	3	E, G, M, NFX, TE
5	2	AMX, M, SXT, TE, V
5	3	AMC, AMX, E, M, SXT
6	1	E, G, M, NFX, SXT, TE
6	1	AMC, E, G, M, NFX, SXT

V = Vancomycin, M = Methicillin, Amx = Amoxicillin, Amc = Amoxicillin-clavulanic acid, G = Gentamycin, E = Erythromycin, SXT = Trimethoprim-sulfamethoxazole, TE = Tetracycline, NFX = Enrofloxacin.

hospital acquired infections (Bonten et al., 2001 and Rice, 2001). The present investigation revealed a high incidence of resistance enterococci isolates to commonly used antimicrobial agents. Most of the resistant strains carried multiple resistance determinants. The most frequent resistance properties were resistance to erythromycin (80% in *E. faecalis* and 82% in *E. faecium*), tetracycline (73% in *E. faecalis* and 75% in *E. faecium*) and methicillin (60% in *E. faecalis* and 73% in *E. faecium*). These high percentages of resistance to

erythromycin and tetracycline, are similar to those found by Kolar et al. (2002) and Ruzauskas et al. (2009) and can be due to the common use of these antimicrobial drugs as prophylactic and growth promoter. Hinton et al., 1985 described the effect of long-term usage of sub therapeutic doses of tetracyclines resulting in the increased level of resistant gut microflora (Hinton et al., 1985). Resistance of the most strains to methicillin matches the intrinsic antibiotic resistance profile of enterococci (Giraffa, 2002). High percentages of

susceptibility to vancomycin (100% in *E. faecalis* and 91% in *E. faecium*), amoxicillin (100% in *E. faecalis* and 89% in *E. faecium*) and amoxicillin-clavulanic acid (100% in *E. faecalis* and 85% in *E. faecium*) also were observed. Although, the presence of 9% vancomycin resistant *E. faecium* isolates is an unfavorable factor in the present study. 60% of *E. faecalis* and 48% of *E. faecium* isolates were resistant to gentamicin. Aarestrup et al. (1998) reported only 1% of the *E. faecalis* isolated from healthy broilers at slaughter were resistant to gentamicin and no gentamicin-resistant *E. faecium* were isolated. Van den Bogaarda et al. (2002) reported high prevalence of gentamicin resistance in faecal samples of two chicken populations: broilers and laying-hens. High-level gentamicin resistance in enterococci also has been reported in several studies from human infections (Schouten et al., 1999; Patterson et al., 1990). The results of the present study, is also confirmed by other studies that have found the prevalence of antibiotic-resistant enterococci in farm animals and their meat to be higher than 60% (Giraffa, 2002; Teuber et al., 1999). The high incidence of antibiotic resistance in avian isolated enterococci, suggests the need for public health intervention, restriction on the use of antibiotics as food additives and rational use of antimicrobial therapy of infections in man and animals. This uncontrolled high usage of antibiotics in poultry may compromise veterinary therapy and also the resistance problem in human medicine will not be solved if there is a constant influx of resistance genes into the human microflora via the food chain.

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