NOTE

Bacteriology

Slime Production and Antibiotic Resistance of *Enterococcus faecalis* Isolated From Arthritis in Chickens

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**ABSTRACT.** Slime factor production and antibiotic resistance of 67 *Enterococcus faecalis* strains isolated from chicken arthritis were investigated in this study. Slime factor productions of enterococci were found as 59.7%. The antibiotic resistances were investigated by testing gentamycin, penicillin, streptomycin, vancomycin, danofloxacin, and enrofloxacin. The resistance rates were found as 62.68%, 76.11%, 67.16%, 13.43%, 47.76%, 43.28%, respectively. For slime factor positive enterococci, the antibiotic resistance rates were found as follows respectively; 82.50%, 87.50%, 92.50%, 17.50%, 72.50%, and 60.00%. In conclusion; the slime factor might play a role as a colonization factor for chicken arthritis and slime factor positive enterococci were found to be more resistant to these antibiotics. The resistance rates between slime factor positive and negative enterococci against the tested antibiotics except for vancomycin were found statistically significant (p<0.05).

**KEY WORDS:** antibiotics, arthritis, *Enterococcus faecalis*, slime factor.

Enterococci are commonly isolated from various poultry environments and are considered as inhabitants of normal microflora of intestinal tract. *Enterococcus* spp. is Gram positive, spherical bacteria which occur singly, in pairs or short chains. They are non-motile, nonspore-forming, facultative anaerobes and catalase negative. Generally avian isolates differ in fermentation abilities of some carbohydrates (mannitol, sorbitol, and L-arabinose) and growth on MacConkey Agar. Enterococci in avian species have been found as responsible for endocarditis, meningitis, ovarian inflammation, fibrinous arthritis, tenosynovitis, and amyloid arthropathy [6, 21]. Studies have recently focused on enterococcal infections in veterinary medicine in parallel with coming out the animal factor in transmission of vancomycin resistant enterococci to humans [14, 38]. The most important infection caused by enterococci in chickens is amyloid arthropathy which effect numerous chickens and results in significant economic losses [24, 48].

Slime factor (biofilm) is a structured community of microorganisms encapsulated within a self-developed polymeric matrix and adhere to various surfaces irreversibly [29]. Production of biofilm has a major role in the pathogenesis of many clinically important pathogens [19]. Biofilm formation increases the resistance of bacteria to killing (phagocytosis) because antigens recognized by immune system may be hidden and key ligands may be repressed. In biofilms, invasion and motility machinery of bacteria are not expressed [29, 35]. Biofilm production has been reported in some enterococcal infections [15, 38, 47]. The major clinical infections have been traditionally caused by *Enterococcus faecalis* and *Enterococcus faecium* and both are capable of producing biofilms [29, 33]. Enterococci with biofilms are more highly resistant to antibiotics than planktonically growing enterococci, thus the potential impact of biofilm formation could be significant [29].

Most enterococci have inherent resistance to various antibiotics such as cephalosporins and semi synthetic penicillinase resistant penicillins, aminoglycosides and clindamycin. Most enterococci are also tolerant to bactericidal effects of cell wall inhibiting agents such as ampicillin and vancomycin, but this property is due to acquire genes mediating resistance to these agents [31, 33].

In this study, determination of antibiotic resistance patterns of *Enterococcus faecalis* strains isolated from chicken arthritis and the relationship between slime production and antibiotic resistance were aimed. Isolates included in this study isolated from 160 synovial fluids of chickens with arthritis in chicken farms in Ankara, Kirikkale and Samsun. Blood Agar (with 7% sheep blood), Enterococcus Presumptive Broth (as an enrichment medium) and D-Coccosel Agar (as a selective medium) were used for isolation of enterococci. Incubation was carried out at 37°C for 24–48 hr aerobically. Turning blue color to yellow in Enterococcus Presumptive Broth was suggested as growth of *Enterococcus* spp. After inoculation from this broth culture to D-Coccosel Agar, occurrence of black colonies on agar plates were evaluated as *Enterococcus* spp. These colonies were examined for Gram reaction, catalase and carbohydrate fermentation (mannitol, sorbitol and L-arabinose) for identification in species level [6].

Congo red agar was used to detect slime production. Black colonies on Congo red agar were evaluated as slime positive, pink or colorless colonies were evaluated as slime
negative [29].

All isolates were tested against streptomycin (300 μg), penicillin (10 μg), vancomycin (30 μg), enrofloxacin (5 μg), danofloxacin (5 μg) and gentamycin (120 μg) using Kirby-Bauer disc diffusion method [32].

Chi-square test was used to determine the relationship between resistance to each antibiotic tested in this study and slime production of *E. faecalis* isolates.

Sixty-seven strains were isolated from all 160 specimens and identified as *E. faecalis*. No *E. faecium* was isolated. The susceptibility patterns of all strains tested by Kirby-Bauer disc diffusion method and slime production of these strains are presented comparatively in Table 1. Forty of 67 (59.7%) *E. faecalis* strains were found to be slime positive and 27 (40.3%) were slime negative. A susceptibility test result of each antibiotic was evaluated according to NCCLS interpretive standards [32]. Resistance to penicillin, a beta lactam antibiotic, detected as 76.11%. Resistance (high level) to 2 aminoglicoside, streptomycin and gentamycin were found as 67.16% and 62.68%, respectively. Vancomycin (glycopeptide), enrofloxacin (quinolone) and danofloxacin (quinolone) resistance were detected as 13.43%, 43.28% and 47.76%, respectively.

According to chi-square analysis, P values for gentamycin, penicillin, streptomycin, enrofloxacin and danofloxacin were <0.05, only for vancomycin it was >0.05.

Enterococci have been considered as harmless inhabitants of normal flora of humans and animals for years. Recent studies have shown increasing resistance of entero cocci to various antimicrobials. Sometimes there is no chance for antimicrobial therapy due to their multiple antibiotic resistances [30].

*E. faecalis* is the most common species among all enterococcal clinical isolates [12, 37]. All isolates in this study were identified as *E. faecalis* in 41.88 percent. Antibiotic resistance in enterococci is either intrinsic or acquired. Enterococci generally express intrinsic resistance to semi synthetic penicillinase resistant penicillins, cephalosporins, low level of aminoglycosides and low level of clindamycin, however express acquired resistance to chloramphenicol, erythromycin, high level of clindamycin, tetracycline, high level of aminoglycosides, penicillin, fluoroquinolones and vancomycin [27]. *Enterococcus* strains isolated from chicken with arthritis in this study tested for resistance to penicillin, amoxicillin, streptomycin, gentamycin, enrofloxacin, danofloxacin and vancomycin.

Because enterococci are not considered as important pathogens in animals, studies on antimicrobial resistance are sparse and mainly have been conducted for the purpose of identifying potential reservoirs of resistant strains. In most studies, it has been focused on antimicrobial resistance to one or a few agents and comparison between studies is difficult [26]. We found that 51 of 67 *E. faecalis* isolates (76.11%) were resistant to penicillin. While Rahangdale et al. [36] have found that 89.43% of all enterococcal isolates were penicillin resistant; Miskeen and Deodhar [28] have reported that 20% of their *E. faecalis* isolates showed penicillin resistance.

High-level aminoglycoside resistance has been reported in food animals, food of animal origin and environment worldwide [26]. Most of the studies in US [20], Denmark [1, 2] and Belgium [10, 11], gentamycin resistance have only been observed at a low rate. We found the gentamycin resistance in *E. faecalis* isolated from chicken arthritis as 62.68% at a higher rate. Streptomyocin resistant enterococci have been isolated from chickens in the US at 66%. However streptomyocin resistant *E. faecalis* has been found in 10 percent in Denmark and 26 percent in Belgium [26]. Although limited studies on aminoglycoside resistance in nonhuman enterococcus have been reported, resistance seems to be widespread and the rates of resistance are similar to those observed among human isolates. Sentürk et al. [42] and Barisic and Punda-Polic [7] have found 46.3% and 52% of streptomyocin resistance in human *E. faecalis* isolates, respectively. In this study, we observed streptomyocin resistance in *E. faecalis* isolates from chicken arthritis at a high level, 67.16%.

Vancomycin, a glycopeptide antimicrobial agent has been used to treat Gram positive infections in humans but resistance to glycopeptides has emerged and spread among clinical isolates. Avoparcin, another glycopeptide has been used as a growth promoter in broilers in several countries, not in the US. This might be responsible for selecting vancomycin resistant enterococci (VRE) in farm animals and VRE may have been transmitted to humans via the food chain. For this reason, the European Community banned the use of avoparcin in animals as of April 1997 [5, 26]. In Turkey, the use of all growth promoter antibiotics was banned in 2006 [44]. In a study, VRE has been reported to remain prevalent three years after this banning [8]. However, after

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Slime positive (n= 40)</th>
<th>Slime negative (n=27)</th>
<th>Total (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamycin</td>
<td>33 (82.50)</td>
<td>9 (33.30)</td>
<td>42 (62.68)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>35 (87.50)</td>
<td>16 (59.25)</td>
<td>51 (76.11)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>37 (92.50)</td>
<td>8 (29.62)</td>
<td>45 (67.16)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>7 (17.50)</td>
<td>2 (7.40)</td>
<td>9 (13.43)</td>
</tr>
<tr>
<td>Danofloxacin</td>
<td>29 (72.50)</td>
<td>3 (11.11)</td>
<td>32 (47.76)</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>24 (60.00)</td>
<td>5 (18.51)</td>
<td>29 (43.28)</td>
</tr>
</tbody>
</table>
the ban, the decreasing in prevalence of VRE in poultry has been reported in several countries [45]. The prevalence of VRE in poultry has been decreased from >80% in 1995 to <5% in 1999 in Denmark [4], from 100% in 1995 to 25% of samples tested by 1997 in Germany [23], from 15% to 8% in Italy [34]. The proportion of VRE has been reported to decrease between 2000 and 2003, from 13.7% to 3.7% for E. faecalis in Taiwan [25]. There are limited reports about the prevalence of vancomycin resistance in enterococci isolated from poultry. Kaya et al. [22] have not found VRE from chicken samples in their study. In this study, vancomycin resistance in E. faecalis isolates was found as 13.43%. This rate is not low when compared with the prevalence rate mentioned above. It may due to persistence of VRE in the environment, especially poultry-house. Thus in recent studies, extensive occurrence of VRE on broiler farms and in broiler flocks after the avoparcin ban has been reported and this has been explained by persistence of VRE in the broiler house environment despite cleaning and disinfection between rotations [18].

The fluoroquinolones are a current antimicrobial class whose use in veterinary medicine is being scrutinized [39]. The significantly higher fluoroquinolone resistance observed in broilers and laying hens is most likely due to the more common use of enrofloxacin and fluomequine [46]. The unconscious use of ciprofloxacin may also be responsible for enrofloxacin resistance due to cross resistance [9], however resistance to fluoroquinolones is caused by chromosomal mutations and is not transferable. The prevalence of ciprofloxacin resistance to enterococci in broilers and layer hens have reported as 28% and 8%, respectively [46]. In another study ciprofloxacin resistance rate have found as 64% [40]. The rate of quinolone resistance including enrofloxacin and danofloxacin in this study was found relatively high (43.78% and 47.76%, respectively). Enrofloxacin and the other fluoroquinolones are used widely to treat the infections of poultry and other animals. In Turkey, no report could found on quinolone resistance to enterococci in poultry. However in a study of quinolone resistance among chicken isolates of campylobacter in Turkey [17] the significant increase in the rates of fluoroquinolone-resistance strains from 1992 (7.1%) to 2000 (92.9%) and in the same study a high-level of cross resistance has been observed among enrofloxacin, ciprofloxacin and nalidixic acid. Quinolone resistance may reach significant levels as long as quinolone use widely in veterinary medicine.

The mechanisms of resistance to antibiotics due to bacterial biofilm formation have based on three main hypotheses [41]. The first is the possibility of slow or incomplete penetration of the antibiotic into the biofilm. The second hypothesis depends on an altered chemical microenvironment within the biofilm. In the surface layers of a biofilm, oxygen concentration can be reduced and develop an anaerobic conditions and in these conditions some antibiotics such as aminoglycosides are less effective than aerobic conditions [43]. Accumulation of acidic waste products and alteration of osmotic environment in biofilm can also contribute the antibiotic resistance. The last mechanism that is still speculative is that a subpopulation of micro-organisms in a slime factor forms a unique, and highly protected, phenotypic state similar to spore formation. It has been reported that there is an association between slime production in S. epidermidis and P. aeruginosa and antibiotic resistance especially to aminoglycosides. Also it has clearly been shown that in the E. faecalis strains, the resistance to the gentamicin is associated with biofilm formation [3]. However, in some studies [13, 16] no correlation between slime productions of S. aureus and resistances has been found. We investigated the relationship between antibiotic resistance of E. faecalis isolates and slime production in this study. The relationship between resistance to five antibiotics, gentamicin, penicillin, streptomycin, enrofloxacin and danofloxacin and slime production were statically important while the relationship between resistance to vancomycin and slime production was statically not important. However the latter may due to low numbers of vancomycin resistant E. faecalis isolates.

In this study, antibiotic resistance profiles of E. faecalis strains isolated from chicken arthritis were presented. This may important both in monitoring the choices of antimicrobial treatment of enterococcal infections in poultry and for awareness of resistant strains which are transmissible from food animals to humans.

REFERENCES


