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DETERMINATION OF ANTIBIOTIC RESISTANCE OF ENTEROCOCCI ISOLATED FROM CHEESE SAMPLES

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Abstract: In this study, the antibiotic resistance properties of enterococcal isolates isolated from traditional cheeses collected from some eastern provinces of Turkey were determined. Within the scope of the study, a total of 20 traditionally produced cheese samples obtained from different places were used for *Enterococcus* isolation. Enterococci isolated from cheeses were used for verification at genus level; Gram staining, catalase and growth tests under different conditions (10°C, 45°C and pH 9.6) were performed. Antibiotic resistance properties of the isolates were determined using the disk diffusion method. It was determined that the 40 enterococcal isolates isolated were generally sensitive to some antibiotics (vancomycin, tetracycline, streptomycin, penicillin, ampicillin, gentamicin, erythromycin). However, as a result of the study, it was determined that cheese produced by traditional methods may contain a small number of antibiotic-resistant bacterial flora, which may pose a risk to public health.
Keywords: Cheese, *Enterococcus* spp., antibiotic resistance.

1. INTRODUCTION

Enterococci are frequently isolated from human and animal digestive systems, soil, plants and various fermented foods. Enterococci are frequently isolated from traditional cheeses made from raw or pasteurized milk in the Mediterranean and some European countries. In some studies conducted on different types of cheese, it was determined that enterococci constitute the dominant flora (Freitas et al., 1996; Öner et al., 2004; Jurkovič et al., 2006; Tuncer, 2009). Enterococci are frequently isolated from foods of animal origin produced from raw materials such as milk and meat, due to their ability to easily adapt to growth conditions such as low and high temperatures, extreme pH and salt concentrations, as well as their resistance to pasteurization temperatures (Lombardi et al., 2008; İşleroğlu et al., 2008). Since enterococci are a part of the natural microflora of dairy and meat products, they are among the natural starter cultures in the fermentation of these products (Giraffa, 2003). Various studies have shown that *Enterococcus* strains in cheese and traditional fermented foods are responsible for initiating fermentation as well as improving the structural and organoleptic properties of the product (Centeno et al., 1996; Yoon et al., 2008). In studies

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conducted by different researchers, it was determined that enterococci had a positive effect on the ripening and aroma development of various traditional cheeses due to their proteolytic and lipolytic activities as well as their production of diacetyl (Foulquié Moreno et al., 2006).

In addition to the positive effects of *Enterococcus* isolates on the ripening and aroma development of traditional cheeses, it has been determined that the various bacteriocins produced by different enterococcal strains have an inhibitory effect on various foodborne pathogenic microorganisms, especially *Listeria monocytogenes* (Ennahar et al., 2001; Galvez et al., 2009).

In studies conducted using dairy products produced in different geographical regions, it has been reported that *Enterococcus faecium*, *Enterococcus faecalis* and *Enterococcus durans* species were isolated with high frequency. These species are thought to play an important role in the development of taste and texture, especially in traditional cheeses produced without the use of starter cultures. It has an important role in the formation of taste and aroma during the ripening of cheese, especially with its lipolytic and proteolytic activities, and therefore in the development of cheese flavor and quality (Dağdemir and Özdemir, 2006).

In addition to the beneficial effects of some strains, enterococci are also known to be hospital-acquired pathogens that cause bacteremia, endocarditis, and infections in the urinary system and other tissues (Toğay and Temiz, 2011). The most striking feature of enterococci today is the increasing resistance rates to antibiotics (Parlak et al., 2014). The use of antibiotics in animal feed has revealed important carriers of transferable antibiotic resistance genes in various ecosystems and, as a result, has brought about the possible transfer of resistant *Enterococcus* species to humans through the food chain (Çetinkaya and Elal, 2010). The possibility of the presence of antibiotic-resistant *Enterococcus* strains in cheeses produced by traditional methods without the use of starter culture poses a risk to consumer health. For these reasons, although fermented foods containing enterococci have long been considered safe, the presence of these bacteria in foods has also created a significant concern for the food industry and consumers (Cariolata et al., 2008). Because these bacteria are held responsible for food spoilage, food intoxications, the spread of antibiotic resistance in the food chain, and nosocomial infections (Valenzuela et al., 2008; Çetinkaya and Elal, 2010).

Within the scope of this study, the resistance to antibiotics of *Enterococcus* strains isolated from traditional cheese samples produced in some eastern provinces of Turkey was evaluated.

2. MATERIAL AND METHOD

2.1. Isolation and Genus-Level Identification of Enterococci

For the isolation of *Enterococcus* strains, 10 g of cheese samples were weighed and homogenized in 90 mL of peptone water. After homogenization, serial dilutions of each cheese sample were prepared up to 10^{-7}. Then, 0.1 mL of the prepared serial dilutions was taken with the help of a micropipette and transferred to Kanamycin esculin azide agar media and spread on the surface with a drigalski spatula. Typical enterococcal colonies in petri dishes left incubated for 48 hours at 37ºC; They were selected according to the criteria of dark brown color and clear zone formation around them.

2.1.1. Morphological diagnosis and catalase test

The microscopic morphologies of the selected colonies were determined by examining the preparations prepared by the Gram staining method under a light microscope. For the catalase test, bacterial colonies grown on MRS agar medium were transferred to the slide and a drop of 3% hydrogen peroxide solution was added and examined under the microscope for gas evolution. The test was considered negative in slides in which no gas was observed.

2.1.2. Confirmation of enterococci at the genus level

Gram staining, catalase and growth tests (at 10°C, 45°C and pH 9.6) were applied to confirm the enterococcal strains isolated from cheese samples at the genus level. As a result of the tests, the isolates that were Gram positive, catalase negative and had a positive reaction in the growth tests were *Enterococcus* spp. (Sinton et al., 1993; Harwood et al., 2004).

2.2. Determination of Antibiotic Resistance of Isolates

Disc diffusion test method was used to determine the antibiotic resistance properties of the isolates (NCCLS, 2006). In the study, gentamicin (10 μg), ampicillin (10 μg), erythromycin (15 μg), penicillin G (10 μg), tetracycline (30 μg), vancomycin (30 μg) and streptomycin (10 μg) commercial discs of antibiotics were used.

The isolates to be tested were revived by sowing on Tryptic Soy Agar medium and incubating at 37°C for 24 hours. A suspension of these cultures with turbidity in accordance with the 0.5 McFarland standard was prepared in tubes containing 5 mL of sterile saline. The prepared suspensions were spread on the surface of Mueller Hinton Agar medium and then antibiotic discs were placed. After 24 hours of incubation at 35-37ºC, the zone diameters formed around the antibiotic discs were measured.

3. RESULTS AND DISCUSSION

All isolates are sensitive to Vancomycin. 1 of the isolates is resistant to ampicillin, the others are sensitive. 6 of the isolates are resistant to gentamicin, the others are sensitive. 1 of the isolates is resistant to tetracycline, the others are sensitive. 10 of the isolates are resistant to streptomycin, 11 are intermediate sensitive, 19 are sensitive. One of the isolates was found to be resistant to penicillin G, while the others were sensitive. Of the isolates, 1 was found to be resistant, 12 were intermediate sensitive, and 27 were sensitive to erythromycin.

Belicova et al. (2007) evaluated the susceptibility of 310 enterococcal isolates isolated from 3 different varieties of Slovak Bryndza cheese to 9 different antimicrobial drugs (vancomycin, teicoplanin, ampicillin, streptomycin, gentamicin, erythromycin, rifampicin, nitrofurantoin, and ciprofloxacin). As a result of the study, it was determined that all enterococcal isolates were sensitive to ampicillin, streptomycin, gentamicin, vancomycin, and teicoplanin by disk diffusion method. There are general similarities in terms of some antibiotics examined in this study. In another study by Giraffa and Sisto (1997), the isolates obtained in their study on dairy products did not show high levels of resistance to vancomycin, which is similar to this study.

4. CONCLUSION

It is thought that the reason for the different antibiotic resistance of cheese-derived enterococcal isolates reported in studies may be differences in traditional production. Therefore, it is thought that organic practices may limit the development and spread of antibiotic resistance in foodborne bacteria. No resistance to vancomycin was detected in the enterococcal isolates obtained within the scope of the study. In the study, a total of 40 enterococcal isolates obtained from traditional cheeses were found to be generally sensitive to 7 different antibiotics tested (vancomycin, ampicillin, gentamicin, tetracycline, streptomycin, penicillin and erythromycin). This can be attributed to the fact that in traditional cheese production, there are no chemical inputs and no antibiotics are used in feed additives.

As a result, the presence of even very small numbers of antibiotic-resistant enterococci in traditionally produced cheeses can pose a significant concern for public health. Therefore, the risks and benefits of enterococci in traditionally produced products should be studied and more detailed research should be conducted.

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