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Short Paper

Determination of antibiotic resistance of lactic acid bacteria isolated from traditional Turkish fermented dairy products

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Summary

In this study, the antibiotic resistance (AR) of lactic acid bacteria (LAB) isolated from traditional Turkish fermented dairy products was investigated. Yogurt, white cheese, tulum cheese, cokelek, camız cream and kefir as dairy products were collected from various supermarkets. Lactic acid bacteria such as *Lactobacillus* spp., *Streptococcus* spp., *Bifidobacterium* spp., and *Enterecoccus* spp. were isolated from these dairy products. *Lactobacillus* spp. were resistant to vancomycin (58%), erythromycin (10.8%), tetracycline (4.3%), gentamicin (28%), and ciprofloxacin (26%). *Streptococcus* spp. were resistant to vancomycin (40%), erythromycin (10%), chloramphenicol (10%), gentamicin (20%), and ciprofloxacin (30%). *Bifidobacterium* spp. were resistant to vancomycin (60%), E 15 (6.6%), gentamicin (20%), and ciprofloxacin (33%). *Enterococcus* spp. were resistant to vancomycin (100%), erythromycin (100%), rifampin (100%), and ciprofloxacin (100%). As a result, LAB islated from dairy products in this study showed mostly resistance to vancomycin.

Key words: Antibiotic resistance, Lactic acid bacteria, Traditional fermented dairy product

Introduction

Lactic acid bacteria (LAB) are a group of Gram-positive bacteria, which excrete lactic acid as a main fermentation product into the medium. The main groups include *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* (Mathur and Singh, 2005; Liu *et al*., 2011).

Lactic acid bacteria are the most commonly used microorganisms in fermented foods. The emergence of antibiotic resistance (AR) is a global threat because it reduces the efficiency of the antibiotic therapy, which is made worse by the horizontal transfer of AR genes between bacteria. Fermented foods may be important vehicles for enormous amounts of living bacteria to enter human body. These bacteria may carry transferable AR which could be transferred to commensal or pathogenic bacteria. Although LAB have a long history of being widely used in the production of fermented foods and were generally recognized as safe, some of them showed intrinsic or acquired AR. Therefore, it is necessary to evaluate the AR of LAB in different fermented foods (Clementi and Aquilanti, 2011; Pan *et al*., 2011).

Various LAB have been identified from different fermented dairy foods. Dairy industry is a prime consumer of various LAB strains such as *Lactobacillus*, *Lactococcus*, and *Leuconostoc* (Clementi and Aquilanti, 2011; Liu *et al*., 2011). Lactic acid bacteria carry AR determinants. It is possible for this resistance to be transmitted to the human population through the food chain (Rodríguez-Alonso *et al*., 2009). Although many strains are not pathogenic, they could constitute a reservoir of genes conferring resistance to antibiotics which might be transferred to pathogenic strains. Cheeses produced from raw milk or with a starter including LAB resistant to antimicrobials may act as vectors in generating resistance to certain antibiotics in bacteria infecting humans. The use of suitable starter cultures and appropriate substrates, like pasteurized milk, for food fermentation together with prudent employment of antibiotics are among the measures that can prevent the distribution of bacteria spreading resistance to antibiotics (Herreros *et al*., 2005).

Identification of relationships between technological traits and antimicrobial resistances would allow improvement of the current production of traditional cheeses and selection of more suitable microorganisms that are safe to use in the dairy industry. The greatest threat to the use of antibiotics is the emergence and spread of resistance in pathogenic bacteria that consequently cannot be treated by previously successful regimens. Development of AR in bacteria is mainly based on two factors, the presence of resistance genes and the selective pressure by the use of antibiotics (Mathur and Singh, 2005).

Bacterial resistance to antibiotics is an emerging and serious public health concern, manifested by the compromised efficacy of antimicrobial agents used in the treatment of infectious diseases. Studies on microbial resistance to antibiotics mainly focus on clinically

relevant bacterial species, but in recent years more attention has been given to nonclinical isolates because it is known that AR is also widespread among such isolates. Among nonclinical isolates, commensal and ubiquitous bacteria represent a reservoir of AR genes with the potential to be transferred to human, animal, and microbial pathogens (Rodríguez-Alonso *et al*., 2009).

The aim of this work was to determine AR of LAB isolated from traditional Turkish dairy products.

Materials and Methods

Sampling procedures

Fifty samples of traditional Turkish dairy products (white cheese, yogurt, tulum cheese, kefir, çökelek and camız cream) were supplied from markets in Turkey. Samples were transported in a cooling box to the Department of Food Engineering at Cukurova University, Adana, Turkey. The samples were kept under refrigeration (4°C) until the next day when microbiological analyses were carried out.

Isolation of lactic acid bacteria

Twenty-five g portions of the sample were homogenized with 225 ml 0.1% (wt/vol) peptone water (bacteriological peptone, Oxoid, UK) in a blender (Waring Commercial Blender-USA). The homogenates were then submitted to serial 10-fold dilutions in 0.1% (wt/vol) peptone water and 0.1 ml of each dilution was plated on selective media. De Man, Rogosa Sharpe (MRS) agar (Merck, Darmstadt, Germany) was used for isolation of *Lactobacillus* and incubated anaerobically at 30°C for 48 h. M17 agar (Merck, Darmstadt, Germany) was used for isolation of *Pediococcus* and incubated aerobically at 37°C for 24 h. Kanamycin Aesculin Azide (KAA) (Merck, Darmstadt, Germany) agar was used for isolation of *Enterococcus* and incubated aerobically at 37°C for 24 h. After plating, colonies were randomly picked from plates at higher dilution (10^{-6}) and transferred into 10-ml test tubes with sterile MRS or M17 broth. The isolates were purified by successive streaking on the appropriate agar media before being subjected to the colony characterisation. The isolates were Gram-stained and tested for catalase reaction (Harrigan and McCance, 1998; Halkman, 2005). Presumptive LAB was selected based on the morphology, Gram-reaction and the catalase test. The remaining isolates were then characterised by their growth at various temperatures (10, 15 and 45°C), tolerance of different salt levels (2, 4, and 6.5% NaCl), production of gas from glucose, dextran from sucrose and hydrolysis of arginine (Harrigan and McCance, 1998). The isolates were stored at -20°C in MRS or M17 broth containing 10% glycerol and transported frozen for further analysis.

Antibiotic resistance test

Antibiotic resistance of isolated strains was performed by the Kirby-Bauer disk diffusion method (according to the CLSI document M2-A9 suggestions). Antimicrobial susceptibility test disks are used for determination of AR of bacteria to antimicrobial agents such as erythromycin (E 15), tetracycline (TE 30), vancomycin (VA 30), teicoplanin (TEC 30), chloramphenicol (C 30), ciprofloxacin (CIP 5), rifampin (RD 5), ampiciline (AMP 10), nitrofurantoin (F 300), and gentamicin (CN 10). After strains were activated on nutrient agar, they were cultivated on Mueller Hinton Agar and then antibiotic discs were located by means of dispenser. After incubation (24 h, 37°C), bacterial strains were evaluated as resistant, mid-grade sensitive and sensitive according to the criteria of the NCCLS document M2-A9 by measuring inhibition zone diameters around the antibiotic discs (Gür, 2007).

Results

Lactic acid bacteria isolation from traditional Turkish dairy products

In this study, 50 dairy products (20 yogurt, 15 white cheese, 10 tulum cheese, 3 cokelek, 1 camız cream, and 1 kefir) were analyzed and 72 LBA were isolated from the samples. Isolated LBA are shown in Table 1.

From 15 white cheese samples, 23 isolates were obtained. It was determined that of these, 11 isolates were *Lactobacillus* spp., 7 isolates were *Streptococcus* spp., 4 isolates were *Bifidobacterium* spp., and 1 isolate was *Enterecoccus* spp.

From 20 yogurt samples, 23 isolates were obtained. Of these isolates, 21 isolates were *Lactobacillus* spp., 1 isolate was S*treptococcus* spp., and 1 isolate was *Bifidobacterium* spp.

From 10 tulum cheese samples, 18 isolates were obtained. Of these isolates, 10 isolates were *Lactobacillus* spp., 6 isolates were *Bifidobacterium* spp., and 2 isolates were *Streptococcus* spp.

From 3 cokelek samples, 5 isolates were obtained. Of these isolates, 3 isolates were *Bifidobacterium* spp. and 2

Table 1: Lactic acid bacteria isolated from different dairy products

Dairy product	Number of isolate	Lactic acid bacteria species
White cheese	23	11 *Lactobacillus* spp., 7 S*treptococcus* spp., 4 *Bifidobacterium* spp., and 1 *Enterecoccus* spp.
Yogurt	23	21 *Lactobacillus* spp., 1 S*treptococcus* spp., and 1 *Bifidobacterium* spp.
Tulum cheese	18	10 *Lactobacillus* spp., 6 *Bifidobacterium* spp., and 2 S*treptococcus* spp.
Cokelek	5	3 *Bifidobacterium* spp., and 2 *Lactobacillus* spp.
Kefir	2	1 *Lactobacillus* spp., and 1 *Bifidobacterium* spp.
Cream	1	1 *Lactobacillus* spp.
Total	72	46 *Lactobacillus* spp., 10 S*treptococcus* spp., 15 *Bifidobacterium* spp., and 1 *Enterecoccus* spp.

Table 2: Antibiotic resistance profile of lactic acid bacteria according to dairy product variety

Dairy product	Number of isolate	VA 30 (%)	E 15 (%)	TE 30 (%)	AMP 10 (%)	C 30 (%)	CN 10 (%)	RD 5 (%)	CIP 5 (%)	F 300 (%)
White cheese	23	52	13	0	0	4.3	22	4.3	30	0
Yogurt	23	52	4.3	4.3	0	0	22	0	22	0
Tulum cheese	18	56	11	4.3	0	0	28	0	33	0
Cokelek	5	80	20	0	0	0	40	0	40	0
Kefir	2	100	50	0	0	0	50	0	0	0
Cream	1	0	0	0	0	0	0	0	0	0
Total	72	57	11	2.8	0	1.4	22	1.4	28	0

Table 3: Antibiotic resistance profile of lactic acid bacteria according to species

Strains	Number of strain	VA 30 (%)	E 15 (%)	TE 30 (%)	AMP 10 (%)	C 30 (%)	CN 10 (%)	RD 5 (%)	CIP 5 (%)	F 300 (%)
Lactobacillus spp.	46	58	10.8	4.3	0	0	28	0	26	0
Streptococcus spp.	10	40	10	0	0	10	20	0	30	0
Bifudobacterium spp.	15	60	6.6	0	0	0	20	0	33	0
Enterococcus spp.	1	100	100	0	0	0	0	100	100	0
Total	72	57	11	2.8	0	1.4	22	1.4	28	0

isolates were *Lactobacillus* spp.

From 1 kefir sample, 2 isolates were obtained and, one of them was *Lactobacillus* spp., and the other was *Bifidobacterium* spp.

From 1 cream sample, 1 isolate was obtained and this isolate was *Lactobacillus* spp.

Antibiotic resistance of LAB isolated from traditional Turkish dairy products

In this study, AR of LAB isolated from different dairy products were observed according to both dairy product variety and LAB species. Antibiotic resistance of LAB according to dairy product variety was shown in Table 2 and AR of LAB according to LAB species was given in Table 3. As can be seen in Table 3, 72 LAB were isolated from dairy products and these isolates have vancomycin (57%), erythromycin (11%), tetracycline (2.8%), chloramphenicol (1.4%), gentamicin (22%), rifampin (1.4%) and ciprofloxacin (28%). From these isolates *Lactobacillus* spp. were resistant to vancomycin (58%), erythromycin (10.8%), tetracycline (4.3%), gentamicin (28%), and ciprofloxacin (26%). From these isolates, *Streptococcus* spp. were resistant to vancomycin (40%), erythromycin (10%), chloramphenicol (10%), gentamicin (20%), and ciprofloxacin (30%). From these isolates, *Bifidobacterium* spp. were resistant to vancomycin (60%), E15 (6.6%), gentamicin (20%), and ciprofloxacin (33%). As seen in Table 1, from white cheese samples only 1 isolate was found as *Enterococcus* spp. This *Enterococcus* spp. was resistant to vancomycin, erythromycin, rifampin and ciprofloxacin. This could be associated with the intensive and extensive usage of such antimicrobials within the country.

Discussion

Erkuş (2007) detected LAB in yogurt. In Erkus' (2007) study, *L. delbrueckii* and *S. thermophilus* were identified in yogurt. Our results were similar to Erkus (2007) with regard to concentration of *Lactobacillus* spp. In another study (Karakaş, 2005), 3 *E. faecium*, 1 *E. durans* and 1 *E. avium* were isolated from 30 dairy products. As can be seen, our results were in agreement with this study because number of isolated enterococcus strains were quite few in both studies.

As a result, in this study, of 72 isolates obtained from 50 dairy products, 64% was *Lactobacillus* spp., 21% was *Bifidobacterium* spp., 14% was *Streptococcus* spp. and 1% was *Enterecoccus* spp.

As can be seen in Table 2, out of cream, lactic bacteria in other all dairy products have vancomycin, erythromycin and gentamicin resistance. Whereas as can be seen in Tables 2 and 3, all isolates in dairy products were susceptible to ampiciline and nitrofurantoin. Mostly, LAB islated from dairy products in this study showed resitance to vancomycin. Various strains of lactobacilli were reported to be resistant to high levels of vancomycin. Natural resistance of lactobacilli to kanamycin, gentamicin, streptomycin and vancomycin was also documented by Danielsen and Wind (2003). In addition to this, Florez *et al*. (2005) invesitigated AR of LAB in Spanish traditional blue-veined Cabrales cheese. It was reported that all lactobacilli and leuconostoc isolates were resistant to high levels of vancomycin and also some strains of *L. lactis*, *Enterococcus* spp., and *Lactobacillus* spp. were resistant to antibiotics, such as chloramphenicol, erythromycin, clindamycin, or tetracycline. These results were similar to ours.

The knowledge of intrinsically coded resistance of LAB to common antibiotics is necessary to recognize acquired resistance traits. Enterococci are intrinsically resistant to cephalosporins and low levels of amino-glycoside and clindamycin. The role of the food chain as a possible source of AR enterococci has been proposed, and recently strains harbouring glycopeptide resistance were detected in various foods in Europe. *Lactobacilli*, *Pediococci* and *Leuconostoc* spp. have been reported to have a high natural resistance to vancomycin, a property that is useful to separate them from other Gram-positive bacteria. Some *Lactobacilli* have a high natural resistance to bacitracin, cefoxitin, ciprofloxacin, fusidic acid, kanamycin, gentamicin, metronidazole, nitro-

furantoin, norfloxacin, streptomycin, sulphadiazine, teicoplanin, trimethoprim/sulphamethoxazole and vancomycin. For a number of lactobacilli a very high frequency of spontaneous mutation to nitrofurazone (10-5), kanamycin and streptomycin was found (Cocconcelli *et al*., 2003; Mathur and Singh, 2005).

In the study of Mathur and Singh (2005), it was determined AR of lactobacillus and enterococ was isolated from yogurt and fermented dairy products. They found that vancomycin and gentamicin resistance were similar to ours, but chloramphenicol resistance was different from ours.

Ammor *et al*. (2007) found similar results to ours in terms of gentamicin resistance and chloramphenicol susceptibility of industrial *Lactobacillus*. However, LAB showed susceptibility to erythromycin and tetracycline in the study of Ammor *et al*. (2007) unlike ours.

In the study of Herreros *et al*. (2005) it was determined AR of LAB isolated from Armada cheese. They reported that none of the strains were totally susceptible to all antibiotics tested and multiple resistance was observed. Also most of the tested strains were resistant to cefotoxin, oxacillin, vancomycin, teicoplanin, nitrofurantoin and trimethoprim. It was found that this study was similar to ours with regard to multiple resistance

In addition to these studies, AR of probiotic bacteria was studied and it was reported that some probiotic bacteria had AR. This generates the possibility of resistance transfer from the probiotic to human bacterial pathogens, either directly or indirectly via the commensal flora (Temmerman *et al*., 2003; D'Aimmo *et al*., 2007).

As can be seen from studies mentioned above, profile of AR can change according to product variety or LAB species. So a detailed study should be done about profile of AR and source of bacteria that showed AR should be determined. Also, these results showed that routine antibiotic susceptibility testing of food-associated bacteria was needed.

The food chain is considered as the main route of transmission of AR bacteria between the animal and human population. More specifically, traditional fermented dairy products that are not heat-treated prior to consumption provide a vehicle for AR bacteria, with a direct link between the animals' indigenous flora and the human gastrointestinal tract.

In the present study, multi-resistant bacterial isolates were detected in dairy products. The results of present study can help to prevent the spread of bacterial resistance. Especially, these traditional dairy products contain a great deal of LAB because of spontanous fermentation. These LAB uncontrollaby form and this situation is a risk factor in spread of AR. Because characteristics of LAB in these traditional dairy products are not known, these dairy products can carry AR LAB. So some preventative measures should be taken about traditional dairy production. These results may help us to understand some aspects linked to the difficulty in finding high-quality traditional dairy products in local markets in Turkey. Moreover, these findings encourage us to consider good quality dairy production.

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