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Research Article

ANTIMICROBIAL EFFECT OF COMMERCIAL *L. RHAMNOSUS* STRAINS ON *VANM*-RESISTANT ENTEROCOCCI IN THE CHICKEN MEAT FILLET MODEL

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ABSTRACT. Enterococci are ubiquitous bacteria and are of critical importance due to their ability to harbor antibiotic-resistance genes and their potential to transfer these genes to other bacteria. Vancomycin-resistant enterococci (VRE) are recognized as significant public health pathogens due to limited treatment options. The prevention of the transmission of vancomycin resistance by *vanM*-resistant enterococci to other pathogens, and the spread of this resistance gene to the environment are very significant for public health. Studies on the antimicrobial effect of Lactic acid bacteria (LAB) cultures on chicken meat are generally limited to *Salmonella*, *Escherichia coli*, and *Listeria monocytogenes*, and studies on inhibiting VRE are insufficient. In this context, the suppressive effect of commercially produced *Lactobacillus rhamnosus* cultures on *vanM*-resistant enterococci was investigated in this study. For this purpose, raw chicken fillet samples were contaminated with 4-6 log cfu/ml VRE, then dipped in a solution containing 9 log cfu/ml *L. rhamnosus*. On the application day (day 0), a decrease of 1.36 and 0.54 log cfu/ml was determined in the enterococcal counts in samples contaminated with 4-6 log cfu/ml VRE, respectively. Furthermore, on the 3rd day following the application, there was a decrease of 4 and 2.3 log cfu/ml in the VRE counts in the samples, respectively. After the application of *L. rhamnosus* solution, it was determined that there was more bacterial inhibition in samples with a bacterial density of 4 log cfu/ml than in samples with 6 log cfu/ml ($p<0.05$). Enterococci counts remained below the initial contamination at the end of the 3rd day following the application to the samples with both contaminations. When the results of the study were evaluated, it can be concluded that the commercially used *L. rhamnosus* cultures can be used to suppress VRE in poultry meat and products.

Keywords: *vanM-resistant E. faecium, LAB bacteria, Vancomycin-variable enterococci, Generally Recognized as Safe, Qualified Presumption of Safety.*

INTRODUCTION

Enterococci are bacteria commonly found in the gastrointestinal tract of both humans and animals. Contamination of food animals with enterococci has important public health consequences. In particular, the potential for antibiotic-resistant enterococci to spread from animals to humans raises concerns about antibiotic resistance gene dissemination. This can lead to reduced effectiveness of antibiotics in treating infections in both animals and humans, ultimately compromising medical interventions [1]. In this context, with the

Commission Implementation Decision No. 2020/1729/EU on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria, *Enterococcus faecalis* and *Enterococcus faecium* isolates are expected to be isolated from cecum samples taken from cattle during slaughter [2].

Vancomycin-resistant enterococci (VRE) are recognized as important public health pathogens due to limited treatment options [3], a serious threat that causes an estimated 54,500 cases and 5,400 deaths in hospitalized patients annually [4]. Vancomycin resistance in enterococci is associated with several *van* genes, including *vanA*/*B*/*C*/*D*/*E*/*F*/*G*/*L*/*M*/*N* [5]. Although analyzing only *vanA* and *vanB* genes in universal routine analyses for genotypic typing of vancomycin resistance in enterococci causes *vanM* -type vancomycin resistance to be unnoticed, the most common vancomycin resistance in *E. faecium* and *E. faecalis* is encoded by *vanA*/*B*/*M* genes, which are of clinical importance due to their transferability between bacteria [3]. On the other hand, it has been reported that only *vanA* and *vanM* genes cause high levels of vancomycin and teicoplanin resistance [5]. These data indicate that the global spread of *vanM*-type VRE is a critical public health problem. Therefore, it is critical to prevent *vanM*-resistant enterococci from transmitting vancomycin resistance to other pathogens and from spreading this resistance gene to the environment [6]. *VanM*-type VREs have so far been reported only from Turkey, China, Japan, and Singapore, and their clinical isolation has increased rapidly in China [7, 8, 9, 10]. It has also been reported that *vanM*-type VRE is more common in Shanghai than *vanA*-type [7]. These data indicate that the global spread of *vanM*-type VRE is a critical public health problem.

On the other hand, lactic acid bacteria (LAB) are responsible for the production of many antimicrobial substances such as organic acids and bacteriocins used by the food industry, and therefore their use as biopreservatives in food products has been reported due to their broad antagonistic properties against pathogens [11]. Increasing consumer demand for food products containing fewer chemicals and more natural biological protection keeps the use of LAB, which effectively combats pathogens in food products, current [12]. Biopreservation with LAB is considered the most promising alternative to chemical preservatives in the meat and dairy industry. This is primarily due to LAB's Qualified Presumption of Safety (QPS) status in the European Union and Generally Recognized as Safe (GRAS) status in the United States. These designations confirm the safety of LAB for consumption, making them a preferred option for natural and sustainable food preservation [13]. There are studies on the preservative application of certain LAB strains or their metabolic products in foods against pathogenic microorganisms in dairy products [14, 15, 16, 17] and meat products [18, 19, 20]. However, it is noteworthy that studies conducted to analyze the antimicrobial effect of commercially used LAB cultures on chicken meat are generally limited to *Escherichia coli, Salmonella*, and *Listeria monocytogenes* microorganisms [11, 21, 22, 23].

This study aimed to examine the suppressive effect of commercially produced *L. rhamnosus* cultures on *vanM*-resistant enterococci, which are considered a public health problem, in a contaminated chicken meat model. The research results were expected to help poultry meat producers in providing quality and safe chicken carcass/chicken meat products.

MATERIALS AND METHODS

Preparation of working groups

Within the scope of the study, raw chicken meat fillets were obtained from local sales points in Ankara on the day of the study and delivered to the microbiology laboratory of Ankara University Faculty of Veterinary Medicine, Department of Food Hygiene and Technology, under aseptic conditions. The study was carried out in three repetitions, and in each repetition, five chicken fillet samples weighing 250-350 g were studied. The examples in the working groups are symbolized as K, 4VRE, 6VRE, 4LR, 6LR. Samples in working groups are symbolized as K, 4VRE, 6VRE, 4LR, 6LR. K represents the sample used for control purposes for enterococcal contamination; 4VRE and 6VRE symbolize samples contaminated with an immersion solution containing 4-6 log cfu/ml VRE with a retention time of 30 min, respectively. 4LR and 6LR represent samples contaminated with a dipping solution containing 4-6 log cfu/ml VRE and then immersed in a solution containing 9 log cfu/ml *L. rhamnosus* for 2 min. Samples were analyzed after a retention time of 30 minutes. Following the first day of application, the samples were stored in refrigerator conditions for three days and were analyzed for *Enterococcus* and *Lactobacillus* spp. on the 1st, 2nd, and 3rd days.

Bacterial cultures

The *vanM*-resistant *E. faecium* S98b isolate (GenBank accession number: CP104083.1) isolated from cecum samples in our previous study [10] used as VRE strain in the study was used from the culture collection of Ankara University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology. Nu-trish® LGG, Batch no: 3627300, in lyophilized form, produced commercially by CHR Hansen, was used as *L. rhamnosus* culture.

Preparation of VRE and L. rhamnosus solutions

To prepare VRE solutions, the *vanM*-resistant *E. faecium* S98b isolate, which was enriched in BHI (Brain Heart Infusion, Merck 110493) broth at 37 °C for 18 hours the day before, was used and VRE solutions containing 4 and 6 log cfu/ml were prepared. To prepare the dipping solution containing 4 log cfu/ml VRE, 5 ml of the dilution of the S98b isolate containing 6 log cfu/ml was taken and added to 500 ml BPW (Buffered Peptone Water, Oxoid CM0509) solution. Similarly, to prepare the dipping solution containing 6 log cfu/ml VRE, 5 ml of the dilution of the S98b isolate containing 8 log cfu/ml was taken and added to 500 ml BPW solution [24].

To prepare *L. rhamnosus* solutions, 10 g of *L. rhamnosus* culture in lyophilized form was weighed and added to 500 ml of drinking water and incubated at 37°C for 24 hours.

Microbiological analysis

Weighed 25 g of each chicken fillet sample and diluted with 225 mL BPW, using a stomacher (Blender easyMIX™, bioMérieux) for 60 s. Serial dilutions prepared from homogenized suspensions were plated on SB (Slanetz and Bartley Medium, Oxoid CM377B) and MRS (De Man, Rogosa and Sharpe Agar, Oxoid CM1153) media for the enumeration of *Enterococcus* spp. and *Lactobacillus* spp., respectively. Total *Enterococcus* and *Lactobacillus* spp. counts were determined after 48 hours of incubation at 37°C under aerobic conditions [25, 26].

Statistical analysis

Microbiological analysis results were expressed in terms of mean and standard deviations using Microsoft Office Excel 2019. Significant differences between means were determined by T-test (SPSS for Windows 11; One-way ANOVA).

RESULTS AND DISCUSSION

In the study, the suppressive effect of LAB bacteria, specifically commercially produced *L. rhamnosus* cultures on *vanM*-resistant enterococci was examined in a contaminated chicken meat model. Within the scope of the study, the average values of the microbiological cultivation results of chicken fillet samples performed for three days are given in Table 1. The counts of enterococci in control samples were found to be below the detection limit (2.3 log cfu/ml).

After immersing the samples contaminated with 4 and 6 log cfu/ml VRE in *L. rhamnosus* solution, on the application day (day 0), a decrease in the counts of enterococci was observed by 1.36 and 0.54 log cfu/ml, respectively. It was determined that there was a statistically significant difference in the microbiological analysis results in all groups, that is, on days 0, 1, 2, and 3rd. This result confirms that *L. rhamnosus* application is effective from the first day in eliminating *vanM*-resistant enterococci. *Lactobacillus* spp. counts in the samples immersed in *L. rhamnosus* solution were determined as 7.47 and 7.34 log cfu/ml, respectively. This study revealed that this concentration of LAB bacteria was sufficient to eliminate the pathogenic bacteria used in the study. Furthermore, it is noteworthy that on the 3rd day following the application, there was a decrease of 4 and 2.3 log cfu/ml in the VRE counts in the samples, respectively. The samples with a bacterial density of 4 log cfu/ml are more exposed to the inhibitory effect of *L. rhamnosus* solution ($p<0.05$). Indirectly, at the end of the 3rd day following the application, the difference in bacterial reduction between the groups immersed in *L. rhamnosus* solution and those not exposed to the solution also increased. This reveals the impact of initial contamination in determining shelf life. In similar studies conducted on the reduction of *Salmonella* spp. and *L. monocytogenes* in raw chicken samples inoculated with LAB, it was stated that a decrease of 0.50-1.3 log cfu/ml was detected, similar to the reduction obtained [21, 23].

Fig. 1 and Fig. 2 represent samples contaminated with 4-6 log cfu/ml VRE and the enterococcal colony counts of these samples after immersion in *L. rhamnosus* solution, respectively. Another important detail that stands out is that the samples contaminated with 6 log cfu/ml VRE did not exceed the VRE counts on the day of application even on the 3rd day after being immersed in *L. rhamnosus* solution. When Fig. 1 and 2 are evaluated, it can be observed that the enterococci counts remained below the initial contamination at the end of the 3rd day following the application to samples with both contaminations. In another study examining the effect of LAB bacteria on *E. coli*, similar results were obtained after 7 days, and it was stated that the counts of *E. coli* colonies at the end of the 7th day after the application of 5 log cfu/ml *L. lactis* was similar to the initial contamination [22].

Table 1. *The enterococci and lactobacilli counts of the study groups symbolized as 4 VRE, 6 VRE, 4LR, 6LR*

	Medium	Day 0	Day 1	Day 2	Day 3
4VRE	SB	4,14	5,14	6,2	7,3
	MRS	-	-	-	-
6VRE	SB	6,14	6,5	7,2	7,9
	MRS	-	-	-	-
4LR	SB	2,78	3,25	3,78	3,3
	MRS	7,47	7,3	7,07	7,9
6LR	SB	5,6	5,38	5,2	5,6
	MRS	7,34	7,3	7,3	8,07

**4VRE: 4 log cfu/ml VRE; 6VRE: 6 log cfu/ml VRE; 4LR: 4 log cfu/ml L. rhamnosus; 6LR: 6 log cfu/ml L. rhamnosus; -: <2,3 log cfu/ml*

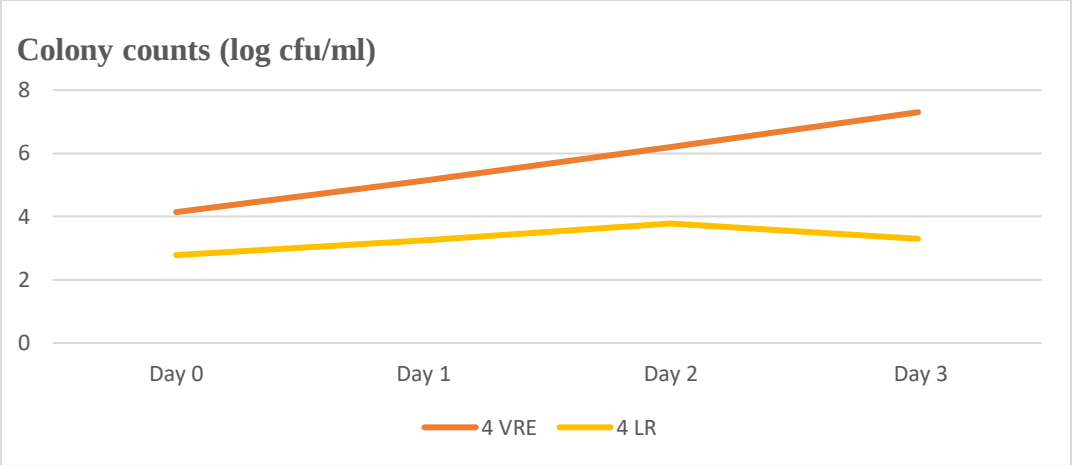

Fig. 1. *Samples contaminated with 4 log cfu/ml VRE and enterococcal colony counts after dipping these samples into L. rhamnosus solution*

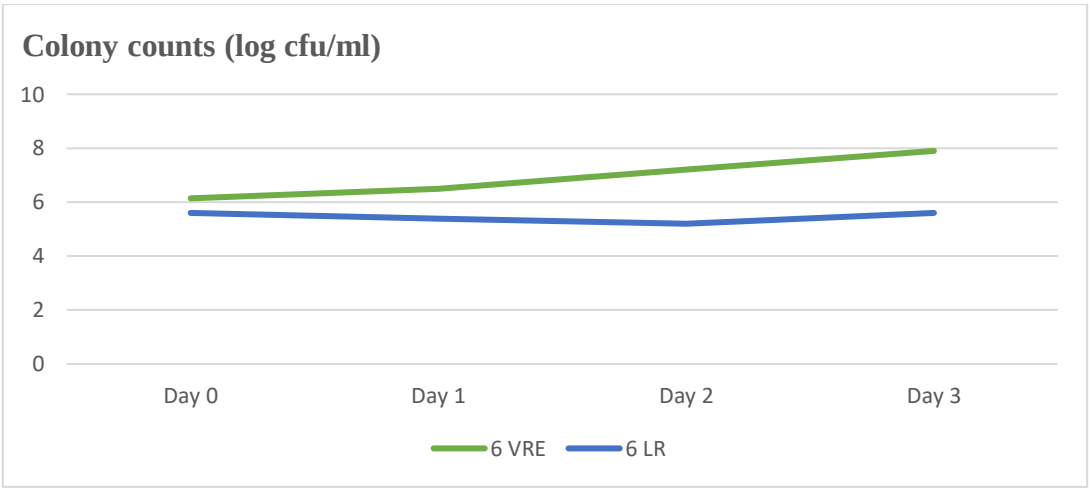

Fig. 2. *Samples contaminated with 6 log cfu/ml VRE and enterococcal colony counts after dipping these samples into L. rhamnosus solution.*

It is noteworthy that studies on the antimicrobial effect of commercially used LAB cultures, especially in chicken meat, are generally limited to *E. coli*, *Salmonella*, and *L. monocytogenes* microorganisms, and studies on the inhibition of VRE are insufficient. Within the scope of the study, the antimicrobial effect of commercially produced *L. rhamnosus* cultures on *vanM*-resistant enterococci, which are considered a public health problem, was determined.

When all these results are evaluated, it is emphasized that commercially used *L. rhamnosus* strains can also be used to suppress VRE in poultry meat processing. The selection of LAB bacteria in meat processing systems is of critical importance. Among the basic requirements for LAB strains to be used in businesses due to their inhibitory properties against pathogenic microorganisms; a) They are in the GRAS category, b) they do not cause any harmful effects on the sensory, chemical and physical properties of the target food, c) they do not lose their viability under adverse conditions, and d) they do not become pathogenic at refrigerator temperatures and their ability to maintain their inhibitory properties against spoilage bacteria [21,27].

CONCLUSION

It is concluded from the study that commercially used *L. rhamnosus* cultures can be used to suppress VRE in poultry meat and its products. It is predicted that *L. rhamnosus* dipping application will be more effective, especially in poultry meat sections with initial contamination of 6 log cfu/g and below bacterial load, and will play an active role in extending the shelf life. It is expected that the research results will help poultry meat producers and consumers in providing quality and safe poultry carcasses or meat products.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: B.O.A Design: B.O.A Data Collection or Processing: E.B., İ.T., Analysis or Interpretation: E.B., İ.T., Literature Search: E.B., İ.T., Writing: B.O.A

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