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Original article

The Antibiotic Susceptibility Pattern and Prevalence of *vanA, vanB,* and *vanC* Genes among *Enterococcus faecalis* Strains Isolated from Consumed Meat

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Abstract

Background: *Enterococci* play an important role in the spread of drug resistant genes and thus resistant strains. The dissemination of vancomycin-resistant *Enterococci* (VRE) strains is one of the crucial issues in hospitals worldwide, especially among those hospitalized patients. This study aimed to assess the antibiotic resistance pattern and the prevalence rates of *vanA*, *vanB*, and *vanC* genes among *Enterococcus faecalis* strains isolated from meat.

Materials and Methods: This cross-sectional study was performed on 181 isolates of *E. faecalis* isolated from consumed meat samples in Borujerd city. Antibiotic susceptibility testing was performed using the disk diffusion method according to CLSI criteria. The prevalence rate of *vanA* and *vanB* genes in vancomycin resistant *E. faecalis* strains was identify by PCR technique.

Results: Of 181 *Enterococci* isolates, 100 strains (55.25%) were *E. faesium*, and 81 strains (44.75%) were *E. faecalis*. About 13 antibiotics were used in this study. The highest resistance was observed against erythromycin, linezolid, vancomycin, and penicillin antibiotics, and the lowest resistance was observed against meropenem; none of the isolates were resistant to nitrofurantoin and cefotaxime. A total of 68 isolates (83.95%) were resistant to vancomycin. Among the isolates, 38 isolates (46.9%) contained *vanA* gene, 21 isolates (25.9%) carried *vanB* gene, and 18 (22.2%) isolates contained *vanA* and *vanB* genes, but *van C* type was not detected in none of the isolates.

Conclusion: The presence of *van* gene in the majority of isolates is an indicator of resistant genes large reservoir in the strains rotation in the community. Furthermore, in order to limit the incidence of VRE, the use of antibiotics for human or animal should be taken with caution.

Key words: Enterococcus faecalis, Antibiotic resistance, Vancomycin, *van A / B / C*

1. Background

Enterococci are Gram-positive fermentative bacteria seen as a chain or single cocci (1). These bacteria have become appropriate strains for research regarding antibiotic-resistant genes transferred to other species, their permanent existence in the human and animal gut as normal flora, their clinical significance in causing infections, their ability to acquire multi-antibiotic resistance, their high capacity for horizontal genes transduction due to mutation, and receiving genetic components through plasmids or transposons transfer (2). These opportunistic pathogens are capable in causing various types of urogenital, endocarditis, meningitis, bloodstream infections, and infection in newborns (3). In addition to warm-blooded animals, *Enterococci* are present in water, soils, and vegetables (4). Studies have shown that 90 and 10% of the enterococcal infections in humans are caused by *E. faecalis* and *E. faecium*, respectively (5). These two *Enterococci* species are mostly found in foods such as cheese, fish, sausages, and cowpeas. Many antibiotics including beta-lactams, macrolides, aminoglycosides, and glycopeptides are used for the treatment of the enterococcal infections (6). The resistance of *Enterococci* to antimicrobial agents is intrinsically (low-level resistance to penicillins, cephalosporins, and aminoglycosides) or acquired (to glycopeptides and high concentrations of aminoglycosides) (7). Due to the spread of multidrug-resistant strains, vancomycin is nowadays as a selective drug for the eradication of drug-resistant enterococcal infections. This drug is one of the last line antibiotics consumed for the treatment of infections caused by Gram-positive multidrug resistant bacteria (8). According to the phenotype and genotype characteristics, there are eight types of vancomycin resistance-acquired genes, including *van A, van B, van C, van E, van D, van G, van M, van L*, and *van N* (9).

In addition, an type of inherent resistance has been identified in *van C* gene (10) as an intrinsic property of motile *Enterococci* (*casseliflavus*, *gallinarum*, *flavescens*). Genes such as *van A, van B, van C, van C2 /* 3, *van G*, and *van L* are responsible for coding resistance to vancomycin, teicoplanin, and avoparcin. The most commonly found resistance is due to *van A* and *van B* genes which are carried on Tn1546 transposon and Tn5382 transposon, respectively (11). Antibiotics used in the livestock (such as avoparcin, bacitracin, spiromycin, tetracycline, and ampicillin) as a growth factor can act as mediators for the increase and spread of VRE in domestic animals; thus, vancomycin-resistant *Enterococci* (VRE) strains have the ability to be transferred to humans via infected food (12). During research in Europe and the United States, VRE strains were identified in pigs, breeding horses, cattle, sheep, chickens, and turkeys (13). Previous studies have shown that humans have a large amount of VRE in their stool and thus are considered as the carriers of which. But researchers were not able to find VRE in stool samples of those who had consumed plants for many years; therefore, the theory confirms that meat is infected with VRE source (14).

2. Objective

The aim of this study was to investigate the antibiotic resistance pattern and the prevalence rates of vanA, vanB, and vanC genes among Enterococcus faecalis strains isolated from meat.

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3. Materials and methods

3.1. Bacterial samples

This cross-sectional study was carried out on 181 *Enterococcus* isolates isolated from collected red meat of slaughterhouses in Borujerd city during 2014-2015 years.

All the samples were cultured in BHI broth (Merck) medium and incubated at 37°C for 24 h. After 24 hours, colonized bacteria were subjected to the followings: staining with gram staining method, catalase test, bile esculin agar, PYR test, growth in BHI broth containing 6.5% sodium chloride, and growth at 45°C. Then, *Enterococci* isolates were identified by the acid production from mannitol, sorbitol, arabinose, and sucrose (1). Performing the related tests, 81 and 100 isolates of *E. faecalis* and *E. faecium* were identified, respectively.

3.2. The antibiotic susceptibility testing

In antibiotic susceptibility testing, the following antibiotics prepared by ROSCO were used: vancomycin (30 µg.disc), ciprofloxacin (5 µg.disc), gentamicin (10 µg.disc), tetracycline (30 µg.disc), chloramphenicol (30 µg.disc), ampicillin (10 µg.disc), penicillin (10 µg.disc), erythromycin(15 µg.disc), linezolid (30 µg.disc), nitrofurantoin (30 µg.disc), streptomycin (30 µg.disc), meropenem (10 µg.disc), and cefotaxime (30 µg.disc). The Kirby-Baur disk diffusion method was performed in the Müller Hinton Agar medium (Merck). Phenotypic resistance was determined according to the CLSI 2015 guidelines. In this study, standard strains of *E. faecalis* ATCC 29212 were used as quality control.

3.3. DNA isolation and PCR reaction

Following determination of the vancomycin-resistant isolates, DNA extraction was performed using a DNA extraction kit (Sinagen). In order to detect *van A*, *van B*, and *van C* genes, the PCR reaction and their specific primers were used (Table 1).

3.4. PCR amplification of van genes

PCR reaction was performed with a final volume of 25 µL, containing 12.5 µL of master mix (Sinagen), 7.5 µL of sterile distilled water, 1 µM of each primer, and 3 ng of template DNA. The PCR reaction was carried out in a thermocycler device (Bio Rad), the temperature and conditions are shown in Tables 2. PCR products were transferred to a 1.5% agarose gel containing ethidium bromide and electrophoresed with 0.5 X TBE buffer (Sina gene). To determine the size of PCR products, a 100-bp molecular marker (Merck) was used. *E. faecium* ATCC 51559 and *E. faecalis* ATCC 51299 were used as a positive control for *van* genes (prepared from the microbial bank of Pasteur Institute of Iran). The standard strain was purchased in the form of liofilization, and its passage culture was carried out in a nutrient broth medium and incubated at 37°C for 24 h.

2.5. Data analysis

Data were analyzed using SPSS software version 21, where ANOVA tests were applied with 95% confidence interval and a significance level of $p<.05$.

4. Results

4.1. Bacterial isolates

A total of 181 isolates of *Enterococci* were isolated from red meat of slaughterhouses in Borujerd city and identified with the conventional biochemical test and confirmed with 16srRNA genes evaluation by PCR method, of which 81 isolates (44.75%) were *E. faecalis*, and 100 isolates were *E. faecium*.

4.2. The antibiotic susceptibility of isolates

All the 81 isolates of *E. Faceless* were enrolled in the antibiotic susceptibility testing with the disc diffusion method. The highest isolates resistance was observed against erythromycin (96.29%, n=78), linezolid (86.41%, n=70), vancomycin (83.95%, n=68), and penicillin (56.79%, n=46), whereas the lowest resistance was observed against meropenem (2.46%, n=2) and likewise, all were susceptible to nitrofurantoin and cefotaxime discs. The isolates antibiotic resistance profile is depicted in Table 3, and the resistance pattern of *E. faecalis* isolates from red meat is shown in Figure 1.

Table1. Primers used for the amplification of *van A*, *van B*, and *van C* genes in this study.

Gene	Amplicon (bp)	Primer Sequence (5'→3')	Reference
van A	734	F: AATACTGTTTGGGGGTTGCT R: CTTTTTCCGGCTCGACTTCCT	1
van B	420	F: GCGGGGAGGATGGTGC R: GGAAGATACCGTGGCTCAAA	1
Van C	485	F:ATGGCTGTATCCAAGGACTG R: AGGCAATGGTGCTGGGAC	15

Table 2. PCR reaction thermal profile for the *van A*, *van B* and *van C*.

Program	*VanA*			*VanB*			*Van*C		
	Tempreture	Time	Cycles	Tempreture	Time	Cycles	Tempreture	Time	Cycles
Initial Denaturation	94 °c	5 min	1	94 °c	4min	1	94°c	5min	1
Denaturation	94 °c	1min		94 °c	1min		94°c	1min	
Annealing	58 °c	1min	35	52 °c	1min	30	60°c	1min	35
Extension	72°c	1min		72°c	1min		72°c	1min	
Final Extension	72°c	10min	1	72°c	7min	1	72°c	10min	1

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Table3. Antibiotic resistance pattern of *E. faecalis* isolates in this study .

The Rate of Resistance, No (%)	Antibiotics Resistance Pattern
14 (28/17%)	Two antibiotics
44 (32.54%)	Three antibiotics
8 (87.9%)	Four antibiotics
7 (64.8%)	Five antibiotics
5 (17.6%)	Six antibiotics
4 (93.4%)	Seven antibiotics
--	All antibiotics
--	Susceptible to all antibiotics

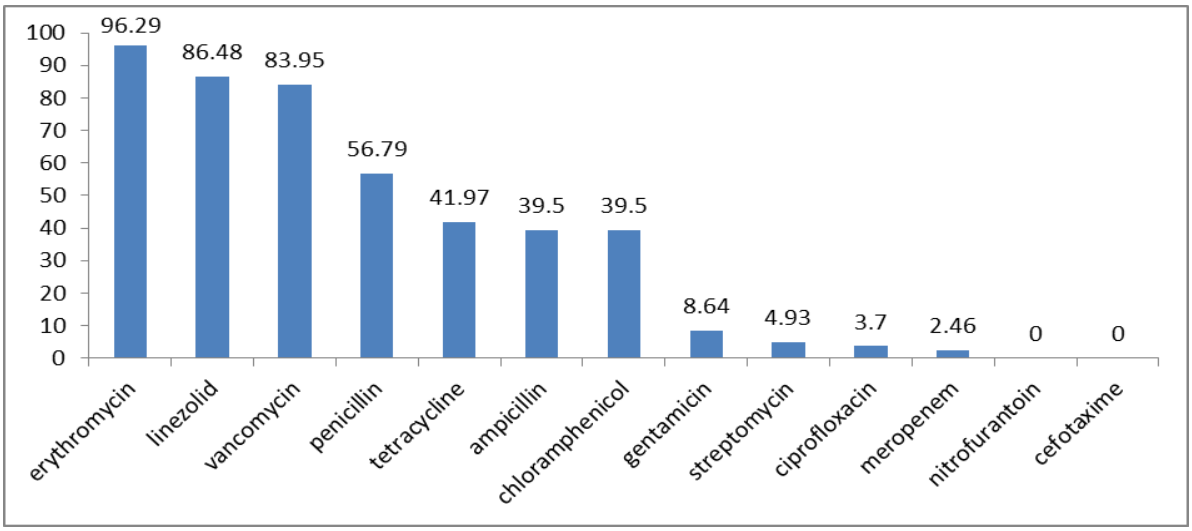

Figure 1. The antibiotic resistance pattern of *E. faecalis* isolates from consumable meat.

4.3. PCR amplification of van A, van B, and van C genes

After examining the extracted genome of vancomycin-resistant *E. faecalis* (VRE), 68 strains (83.95%) were resistant to vancomycin, 38 (46.9%) amplified *van A* gene, 21 strains (25.9%) contained *van B* gene, and in 18 isolates (22.2%) both *van A* and *B van* genes were detected, but *van C* phenotype was not amplified in any of the strains by PCR (Figures 2 and 3).

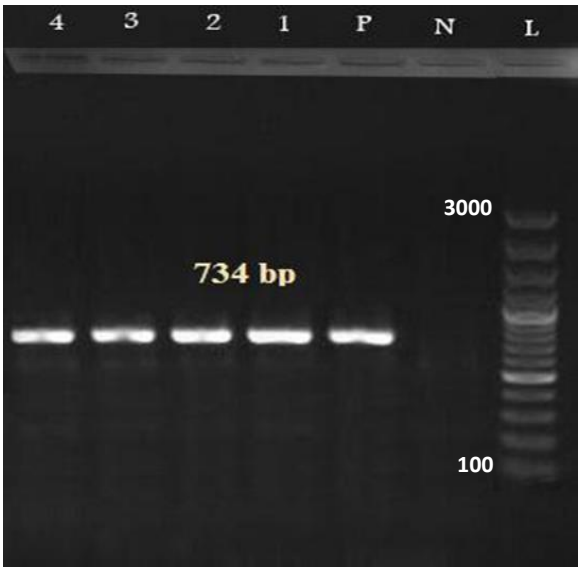

Figure 2. Electrophoresis of the PCR product of van A gene, L: 100bp ladder/marker, N: negative control E. faecalis ATCC51299, P: positive control E. faeciumATCC51559, and columns 1-4: van A gene.

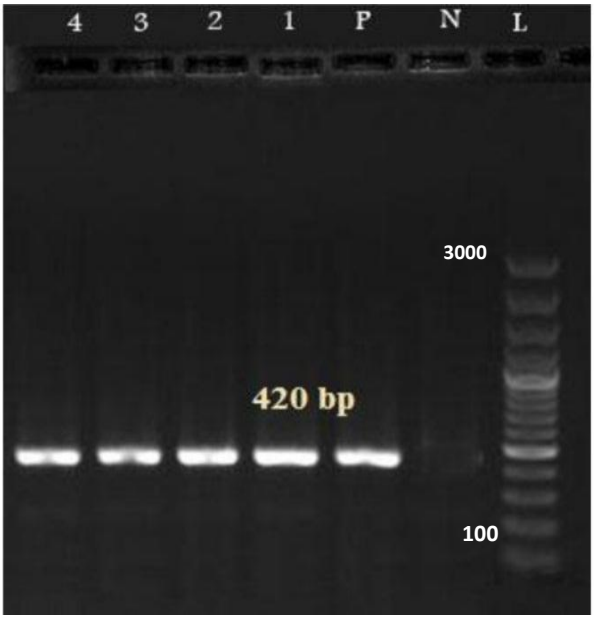

Figure 3. Electrophoresis of PCR product of the *van B* gene, L: 100 bp marker, N: negative control *E. faecium* ATCC51559, P: positive control *E. faecalis* ATCC51299, and columns 1-4: the *van B* gene.

5. Discussion

Food is one of the leading reservoirs for the transmission of antibiotic-resistant bacteria among human and animal populations (16). In this process, those antibiotic resistance-encoding genes can be transmitted from microbial flora into

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human pathogens (17). *Enterococci* spp are able to colonize in healthy animals, which is a concern due to the presence of strains carrying genes encoding antibiotic resistance and the possibility of spread of non-susceptible *Enterococci* species (18). Various surveys have demonstrated that primary vancomycin-resistant *Staphylococcus aureus* (VRSA) strains have probably received vancomycin-resistant gene (*vanA*) from *Enterococcus* strains which had contaminated food (19).

The consumption of antibiotics by humans, agricultural industries, and veterinary medicines used for livestock and poultry cultivated for human nutrition increase the resistance patterns and the emergence of multidrug resistant isolates and have made these strains as an epidemiological problem all over the world (20).

In this study, in addition to the prevalence rate of *van* gene, the resistance pattern and multiple resistance pattern of *Enterococci spp* isolates were investigated. *Enterococcus* isolates depicted the highest resistance to erythromycin 96.29% (78 samples) and the lowest resistance to meropenem (2.46%, n=2). This finding indicates the most commonly used antibiotic intaked in one area, and that erythromycin has probably been used far more than other antibiotics in livestock breeding. Drug resistance pattern in various regions of Iran and the world is not the same due to such factors as genetic alterations in the isolates, difference in the amount of antibiotics used, and the difference in the antibiotics availability.

In this study, 82.9% of the isolates were MDR, and 6% of them demonstrated extensively drug resistant (XDR) phenotype. This phenotype is a potential crisis in the treatment of infections caused by these strains in humans.

Chingwaru and colleagues (2003) isolated *E. faecalis* strains from 46.1% of the meat samples, consistent with the frequency of this bacterium in the present study. In their study, although the prevalence of the residual pattern obtained by disc diffusion method showed 96% resistance to ampicillin, resistance to vancomycin was observed in 39.7% of the isolates (21), which was inconsistent with the present study results in which 83.95% resistance to vancomycin was an enhancing trend. In another study, the MIC method used for *E. faecalis* strains isolated from pork showed a higher concentration for the isolates than what is recommended for antibiotics like vancomycin (22).

Considering the similarity between the resistance pattern of the isolates such as *Enterococci* spp in hospital settings and environment, it is possible to reduce the risk of clinical resistance by controlling and preventing environmental contamination.

Rozanska et al. (2015) showed that 94 (119.68%) *E. faecalis* strains isolated from red meat were resistant to vancomycin, which is consistent with our results (23).

In a research conducted by Johnston (2004) in the United States on fresh meat products, 34% of the *E. faecalis* isolates were multidrug resistant (24) while in our research, the amount of multidrug resistant isolates were significantly higher (2.5 fold), highlighting the irrational use of antibiotics in the food industry.

6. Conclusion

The emergence of MDR enterococcal strains has led to a crisis in the medical community. Therefore, the following steps are recommended to be taken: the use of control methods for preventing the horizontal transmission of genes, the proper use of antibiotics, and taking into account the important role of livestock specimens as the reservoirs of resistance indices because these factors have a significant effect on decreasing trend of the resistance phenotype. Due to the transferability of vancomycin-resistant genes among animals, humans, and the environment, the contamination of consumable meat by VRE is a serious threat for the transfer of these resistant strains to humans. Resistance to glycopeptides, such as vancomycin and teicoplanin, limits the therapeutic selections, and there is another risk of resistance genes transfer to other bacteria such as *Staphylococci* strains. In order to limit the incidence of VRE, caution should be exercised in medicines consumption both by animal and human; also, precaution should be taken against the spread of *Enterococci* species by carefully monitoring the health and nutrition systems.

Conflict of interest

None to declare by the authors.

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Authors Contribution

MR.Mehrabi designed the research, helped in data analysis and writing the paper; E.Madanipour performed experiments, wrote the paper, and analyzed the data; M.Mirzaee advised for PCR technique, extracted DNA.

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References

1. Khan SA, Nawaz MS, Khan AA, Hopper SL, Jones RA, Cerniglia CE. Molecular characterization of multidrug-resistant *Enterococcus* spp. from poultry and dairy farms: Detection of virulence and vancomycin resistant gene markers by PCR. Mol Cell Probes. 2005;19(1):27-34.
2. Bjørkeng EK. On mobile genetic elements in enterococci: Adding more facets to the complexity. 2010.
3. Kolar M, Urbanek K, Vagnerova I, Koukalova D. The influence of antibiotic use on the occurrence of vancomycin-resistant *Enterococci.* J Clin Pharm Ther. 2006; 31(1): 67-72
4. Jones RN, Marshall SA, Pfaller MA, Wilke WW, Hollis RJ, Erwin ME, et al. Nosocomial enterococcal blood stream infections in the SCOPE program: Antimicrobial resistance, species occurrence, molecular testing results, and laboratorytesting accuracy. Diagn Microbiol Infect Dis. 1997; 29(2): 95–102.
5. Gordon S, Swenson J, Hill B, Pigott N, Facklam R, Cooksey RC, et al. Antimicrobial susceptibility patterns of common and unusual species of *Enterococci* causing infections in the United States. J Clin Microbiol. 1992; 30(9):2373-8.
6. Pootoolal J, Neu J, Wright GD. Glycopeptide antibiotic resistance. Annu Rev Pharmacol Toxicol. 2002; 42(1):381-408.
7. Liassine N, Frei R, Jan I, Auckenthaler R. Characterization of glycopeptide-resistant *Enterococci* from a Swiss hospital. J Clin Microbiol. 1998; 36(7):1853-8.
8. Ghaffarpasand I, Moniri R, Kheradi I. The prevalence of fecal carriage of antibiotic resistant *Enterococci* among hospitalized patients in Shahid Beheshti hospital, Kashan, Iran at 2007..
9. Macove L, and Zurek L. Ecology of antibiotic resistance genes: Characterization of *Enterococci* from house flies collected in food setting. Appl Environ Microbiol. 2006; 71(6):4028-35.
10. Courvalin P. Vancomycin resistance in gram-positive cocci. Clin Infect Dis. 2006; 42(Suppl 1):S25-34.
11. Appleman MD, Citron DM, Kwok R. Evaluation of the velogene genomic assay for detection of *van A* and *van B* gene in vancomycin resistant *Enterococcus* species. J Clin Microbial. 2004;42(4):1751-2
12. Ballard SA, Pertile KK, Lim M, Johnson PDR, Grayson ML. Molecular characterization of *van B* elements in naturally occurring gut anaerobes. Antimicrob Agents Chemother. 2005;49(5):1688-94.
13. Vidyalakshmi PR, Gopalakrishnan R, Ramasubramanian V, Abdul Ghafur K, Senthur Nambi P,Thirunarayana MA. Clinical, epidemiological, and microbiological profile of patients with vancomycin-resistant *Enterococci* from a tertiary care hospital. J Glob Infect Dis. 2012; 4(2):137–138

[DOR: 20.1001.1.25884107.2017.3.4.4.7]

14. Naas T, Fortineau N, Snanoudj R, Spicq C, Durrbach A, Nordmann P. First nosocomial outbreak of vancomycin-resistant *Enterococcus faecium* expressing a *vanD*-like phenotype associated with a *van A* genotype. J Clin Microbiol. 2005; 43(8):3642-9.
15. Sun M, Wang Y, Chen Z, Zhu X, Tian L, Sun Z. The first report of the vanC1 gene in Enterococcus faecium isolated from a human clinical specimen. Memórias do Instituto Oswaldo Cruz. 2014;109(6):712-5.
16. Giraffa G. *Enterococci* from foods. FEMS Microbiol Rev. 2002; 26(2):163-71.
17. Fisher K, Phillips C. The ecology, epidemiology, and virulence of *Enterococcus*. Microbiology. 2009; 155(Pt 6):1749-57.
18. Mozafari NA, Forouhesh Tehrani H, Salek Moghadam A. The prevalence of Enterobacteriaceae producing heat-stable enterotoxin in food sent to the microbiology laboratory of food science research and educational center lab. Pajouhesh dar Pezeshki (J Res Med Sci). 2002; 26(1):65-69.
19. Normanno G, La Salandra G, Dambrosio A, Quaglia NC, Corrente M, Parisi A, et al. Occurrence,characterization, and antimicrobial resistance of *enterotoxigenic Staphylococcus aureus* isolated from meat and dairy products. Int J Food Microbiol. 2007; 115(3):290-6.
20. McGowan LL, Jackson CR, Barrett JB, Hiott LM, Fedorka-Cray PJ.Prevalence and antimicrobial resistance of *Enterococci* isolated from retail fruits, vegetables, and meats. J Food Prot. 2006; 69(12): 2976-82.
21. Chingwaru W, Mpuchane S, Gashe B. *Enterococcus faecalis* and *Enterococcus faecium* isolates from milk, beef, and chicken and their antibiotic resistance. J Food Prot. 2003; 66(6):931-6.
22. Rizzotti L, Rossi F, Torriani S. Biocide and antibiotic resistance of *Enterococcus faecalis* and *Enterococcus faecium* isolated from the swine meat chain. Food Microbiol. 2016; 60:160-4.
23. Różańska H, Lewtak-Piłat A, Osek J. Antimicrobial resistance of *Enterococcus faecalis* isolated from meat. Bulletin of the Veterinary Institute in Pulawy. 2015; 59(2):229-33.
24. Johnston LM, Jaykus L-A. Antimicrobial resistance of Enterococcus species isolated from produce. Appl Environ Microbiol. 2004; 70(5):3133-7

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