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Research article

DISTRIBUTION OF ANTIBIOTIC RESISTANCE GENES IN *ENTEROCOCCUS* SPP. ISOLATED FROM MASTITIS BOVINE MILK

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In this study, determination of enterococcus species that were isolated from mastitic milk samples, investigation of their susceptibilities to antibiotics and identification of the existence of resistance genes in resistant strains were conducted. The specimens consist of 600 mastitic milk samples that were collected from 242 cows. Isolation of enterococcus was carried out in selective media and 94 (15.6%) *Enterococcus* spp. were isolated. A total of 94 species of Enterococci were identified using both sequencing and polymerase chain reaction (PCR). *Enterococcus* spp. isolates belong to 5 different species (*E. faecalis, E. faecium, E. durans, E. hirae, E. mundtii*) in sequence analysis and 4 different species (*E. faecalis, E. faecium, E. durans, E. hirae*) were identify by PCR method with specific primers. Analyzing 94 enterococcus strains by antibiotic sensitiveness test a high rate of resistance to tetracycline in 77 (81.9%) isolates was shown. The *tet* resistance genes were identified as follows: 54 were *tet*M positive, 23 were *tet*K positive and 17 were positive on *tet*M and *tet*K. Resistance to erythromycin was established in 27 (28.7%) isolates (25 *erm*B) while the chloramphenicol resistance gene was found in 10 (10.7%) of isolates and the *cat* gene was identified in nine samples and one isolate was resistant to vancomycin (1.06%) with the *Van*A gene confirmed. In conclusion, it was shown that *E. faecalis* has the biggest role in *enterococcus* originated mastitis and these strains were found to be mostly resistant to tetracycline. One vancomycine resistant isolate that had the *Van*A gene was also determined.

Key words: Antibiotic resistantance genes, *Entrococcus*, Mastitis, PCR, *van*A

INTRODUCTION

Enterococci can cause many economically important animal diseases including bovine mastitis [1]. Mastitis reduces milk yield, increases health cost and makes milk less suitable for both consumption and processing [2]. *Enterococcus* can enter from the teat surface (if it is not disinfected properly) to the teat canal and might cause mastitis. Enterococci can be transmitted between the environment and the animal rather than

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from animal to animal [3]. When data were analyzed, the incidence of Enterococcus spp.as aetiological agents of bovine mastitis varied from 0 [4] to 46% [5,6].

In addition to their importance in disease, enterococci can generate antibiotic resistance against a number of antibiotics. This resistance evolves in the bacteria that have resistance genes. Enterococci may harbor multi-drug resistance for antimicrobial agents such as cephalosporins and aminoglycosides and the *van* gene can be transfered to other gram positive bacteria. Vancomycin-resistant enterococci (VRE) were first reported in 1998 and their incidence has increased rapidly through the world after that [7]. VRE strains have been mentioned in human sciences and subsequently in veterinary sciences in previous studies, but the gene has not been discovered in our province until now.

In addition to their present resistance to antibiotics at, bacteria can generate antibiotic resistance with remarkably new mechanisms and can transfer this resistance to each other [8,9]. Resistance genes are often easily transferred to other species through conjugative transposons and plasmids showing the broad host profile [10].

In this study, determination of enterococcus species that were isolated from mastitic milk samples, investigation of their susceptibilities to antibiotics and identification of the resistance genes was conducted.

MATERIALS AND METHODS

Sample Collection

In this study, 600 mastitic milk samples that were collected from 242 cows in 38 different private dairy cattle farms in Aydin were included in the study. Clinical mastitis was diagnosed according to the presented changes in the udder and milk by veterinary practitioners. Changes in the udder included pain, swelling, warmth and abnormal appearance of milk (blood tinged milk, watery secretions, clots, pus). Cows that did not have clinical mastitis were subjected to further investigation for subclinical mastitis using California Mastitis Test (CMT). The procedures and interpretations have been described previously [11]. For collection of milk samples, the teat tips were cleaned using 70% alcohol moistened swabs and allowed to dry. After discarding the first few milk jets, 2-5 ml of the milk samples were collected into sterile 5 ml glass flasks. Approximately 6-8 mastitic cattle were sampled and tested for the presence of enterococci, on each of 38 dairy farms from end of 2012 to 2014.

Isolation and Identification of Enterecocci

For isolation, one full loop of mastitic milk sample was inoculated to selective Chromocult® Enterococci Broth (Merc, Darmstadt, Germany). The enrichment broth was incubated for 18–24 h at 37°C. Colour change was evaluated as an indicator of enterococci growth in the medium. Positive cultures were transferred to BBL™ Enterococcosel Agar (EA) (Becton Dickinson, Heidelberg, Germany) for the isolation

of enterococci. Plates were incubated overnight at 37°C. At the end of the period black and dark brown colonies were subcultured onto Difco mEnterococcus (Difco, Heidelberg, Germany) agar plates and incubated at 37°C for 48 h. [12]. For the separation at the level of the genus Enterococcus, the presumptive positive colonies Gram stain, catalase tests on slide was performed as well as growth ability in nutrient broth (Merc, Darmstadt, Germany) containing 6.5% NaCl were made [13]. The purified and confirmed (salt tolerant and catalase-negative) enterococci were inoculated into Enterococcosel Agar (Becton Dickinson, Heidelberg, Germany) plates and incubated overnight at 37°C. All suspicious colonies were stored in Brain Heart Infusion Broth (Oxoid, Hampshire, UK) with 20% glycerin at -20ºC until identified using molecular methods.

Antimicrobial Susceptibility Testing

The antimicrobial sensitivity phenotypes of bacterial isolates were determined using a Kirby-Bauer disk diffusion assay according to the standards and interpretive criteria described by Clinical and Laboratory Standards Institute [14]. The following antibiotics were used: ampicillin (AMP), 10 μg; tetracycline (TET), 30 μg; streptomycin (STR), 10 μg; vancomycin (VA), 30 μg; Teicoplanin (TEC), 30 μg; erythromycin (E), 15 μg; ciprofloxacin (CIP), 5 μg; chloramphenicol (C), 30 μg; gentamicin (CN), 120 μg; The disks were purchased from Oxoid (Hemakim, Izmir, Turkey) and the results were recorded based on CLSI guidelines [14]. The reference strains *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were used as the quality control.

Molecular Methods

The enterococus suspicious colonies were confirmed genetically using genus [15] and species [16] specific primers for the PCR. The Uniplex PCR was used for the detection of antibiotic resistance genes in resistance strains. All Oligonucleotide primers used to in the study are listed in Table 1.

DNA extraction

Genomic DNA was isolated by the commercial extraction kit (InstaGene Matrix, Germany). The integrity of DNA isolated by electrophoresis was shown to be complete [17]. Quantitative determinations of the isolated DNAs were performed by nano spectrophotometer (Thermo NanoDrop 2000/2000c). DNA quantity was measured with regard to absorbance value, in conclusion A260 / A280 ratios between 1.8 and 2 DNAs were considered useful [18].

Polimerase Chain Reaction (PCR)

All the PCR reactions were carried out in a final volume of 30 μl containing 1X PCR buffer, 2 mM $MgCl_2$, 200 mM each of the four dNTPs, 0.5 mM of each primer and

1.25 units of Taq DNA polymerase. The cycles used were 95°C for 5 min, 95°C for 30 s, 51 °C (*ddlE* $_{faecium}$), 53°C (*ddl*$_{E.faecalis}$) 55°C (*ddl*$_{E.faecalis}$) 57°C (*ddl*$_{hirae}$ and *ddl*$_{durans}$), 48 °C for (*tet*K, *cat*, *erm*B), 51 °C (for *tet*M) and 56 °C (for *van*A) 30 s and 72°C for 60 s for the next 35 cycles, 72°C for 15 min were used fort the last cycle. The amplification products were analysed by electrophoresis on 1.5% agarose gel at 100 V for 30 min in Tris-acetate-EDTA buffer and revealed in ethidium bromide (20 μg/ml). Amplicons, which were taken from bacterias, sequenced to identification.

Table 1. Oligonucleotide primers used to in the study

	Primer	Sequence (5'-3')	Amplicon sizes (bp)	Reference
1	Ent1 Ent2	TACTGACAAACCATTCATGATG AACTTCGTCACCAACGCGAAC	112	[15]
2	DDF DDR	CACCTGAAGAAACAGGC ATGGCTACTTCAATTTCACG	475	[16]
3	FAC11 FAC21	GAGTAAATCACTGAACGA CGCTGATGGTATCGATTCAT	1.091	
4	DU1 DU2	CCTACTGATATTAAGACAGC TAATCCTAAGATAGGTGTTTG	295	[12]
5	HI1 HI2	CTTTCTGATATGGATGCTGTC TAAATTCTTCCTTAAATGTTG	187	
6	*van*A	GGG AAA ACG ACA ATT GC GTA CAA TGC GGC CGT TA	732	[17]
7	*tet*M	GTGGACAAAGGTACAACGAG CGGTAAAGTTCGTCACACAC	406	[18]
8	*tet*K	CAATACCTACGATATCTA TTGAGCTGTCTTGGTTCA	352	[19]
9	*erm*B	GAAAAGTACTCAACCAAATA AGTAACGGTACTTAAATTGTTTAC	639	[20]
10	*cat*TCF *cat*TCR	CATATCAAATGAACTTTAATA CGTTTTGTGAAGTAGTACACT	718	[21]

Sequence

The amplicons with the expected size were sent to Macrogen Korea in 96 well plates for sequence analysis (Macrogen Inc., Seoul, Korea). Sequence analysis was done after purification using ABI Primse sequencing system by Macrogen Inc.The obtained sequences were compared to the gene bank using Nucleotide-Nucleotide BLAST software at National Centre of Biotechnology Information web page (http://www.ncbi.nlm.nih.gov).

RESULTS

Isolation and Identification

In this study, 94 (15.6%) *Enterococcus* spp. were isolated from 600 mastitis milk samples that were collected from 242 cows. Isolation of enterococcus was carried out in

selective media. 1371 bp length of amplicons were obtained by 16S PCR to control genus specific PCR with sequence analysis and existence of bacterial DNA in samples.

Thus, it was determined that DNA was extracted in all 94 isolated samples. All the microorganisms grown in EA are *Enterococcus* spp. were also confirmed by sequence analysis. PCR was carried out by specific primers to determine enterococcus isolates at the genus level accurately. The isolated and identified 94 isolates were specified as *Enterococcus* spp. by using genus and species level PCR (Figure 1). 56 of them were identified as *E. faecalis* while 20 of them were *E. faecium,* 11 of them were *E. hirae* and 7 of them were *E. durans* (Figure 2)

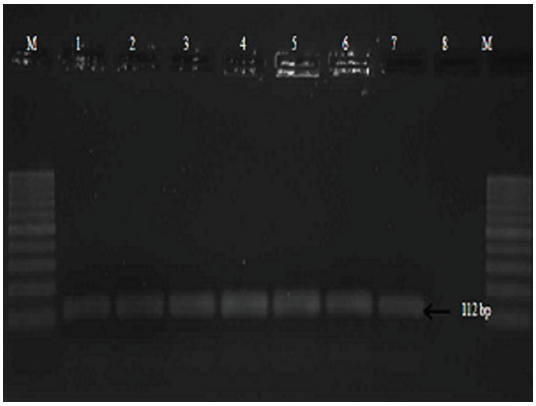

Figure 1. *Enterococcus* spp. PCR electrophoresis image **1-6:** *Enterococcus* spp. field isolates **7**: Positive control (*E. faecalis* ATCC 29212) **8:** negative control (*E. coli* ATCC 25922) **M:** 100 bp DNA ladder (Vivantis, Selangor Darul Ehsan, Malaysia)

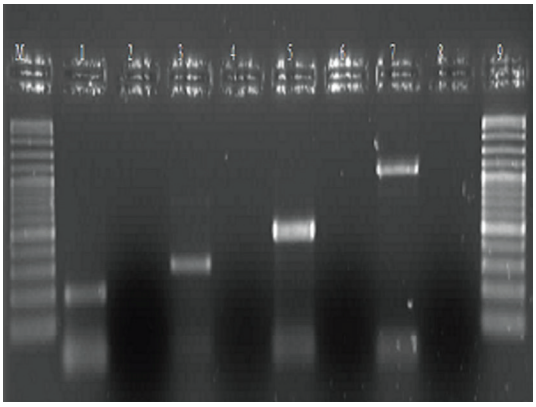

Figure 2. *Enterococcus* species specific PCR electrophoresis image.
1. *E. hirea* (188 bp) **2.** *E. hirae* PCR negative control **3.** *E. durans* (295 bp) **4.** *E. durans* negative control **5.** *E. faecalis* PCR (475 bp) **6.** *E. faecalis* PCR negative control **7.** *E. faecium* (1091 bp) **8.** *E. faecium* negative control

Antibiotic Susceptibility

In total, from the identified 94 enterococci, 77 (81.9%) tetracycline, 27 (28.7%) erythromycin, 10 (10.7%) chloramphenicol and 1 (1.06%) vancomycin-resistant strains were determined according to Kirby-Bauer disk diffusion test (Table 2).

Antibiotic Resistant Genes

Results of the examination of the antibiotic resistance genes showed that, 54 of tetracycline resistant isolates carry *tet*M gene, 23 of them carry *tet*K, 17 of them carry both *tet*M and *tet*K genes. It was also determined that 25 of 27 erythromycin resistant isolates carry *erm*B gene while 9 of 10 chloramphenicol resistant isolates carry *cat* gene and 1 vancomycin resistant isolate carry *van*A gene (Table 2) (Figure 3).

Table 2. Antibiotic resistance ratios and distribution of resistant genes

Antibiotic	Resistance Ratio (n:94)	Distribution of resistant genes
Tetracycline	77 (81.9%)	54 tetM 23 tetK 17 tetM and tetK
Erythromycin	27 (28.7)	25 ermB
Chloramphenicol	10 (10.7)	9 cat
Vancomycin	1 (1.06)	1 vanA

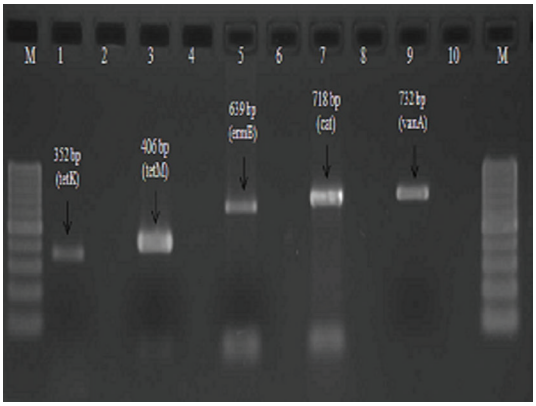

Figure 3. PCR electrophoresis image of antibiotic resistance genes.
1. *tet*K (352 bp) **2.** *tet*K negative control **3.** *tet*M (406 bp) **4.** *tet*M negative control **5.** *erm*B (639 bp) **6.** *erm*B PCR negative control **7.** *cat* PCR (718 bp) **8.** *cat* negative control **9.** *van*A PCR (732 bp) 10. *van*A negative control

DISCUSSION

Enterococcus species are bacterial agents that exist as a natural flora in the intestinal tract of human and animals and they can cause mastitis in cows. It has been known

that enterococcus is resistant to many antibiotics and that resistant traits could be easily transferred to other bacteria. In this study, species of isolates and resistance genes in antibiotic resistant strains were determined by PCR using specific primers.

As a result, 94 (15.6%) of the *Enterococcus* spp. were isolated and identified from analyzed 600 mastitic milk samples. *E. faecalis* was the dominant species among the isolates and it was found in 59.6% of samples. The *E. faecium* was detected in 21.3% of the samples. Obtained results are similar to other studies on the prevalence of *Enterococcus* in milk from cows with mastitis [19-22].

Enterococcus species were encountered in many mastitic milk studies in Turkey at different rates, such as 4.22% by Macun *et al.* [23], 3.73% by Yesilmen *et al.* [24] and 10.9% by Kuyucuoglu [25], however, *Enterococcus* were isolated at a higher rate (15.6%) in this work. The differences between laboratories could be related to specific selective media used for *Enterococcus* isolation from mastitic milk samples.

Enterococci were determined to have resistance to many antibiotics such as tetracycline, erythromycin, cephalosporin, aminoglycoside and clindamycin in clinic applications [26] More than one gene were found responsible for vancomycin resistance in *Enterococci*. *Van*A resistant strains were also highly resistant to vancomycin and teicoplanine [27].

From 94 *Enterococcus* strains isolated in this work, 77 of them (81.9%) were resistant to tetracycline, 27 of them (28.7%) were resistant to erythromycin, 10 of them (10.7%) were resistant to chloramphenicol and 1 isolate was resistant to vancomycin (1.06%).

Genetic characterization of resistance genes from these strains, has shown that 54 carry *tet*M gene while 23 of them were *tet*K positive and 17 of them have both *tet*M and *tet*K. It was also determined that 25 of 27 erythromycin resistant isolates carry the *erm*b gene while 9 of 10 chloramphenicol resistant isolates carry the *cat* gene and 1 vancomycin resistant isolate carries the *van*A gene. Vancomycin resistant *E. faecalis* isolate were also detected to have resistance to tetracycline and erythromycin by carrying *tet*M and *erm*b genes.

In a study carried out by Nam *et al.* [22], all of the 105 *Enterococcus* isolates were found susceptible to ampicillin, gentamycin and vancomycin, but resistance to tetracycline was described at a rate of 69.5%. Multiple drug resistance was observed in 30.4% of all strains with penicillin, tetracycline and erythromycin.

In another study [25], 43 *Enterococci* were isolated from 392 CMT (California Mastitis Test) positive samples and these strains were identified as *E. faecalis* (53.4%) and *E. faecium* (18.6%). *E. faecalis* strains were found resistant to tetracycline, erythromycin, penicillin and vancomycin, at a rate of 91.3%, 82.6%, 78.2% and 4.3%, respectively. Results from research work from other authors implicate that the resistance to tetracycline antibiotics is based on the intensive use of these antibiotics in field conditions.

Findings about resistance to important antibiotics from isolated enterococci in our study were mostly found compatible with previously published results [22,23,25]. Resistance

rates of tested antibiotics were low except for tetracycline and the encountered genes were resistance genes that normally exist in enterococci.

*van*A and *van*B type resistances were found to be the most common resistances in *E. faecium* strains that were isolated from humans in Europe [28]. *E. faecium* is a common isolate and it carries *van*A gene as it was determined by Unal *et al.* [29] when they analyzed in the isolated VRE originating from patients in our country. Reinert *et al.* [30] identified 1.5% vancomycin resistance in 730 enterococcus strains isolated from various materials. Aarestrup *et al.* [31] compared the antibiotic resistant genes and their phenotypes in humans, broilers and pigs and results were found quite similar. Vancomycin resistant enterococcus were isolated from turkey's feces, worker's stool, turkey's slaughterhouse and the people in the residential area close to the slaughterhouse at the turkey farm, by a rate of 50%, 39%, 20% and 14%, respectively [32]. Three of 16 enterococcus strains isolated from mastitic milk samples were identified vancomycin resistant [5]. Two of these 3 strains were *E. gallinarum* and the other one was *E. faecalis*, as in our study. According to the data published by EFSA [33], vancomycin resistance in *E. faecalis* was just determined in The Netherlands (at a rate of 1%), as well as in all the research studies conducted in European Countries.

Seputiene *et al.* [34] investigated the antibiotic resistant genes and virulence factors in *E. faecalis* and *E. faecium* strains isolated from sick farm animals (pigs, poultry and cattle). 83% of all erythromycin resistant *E. faecium* and all *E. faecalis* were observed to have *erm*A and *erm*B genes. Tetracycline resistant 47% of *E. faecalis* and 19% of *E. faecium* were found to carry only tetM, while *E. faecalis* (8%) and *E. faecium* (23%) had only *tet*L. Both *tet*M and *tet*L gene combination was at a rate of 42% for *E. faecalis* and 52% for *E. faecium*. Vancomycin resistant enterococcus was not identified in this study. Results of this study showed parallelism with our study in terms of finding *tet*M in tetracycline resistant *E. faecalis*. It was concluded *tet*M gene has a more dominant character.

According to our study, isolates of *E. faecalis* were identified resistant to tetracycline and erythromycin and carry *van*A gene. From this point of view *van*A gene has great importance for having the ability to transfer this resistance to Gram (-) and Gram (+) bacteria.

In conclusion, *E. faecalis* has a huge part in enterococcus originated mastitis and these strains show resistance to tetracycline and erythromycin. Determining antibiotic resistance genes in enterococcus isolates signify that there is a possibility of genetic transfer of resistant genes to other pathogenic and commensal bacteria. Importantly we detected vancomycin resistance in one isolate. The *van*A gene was detected in this particulate isolate. It is thought that enterococcus species that carry the *van*A gene can transfer the resistance gene to animals and/or humans thus creating a potential risks for human health.

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Authors' contributions

EG and PU carried out the molecular studies. UN and EM did sample collections and laboratory studies. TS and KO participated in the design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Devriese LA, Laurier L, De Herdt P, Haesebrouck F: *Enterococcal* and *streptococcal* species isolated from faeces of calves, young cattle and dairy cows. J Appl Bacteriol 1992, 72:29–31.
2. Cengiz S, Dinc G, Sogut MÜ: Detection of several virulence properties, antibiotic resistance and phylogenetic relationship in *E. coli* isolates originated from cow mastitis. Acta Vet Beograd 2014, 64 (4):413-425
3. Rossitto PV, Ruiz L, Kikuchi Y, Glenn K, Luiz K, Watts JL, Cullor JS: Antibiotic susceptibility patterns for environmental *streptococci* isolated from bovine mastitis in central California dairies. J Dairy Sci 2002, 85:132–138.
4. Osteras O, Solverod L, Reksen O: Milk culture results in a large Norwegian survey – effects of season, parity, days in milk, resistance, and clustering. J Dairy Sci 2006, 89: 1010–1023.
5. Kateete DP, Kabugo U, Baluku H, Nyakarahuka L, Kyobe S, Okee M, Najjuka CF, Joloba ML: Prevalence and Antimicrobial Susceptibility Patterns of Bacteria from Milkmen and Cows with Clinical Mastitis in and around Kampala, Uganda. Plos One. [www.plosone.org] 2013, May V:8 Issue 5, e63413.
6. Gurler H, Findik A, Gultekin N, Ay SS, Ciftci A, Koldas E, Arslan S, Findik M: Investigation on the Etiology of Subclinical Mastitis in Jersey and Hybrid Jersey Dairy Cows. Acta Vet Beograd 2015, 65 (3):358-370
7. Kang HM, Jung BY, Moon JS, Kang MS, Kim JM, Chung I: Distribution of Vancomycin-resistant (VRE) and Van Gene Types in Domestic Animals. [http://210.101.116.28/W_files/kiss3/0b700851_pv.pdf], 12 Feb 2015.
8. Murray BE: The life and times of the Enterococcus. Clin Microbiol Rev 1990, 3:46–65.
9. Klare I, Werner G, Witte W: *Enterococci*. Habitats, infections, virulence factors, resistances to antibiotics, transfer of resistance determinants. Contrib Microbiol 2001, 8, 108–122.
10. Coleri A, Cokmus C: Molecular Mechanisms of Resistance to Glycopeptide Antibiotics in *Enterococcus* Species and Modes of Gene Transfer Turk Hij Den Biyol Derg 2008, 65 (2):87-96.
11. Quinn PJ, Carter ME, Markey B, Carter GR: Clinical Veterinary Microbiology. Mosby-Year Book Europe Limited, Lynton House, London, England; 1994, 40-190.

12. Jackson CR, Fedorka-Cray PJ, Barrett JB: Use of a genus- and species-specific multiplex PCR for identification of *enterococci*. J Clin Microbiol 2004, 42:(8):3558–3565.
13. Lanthier M, Scott A, Lapen D, Zhang Y, Topp E: Frequency of virulence genes and antibiotic resistances in *Enterococcus spp.* isolates from wastewater and feces of domesticated mammals and birds, and wildlife. Can J Microbiol 2010, 56:715–729.
14. CLSI: Performance standards for antimicrobial susceptibility testing. Twenty-First Informational Supplement. Clinical and Laboratory Standards Institute 2011, M02-A10 and M07-A08 vol. 31Wayne, PA.
15. Ke D, Picard FJ, Martineau F, Ménard C, Roy PH, Ouellette M, Bergeron MG: Development of a PCR assay for rapid detection of enterococci. J Clin Microbiol 1999, 37: 3497-503.
16. Vilela MA, de Souza SL, Palazzo ICV, Ferreira JC, de Morais Jr. MA, da Costa Darini AL, de Morais MMC: Identification and molecular characterization of Van A-type vancomycin-resistant *Enterococcus faecalis* in Northeast of Brazil. Mem Inst Oswaldo Cruz 2006, 101(7):715-719.
17. Sambrook J, Fritsch EF, Maniatis T: Molecular cloning: A laboratory manual. 2nd edt. Cold Spring Harbor, New York: Cold Spring Harbor Press; 1989, 2.60-2.80.
18. Turner PC, Mclennan AG, Bates AD, White MRH: Instant Notes in Molecular Biology. Biologia Plantarumn1999, 42(3):462-462.
19. Watts JL: Characterization and identification of Streptococci isolated from bovine mammary glands. J Dairy Sci 1988, 71:1616-1624.
20. Jayarao BM, Dore JJE, Oliver SP: Restrictionfragment length polymorphism analysis of 16S ribosomal DNA *Streptococcus* and *Enterococcus* species of bovine origin. J Clin Microbiol 1992, 30:2235- 2240.
21. Devriese LA, Vancanneyt M, Descheemaeker P, Baele M, van Landuyt HW, Gordts B, Butaye P, Swings J, Haesebrouck F: Differentiation and identification of *Enterococcus durans, E. hirae*, and *E. vitillorum*. J Appl Microbiol 2002, 92:821-7.
22. Nam HM, Lim SK, Moon JS, Kang HM, Kim JM, Jang KC, Kim JM, Kang MI, Joo YS, Jung SC: Antimicrobial resistance of *enterococci* isolated from mastitic bovine milk samples in Korea. Zoonoses Public Health 2010, 57(7-8):e59-64.
23. Macun HC, Yagci IP, Unal N, Kalender H, Sakarya F, Yildirim M: Agent Isolation and Antibiotic Resistance in Dairy Cows with Subclinical Mastitis in Kırıkkale. J Fac Vet Med Univ Erciyes 2011, 8(2):83-89.
24. Yesilmen S, Ozyurtlu N, Bademkiran S: The Isolation of Subclinical Mastitis Agents and Determination of the Sensitive Antibiotics in Dairy Cows in Diyarbakır Province. Dicle Üniv Vet Fak Derg 2012, 1(4):24-29.
25. Kuyucuoglu Y: Antibiotic resistances of enterococci isolated from bovine subclinical mastitis. Eurasian J Vet Sci 2011, 27(4):231- 234.
26. Urbaskova P: Antibiotic Resistance Bacteria (Rezistence bakterií k antibiotikům). Vybrané metody. 1998, ISBN 80-238-3106-2. Trios Praha.
27. Cetinkaya Y, Falk P, Mayhall CG: Vancomycin-Resistant *Enterococci*. Clinical Microbiology Reviews 2000, 686-707.
28. Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, Klare I, Kristinsson KG, Leclercq R, Lester CH: Emergence and spread of vancomycin-resistance among *enterococci* in Europe. Eurosurveillance 2008, 13:1-11.
29. Unal N, Dilik Z, Yildirim M: Isolation of a vanA Positive *Enterococcus faecium* from Commercial Broiler Farms in Turkey. Kafkas Univ Vet Fak Derg 2010, 16 (1): 127-129.

30. Reinert RR, Conrads G, Schlaeger JJ, Werner G, Witte W, Luetticken R, Klare I: Survey of antibiotic resistance among enterococci in North Rhine-Westphalia, Germany. J Clin Microbiol 1999, 37:1638-1641.
31. Aarestrup FM, Bager F, Andersen JS: Association between the use of avilamycin for growth promotion and the occurrence of resistance among *Enterococcus faecium* from broilers epidemiological study and changes over time. Microb Drug Resist 2000, 6:71-75.
32. van den Bogaard AE, Mertens P, London NH, Stobberingh EE: High prevalence of colonization with vancomycin- and pristinamycin-resistant *enterococci* in healthy humans and pigs in the Netherlands: is the addition of antibiotics to animal feeds to blame? J Antimicrob Chemother 1997, 40:454-456.
33. EFSA: EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. EFSA Journal 2015, 13(2):4036.
34. Seputiene V, Bogdaite A, Ruzauskas M, Suziedeliene E: Antibiotic resistance genes and virulence factors in *Enterococcus faecium* and *Enterococcus faecalis* from diseased farm animals: pigs, cattle and poultry Pol J Vet Sci 2012, 15(3); 431-438.

DISTRIBUCIJA GENA REZISTENCIJE NA ANTIBIOTIKE KOD *ENTEROCOCCUS* SPP. IZOLOVANIH IZ UZORAKA MLEKA KRAVA SA MASTITISOM

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U ovoj studiji izvršeno je određivanje *Enterococcus* spp u uzorcima mleka krava sa mastitisom, kao i određivanje njihove osetljivosti na antibiotike i identifikacija gena rezistencije kod izolovanih rezistentnih sojeva. Uzeto je 600 uzoraka mleka od 242 krave sa mastitisom. Izolacija enterokoka je izvršena na selektivnim podlogama, pri čemu je izolovano 94 (16,6%) *Enterococcus* spp. Primenom sekvencioniranja i PCR-om ukupno je izolovano 94 *Enterococcus* spp. Sveukupno 5 vrsta (*E. faecalis, E. faecium, E. durans, E. hirae, E. mundtii*) je izolovano sekvencioniranjem, a 4 (*E. faecalis, E. faecium, E. durans, E. hirae*) je identifikovano pomoću specifičnih prajmera metodom PCR. Ispitivanjem antibiotske rezistencije 94 sojeva izolovanih enterokoka visoka stopa rezistencije na tetracikline je određena u 77 izolata (81,9%). Tet gen je utvrđen sa sledećom stopom: 54 tetM pozitivno, 23 tetK pozitivno i 17 tetM i tetK pozitivno. Rezistencija na eritromicin je ustanovljena kod 27 (28,7%) izolata (25 ermB), dok je gen rezistencije na hloramfenikol zabeležen u 10 (10,7) izolata. Cat gen je identifikovan kod 9 uzoraka, a jedan izolat je bio rezistentan na vankomicin (1,06%) uz potvrdu VanA gena.

Možemo zaključiti da *E. faecalis* ima ključnu ulogu u nastanku enterokoknog mastitisa pri čemu su izolovani sojevi bili rezistentni na tetraciklin. Jedan vankomicin rezistentan izolat je istovremeno imao izolovan VanA gen.