Molecular detection of virulence and resistance genes of Enterococci spp isolated from milk and milk products in Egypt.

Ashraf A. Abd El-Tawab[1], Sahar R. Mohamed[2] and Mohamed A. M. Kotb[3]

[1]Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University. Egypt
[2] Animal Health Research Institute, Dokki, Giza, Egypt
[3] Animal Health Research Institute, Tanta Branch, Egypt
ashrafabdeltwab@yahoo.com, saharroshdy12@yahoo.com, mohamedabdelhady923@yahoo.com

Abstract: In this study, two hundred random milk and milk products samples including raw milk, mastitic milk, karish cheese, feta cheese, yoghurt and ice cream were collected from different sources in El– Gharbia governorate, Egypt. Enterococcus spp were detected in milk and milk products with a total incidence 60%. Enterococcus isolates were identified and further tested for antimicrobial susceptibility.92% of tested Enterococci isolates were resistant to cefotaxime, 78% to ampicillin, 66% to rifampin, 58% to erthomycin, 54% to gentamicin, 18% to vancomycin, 18% to ciprofloxacin and 14% to doxycycline. Polymerase chain reaction (PCR) was applied for detection of enterococcal virulence genes (*esp, gelE, asa1* and *ace*) and resistance genes (*vanA, ermB* and *tetM*). Enterococcus isolates were positive mainly for*asa1*(91.7%), *ace* (83.3%) and *esp* (66.7%) while *gelE* was detected in 6(50%) isolates only. *vanA*was detected in three isolates (25%), while *ermB* was detected in 6 isolates (50%). All tested isolates were negative for *tetM.*

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1. Introduction:

Enterococci are Gram-positive, catalase-negative, non spore-forming, facultative anaerobic bacteria, which usually inhabit the alimentary tract of humans in addition to being isolated from environmental and animal sources **(Fisher and Phillips, 2009)**. Enterococci resist to adverse environmental conditions such as low pH, high salinity and high temperatures so this takes account for their ability to colonize different habitats and for their potential for easy spreading through the food chain **(Fracalanzza et al., 2007)**.Enterococci have been proposed as a part of defined starter cultures in some ripened cheeses and are used in production of some dairy products due to their important role in flavor development and fermentation **(Trivedi et al., 2011)**.

Within the genus Enterococcus, two species, *E. faecalis* and *E. faecium*, have emerged as opportunistic pathogens and are responsible for an increasing percentage of nosocomial infections, including bacteremia and intra-abdominal and urinary tract infections **(Conde-Estevez et al., 2011)**.

Enterococci are intrinsically more resistant than many other bacteria to antimicrobial agents commonly used in hospitals as ampicillin, tetracycline, erythromycin and vancomycin. However, wide spread use of vancomycin in hospitals likely contributed to the emergence and dramatic increase of vancomycin-resistant enterococci (VRE) over the past 20 years **(McGowan et al., 2006)**.

Additionally, enterococci may carry various genes directly or indirectly contributing in their virulence **(Franz et al., 2001)**. Genes encoding virulence factors such as aggregation substance (*asa*), enterococcal surface protein (*esp)*, gelatinase (*gel*) and adhesion collagen protein (*ace*) have been described in enterococci isolated from foodstuffs **(Trivedi et al., 2011)**.

Therefore, the aim of this study was to monitor incidence of Enterococcus spp in milk and milk products in addition to monitoring distribution of virulence and resistance genes in these isolates.

2. Material and methods:

2.1. Samples collection:

A total of 200 random milk and milk products samples including raw milk (75), mastitic milk (25), karish cheese (25), feta cheese (25), yoghurt (25) and ice cream (25) were collected from different sources in El – Gharbia governorate, Egypt. Samples were aseptically put into sterile container, kept in ice box and transferred to laboratory as soon as possible.

2.2Isolation and Identification of Enterococci:

For milk samples, 10 ml of the samples were centrifuged at 5000 rpm for 10 minutes, and then the supernatant was discarded. Solid samples (milk product), 3 gm portion of the sample were

homogenized with 27 ml of physiological saline (**Devriese et al., 1995** and **Gomes et al., 2008)**.

Aloopfull from prepared samples were streaked into the surface of Slantez and Bartly medium plates then incubated at 37°C for 24-48 hrs. The enterocoocus isolates were then examined for observing their characteristic colonies **(Slantez and Bartley, 1957)**.Presumptive identification of enterococci was made based on colony morphology, Gram staining, growth on bile esculin agar medium, catalase test and growth in brain-heart infusion broth with 6.5% NaCl **(Cruickshank et al., 1975; Facklam and Moody, 1970** and **Qadri et al, 1978)**. Vitek2 compact system was used as a confirmatory biochemical tool and it was done according to the manufacturer's instructions **(BioMe'rieux, 2006)**.

2.3 Antimicrobial susceptibility testing:

The antibiotic susceptibility testing was performed by standard disc diffusion method in accordance with the Clinical and Laboratory Standards Institute guidelines **(CLSI, 2014)**using Muller Hinton agar and the following antibiotic discs: vancomycin (30), ampicillin (10), doxycycline (30), erythromycin (15), ciprofloxacin (5), rifampin (15), cefotaxime (30mg) and gentamicin (10mg), (Oxoid).Diameters of inhibition zones were observed around the antibiotic discs.

2.4 Molecular screening of enterococcal virulence and resistance genes:

E.faecalis isolates were confirmed using Vitek 2 system and conventional PCR (**Zoletti et al., 2006)**. In The current study, PCR assay was applied for investigation of virulence and resistance genes among 12*E.faecalis* isolates. DNA extraction was done according to QIAamp DNA mini kit instructions. Oligonucleotide primers have specific sequence and amplify a specific product (Table, 1). The amplification products were analysed by electrophoresis on 1.5% agarose gel.

Table (1): Oligonucleotide primers sequences used for genotyping of virulence and resistance of enterococci isolates:

Gene	Primer Sequence 5'-3'	Amplified product	References
esp	AGATTTCATCTTTGATTCTTGG	510 bp	**Vankerckhoven *et al*., 2004**
	AATTGATTCTTTAGCATCTGG		
gelE	TATGACAATGCTTTTTGGGAT	213 bp	
	AGATGCACCCGAAATAATATA		
asa1	GCACGCTATTACGAACTATGA	375 bp	
	TAAGAAAGAACATCACCACGA		
ace	GGAATGACCGAGAACGATGGC	616 bp	**Creti *et al*., 2004**
	GCTTGATGTTGGCCTGCTTCCG		
vanA	CATGACGTATCGGTAAAATC	885 bp	**Patel *et al*., 1997**
	ACCGGGCAGRGTATTGAC		
ermB	CATTTAACGACGAAACTGGC	425 bp	**Schlegelova *et al*., 2008**
	GGAACATCTGTGGTATGGCG		
tetM	GTGGACAAAGGTACAACGAG	405 bp	**Morvan *et al*., 2010**
	CGGTAAAGTTCGTCACACAC		

3. Results:

3.1 Prevalence of Enterococci in milk and milk products:

Out of 200 milk and milk products samples collected from different sources in El-Gharbia governorate, Egypt, 120 Enterococcus isolates were detected with an incidence 60%. The incidence of Enterococci in raw milk, mastitic milk, karish cheese, feta cheese, yoghurt and ice cream was 77.3%, 72%, 80%, 24%, 28% and 44%, respectively.

3.2 Identification of Enterococci:

Enterococci isolates grew on Slantez and Bartley medium with pink or dark red colonies, while on bile esculin agar, blackening of medium was detected. Enterococci appeared as Gram positive cocci, mostly in pairs or in short chains, catalase negative, tolerated to high salinity (grew on BHI broth with 6.5 % NaCl which was indicated by growth accompanied with a colour change of purple to yellow) and grew at pH 9.6 and at variable degree of temperature (10-45°C). Vitek 2 compact system confirmed random isolates as E. faecalis with percentage 93.33%.

3.3 Antimicrobial resistance for Enterococci:

Out of one hundred and twenty isolates, 50 enterococci isolates were selected and tested for their susceptibility to different antimicrobial agents of several groups. Avery different prevalence of antibiotic resistance against the antibiotics tested was detected among Enterococcus isolates (Table, 2). Enterococci isolates were highly resistant to cefotaxime (92%), followed by ampicillin (78%), rifampin (66%), erythromycin (58%) and gentamicin

(54%). Enterococci isolates were highly sensitive to doxycycline (70%) and vancomycin (56%).

It was found that 96% of enterococci isolates exhibited resistance to at least two antibiotics. Multidrug resistance (MDR) was recorded for enterococci isolates resistant to three drugs or more. The highest MDR percentage was (34%) to 4 drugs. The total percentage of MDR of enterococci was 80%.

Table (2): Antimicrobial resistance of Enterococci isolated from milk and milk products:

Antimicrobial agent	Symbol	Disk potency (µg)	No. of resistant strains	%
Vancomycin	VA	30	9	18
Ampicillin	AMP	10	39	78
Doxycycline	DO	30	7	14
Erythromycin	E	15	29	58
Ciprofloxacin	CIP	5	9	18
Rifampin	RA	15	33	66
Gentamicin	CN	10	27	54
Cefotaxime	CTX	30	46	92

% acc. to number of resistant strains. (Total number of tested isolates= 50)

3.4 Investigation of virulence and resistance genes of Enterococcus isolates:

The current results revealed that 91.7% of tested *E. faecalis* strains (12)were harbored the gene of aggregation substance (*asa1*), 83.3% were positive for adhesion collagen protein gene *(ace)* while gene of enterococcal surface protein (*esp*) and gelatinase (*gelE*) were detected in 66.7% and 50% of isolates, respectively. All isolates were negative for *tetM* while *ermB* was detected in 6 isolates (50%). Vancomycin resistant gene (*vanA*) was found in three isolates (25%) (Table, 3).

Table (3): Incidence of virulence and resistance genes in *E. faecalis* isolates:

Type	Gene	No. of +ve isolates	%
Virulence genes	*asa1*	11	91.7
	esp	8	66.7
	ace	10	83.3
	gelE	6	50
Resistance genes	*vanA*	3	25
	ermB	6	50
	tetM	0	0

% acc. to number of positive results. (Total number=12)

4. Discussion:

Enterococci are considered not only potential pathogens, but also a reservoir of genes encoding antibiotic resistance which can be transferred to other microorganisms (**Pesavento et al., 2014**). In the present study, the overall incidence level of enterococci isolates from all examined milk and milk products samples (60%) was higher than that reported in Brazil (52.5%) (**Gomes et al., 2008)**and in France (44%) **(Jamet et al., 2012)**,while it was lower than that obtained in Czech (100%) (**Trivedi et al., 2011)**.

In the current study, the incidence of enterococci in raw milk (77.3%) was agreed with that recorded in Croatia (73.7**) (Zdolec et al., 2016)**, while it was lower than that obtained in Australia (96%) **(McAuely et al., 2015)** and higher than that reported in Korea (25.8%) (**Kim et al., 2012)**.In this study, the incidence of enterococci in mastitic milk samples was 72%. Low percentages of isolation were reported by **Erbas et al., (2016)** and **Wu et al., (2016).**

Herein, Enterococcus spp were found in 80% of karish cheese samples. Very similar results (78.6%) were observed in earlier study by **El Malt (2015)**, while higher results were obtained by **Hammad et al., (2015)** who stated that Enterococcus spp were found in all examined karish cheese samples. The incidence of enterococci in soft (feta) cheese samples (24%) was completely agreed with **Pesavento et al., (2014)**,while **Furlaneto- Mia et al., (2014)** stated that all examined soft cheese samples were contaminated with Enterococcus spp.

In this work, the incidence of enterococci in yoghurt was 28%, which was similar to that obtained by **Gorgy et al., (2016)**, while higher result (83%) was recorded by **El Malt (2015)**.In this study, the incidence of *enterococci* in ice cream was 44%, which were agreed with results obtained in Turkey (40%) **(Gundogan et al., 2013)**.Lower results were reported in Botswana (1.33%) **(Mathews et al., 2013)**.

Vitek2compact system appeared a fast and reliable method for identification and detection of glycopeptide-resistant enterococci **(Abele-Horn et al., 2006)**. In the current study, Vitek 2 system was applied for more accurate identification,93.33% of E. faecalis isolates correctly identified to the species level, which was very similar to that recorded in Italy (**Ligozzi et al., 2002)**.

Enterococci are intrinsically resistant to a wide range of antimicrobials of therapeutic use and are capable of acquiring resistance genes from different bacterial species **(Mannu et al. 2003)**. In our study, mostly used antimicrobial agents in Egypt were tested against Enterococci isolates to screen their resistant patterns. Enterococci isolates were highly resistant to cefotaxime (92%), which was higher than that obtained by **El Malt (2015)** (67.6%).Additionally, a higher frequency of ampicillin resistant enterococcus isolates was recorded (78%) that was higher than that reported by **Wu et al., (2016)** (15%),while **Tuncer et al., (2013**) mentioned that all enterococcal spp were sensitive to ampicillin.

In this study, the frequency of rifampin resistant enterococci was 66%, which is lower than that detected in Turkey (73%) (**Citak et al., 2006)**.The erythromycin resistance level observed in enterococci isolates (58%) is similar to those reported by **(Nam et al., 2010**;**Gundogan et al., 2013**and **Gaglio et al., 2016))** and higher than that obtained in Serbia (**Bulajić et al., 2015)** and in Slovakia **(Krockoet al., 2011)** but it is lower than that was stated by **Pereira et al., (2017)**.Moreover, **Krockoet al., (2011)** reported enterococci resistant to gentamicin (25%) which are lower than the frequencies observed in this study (54%), while similar results was recorded by **WU et al., (2016)** (50%).

Herein, 18% of enterococci isolates were resistant to vancomycin, which completely agreed with result obtained by **Chingwaru et al., (2003)** and similar to that recorded by **Fabinova et al., (2010)** (15.2%). Higher level of vancomycin resistance (33.7%) was reported by **Oguntoyinbo and Okueso (2013)**, while **Pesavento et al., (2014)** stated Low percentage (3.53%) of enterococci were resistant to vancomycin. In the current study, 18% of enterococcus isolates were resistant to ciprofloxacin, which was lower than that mentioned by **Gaglio et al., (2016)** (35%), while some studies proved that all investigated enterococcal strains were sensitive to ciprofloxacin **(Belicova et al., 2007**).

In the present study, multiple drug resistance (MDR) was detected to three or more drugs with a percentage of 80%, which is lower than that obtained in Czech (0%) (**Trivedi et al., 2011)** and in Egypt (67%) **(El Malt, 2015)**.

Although enterococci don't produce potent toxins like some other bacteria, they possess virulence factors in form of aggregation substance, enterococcal surface protein, gelatinase and antibiotic resistance genes **(Sava et al., 2010)**.Herein, twelve *E.faecalis* isolates that were previously identified by Vitek2 system and PCR were surveyed for presence of *asa1, esp, gelE* and ace virulence genes.*asa1*was the most predominant virulence gene with percentage (91.7%). Similar results was observed by **Moraes et al., (2012)**while **Elmali and Can (2018)** found that *gelE* was the predominant (37.8%) virulence factor in isolates followed by asa1(33.3%).

In this study, *esp* was detected with an incidence 66.7% that was higher than that recorded by **WU et al., (2016)** (20%) but it is lower than that stated by **Olawale et al., (2014)** (90%). On the other side, 50% of tested *E.faecalis* isolates in this work were positive for *gelE*, which are similar to that were found in United Kingdom (56%) (**Eaton and Gasson, 2001**), while it was lower than that noticed in Brazil (95%) (**Gomes et al., 2008)** but it is higher than that stated by **Elmali and Can (2018)**. *Ace* gene was detected with an incidence 83.33%, which was similar to that reported in Spain (79%) (**Abou elnaga et al., 2016**).

In this study, *E. faecalis* were also screened for the most common resistance genes (*vanA*, *ermB* and *tetM*). 3(25%) isolates were harbored *vanA* gene, This result was higher than that obtained by **Moraes et al., (2012)** and **Erbas et al., (2016).** On the other side**, Furlaneto-Maia et al., (2014)** found that the *vanA* gene was detected in 100% of vancomycin resistant enterococci, On the contrary to our results, none enterococcal isolates carried vanA that was reported by some authors (**Morandi et al., 2006; Belicova et al., 2007** and **Franciosi et al., 2009).**

Additionally, *E. faecalis* isolates were also screened for *ermB* and *tetM*. 6 out of 12 *E.faecalis* strains were positive for *ermB*, which is lower than recorded in Germany (81.8%) **(Hummel et al., 2007),** in Switzerland (95%) **(Templer and Baumgartner, 2007)**, in Korea (97.4%) (**Kim et al., 2012)**, in France (96 %) (**Jamet et al., 2012)**, in India (91.2%) **(Thumu and Halami, 2012)** and in Turkey (92.6) (**Erbas et al., 2016)**, but it was higher than that reported by **Hammad et al., (2015)** (0.83%). While the *ermB* gene was not detected in any erythromycin-resistant strain **(Bulajić et al., 2015** and **Gaglio et al., 2016).**

Finally, none of all tested *E.faecalis* isolates carried *tetM* gene. In the contrast of our result, **Templer and Baumgartner, (2007)** and **Erbas et al., (2016)** reported that all testes Enterococcus isolates were positive for *tetM*. On the other side, *tetM* was found in one isolate only (**Thumu and Halami, 2012)**, while higher results were recorded by **Jamet et al., 2012** (90%).

In conclusion, Enterococci were resistant to various antimicrobial agents in addition to carrying potential virulence genes that could potentially contribute to bacterial colonization and pathogenesis of enterococci. Antibiotic policies coupled with greater adherence to infection control measures should be enforced to prevent emergence and spread of multi-drug resistant enterococci.

References:

1. Abele-Horn, M.; Hommers, L.; Trabold, R. and Frosch, M. (2006): Validation of Vitek 2 version 4.01 software for detection, identification, and classification of glycopeptide-resistant Enterococci. J. Clin. Microbiol, 44(1): 71–76.
2. Abou elnaga, M.; Lamas, A.; Baluja, M. Q.; Osman, M.; Miranda, J. M.; Cepeda, A. and Franco, C. M. (2016): Evaluation of the extent of spreading of virulence factors and antibiotic resistance in Enterococci isolated from fermented and unfermented foods. J. Ann. Microbiol., (66): 577–585.
3. Belicova, A.; Krizkova, L.; Krajcovic, J.; Jurkovic, D.; Sojka, M.; Ebringer, L. and Dusinsky, R. (2007): Antimicrobial susceptibility of *Enterococcus* species isolated from Slovak Bryndza cheese. J. Folia Microbiol.,52 (2), 115–119.
4. BioMe'rieux (2006): Vitek2 product information, document: 5107694EN1. bioMe'rieux, Inc., Durham, NC.
5. Bulajić, S.; Tambur, Z.; Opačić, D.; Selimović, B. S.; Doder, R. and Milošević, D. C. (2015): Characterization of antiboitic resistance phenotypes and resistance genes in Enterococcus spp. isolated from cheeses. Arch. Biol. Sci., Belgrade, 67 (1): 139-146.
6. Chingwaru, W.; Mpuchane, S. F. and Gashe, B. A. (2003): Enterococcus faecalis and Enterococcus faecium isolates from milk, beef, and chicken and their antibiotic resistance. J. of Food Protection, 66(6): 931–936.
7. Citak, S.; Yucel, N. and Mendi, A. (2006): Antiboitic resistance of Enterococcal isolates in raw milk. Journal of Food Processing and Preservation, 29: 183–195.
8. CLSI (Clinical and Laboratory standards Institute) (2014): Performance standards for antimicrobial susceptibility testing. Twenty-Fourth Informational supplement. M100 –S24, Vol. 34 (1).
9. Conde-Estevez, D.; Grau, S.; Albanell, J.; Terradas, R.; Salvado, M. and Knobel, H. (2011): Clinical characteristics and outcomes of patients with vancomycin-susceptible Enterococcus faecalis and Enterococcus faecium bacteraemia in cancer patients. European Journal of Clinical Microbiology and Infectious Diseases, 30: 103-108.
10. Creti, R.; Imperi, M.; Bertuccini, L.; Fabretti, F.; Orefici, G.; Di Rosa, R. and Baldassarri, L. (2004): Survey for virulence determinants among Enterococcus faecalis isolated from different sources. J. Med. Microbiol., 53(1):13-20.
11. Cruickshank, R.; Duguid, J. P.; Marmion, B. R. and Swain, R. H. A. (1975): "Medical Microbiology, 12th Ed., Living stone, London, New York.
12. Devriese LA, Pot B, Van Damme L, Kersters K and Haesebrouck F (1995): Identification of Enterococcus species isolated from foods of animal origin. International Journal of Food Microbiology, 26 (2): 187-197.
13. Eaton, T. J. and Gasson, M. J. (2001): Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. Applied and Environmental Microbiology, 67 (4): 1628-1635.
14. Elmali, M. and Can, H. Y. (2018): The prevalence, vancomycin resistance and virulence gene profiles of Enterococcus species recovered from different foods of animal origin. Veterinarski Arhiv 88 (1), 111-124.
15. El-Malt, R. M. S. A. (2015): Molecular studies on esp gene in multiple antibiotic resistant enterococci. A thesis (M. V. Sc), Bacteriology, Immunology and Mycology department, Faculty of Vet. Medicine, Zagazig University.
16. Erbas, G.; Parin, U.; Turkyilmaz, S.; Ucan, N.; Ozturk, M. and Kaya, O. (2016): Distribution of antibiotic resistance genes in Enterococcus spp. isolated from mastitis bovine milk. J. Acta Veterinaria-Beograd, 66 (3): 336-346.
17. Fabianova, J.; Duckova, V.; Canigova, M. and Krocko, M. (2010): Presence of Enterococci in cow milk and thier antiboitic resistance. Potravinarstvo, 4(2): 17-21.
18. Facklam R. R. and Moody M. D. (1970): presumptive identification of group D streptococci: the bile- esculin test. Appl. Microbiol., 20 (2): 245-250.
19. Fisher, K. and Phillips, C. (2009): The ecology, epidemiology and virulence of Enterococcus. Microbiology, 155: 1749-1757.
20. Fracalanzza, S. A.; Scheidegger, E. M.; Santos, P. F.; Leite, P. C. and Teixeira, L. M. (2007): Antimicrobial resistance gene profiles of Enterococci isolated from poultry meat and pasteurized milk in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz, 102(7): 853-859.
21. Franciosi, E.; Settanni, L.; Cavazza, A. and Poznanski, E. (2009): Presence of Enterococci in

raw cow's milk and "Puzzone di Moena" cheese. Journal of Food Processing and Preservation, 33: 204–217.
22. Franz, C. M. A. P.; Muscholl-Silberhorn, A. B.; Yousif, N. M. K.; Vancanneyt, M.; Swings, J.; Holzapfel, W. H. (2001): Incidence of virulence factors and antibiotic resistance among enterococci isolated from food. Applied and Environmental Microbiology, 67: 4385–4389.
23. Furlaneto-Maia, L.; Rocha, K. R.; Henrique, F. C.; Giazzi, A. and Furlaneto, M. C. (2014): Antimicrobial resistance in *Enterococcus* spp isolated from soft cheese in Southern Brazil. J. Advances in Microbiology (4):175-181.
24. Gaglio, R.; Couto, N.; Marques, C.; Lopes, M. F. S.; Moschetti, G.; Pomba, C. and Settanni, L. (2016): Evaluation of antimicrobial resistance and virulence of enterococci from equipment surfaces, raw materials, and traditional cheeses. J. Food Microbiology (236):107–114.
25. Gomes, B. C.; Esteves, C. T.; Palazzo, I. C. V.; Darini, A. L. C.; Felis, G. E.; Sechi, L. A.; Franco, B. D. G. M. and De Martinis, E. C. P. (2008): Prevalence and characterization of Enterococcus spp. isolated from Brazilian foods. J. Food Microbiology, 25: 668– 675.
26. Gorgy, S. F.; El Asuoty, M. S.; Saber, A. S. and Ali, A. H. (2016): Prevalence of Enterococci and Streptococci in raw milk and some dairy products and the subsequent alteration on quality. Egypt. J. Chem. Environ. Health, 2 (2):500-515.
27. Gundogan, N.; Ataol, O. and Torlak, F. O. (2013): Determination of some virulence factors in Staphylococcus aureus, Enterococcus faecalis and Enterococcus faecium isolated from meat and milk products. Journal of Food Safety, 33: 387–393.
28. Hammad, A. M.; Hassan, H. A. and Shimamoto, T. (2015): Prevalence, antibiotic resistance and virulence of Enterococcus spp. in Egyptian fresh raw milk cheese. J. Food Control, 50: 815-820.
29. Hummel, A.; Holzapfel, W. H. and Franz, C. M. A. P. (2007): Characterisation and transfer of antibiotic resistance genes from enterococci isolated from food. Systematic and Applied Microbiology 30: 1–7.
30. Jamet, E.; Akary, E.; Poisson, M. A.; Chamba, J. F.; Bertrand, X. and Serror, P. (2012): Prevalence and characterization of antibiotic resistant Enterococcus faecalis in French cheeses. J. Food Microbiology (31):191-198.
31. Kim, J.; Lee, S. J. and Choi, S. S. (2012): Copper resistance and its relationship to erythromycin resistance in *Enterococcus* isolates from bovine milk samples in Korea. J. Microbiol., 50, (3): 540–543.
32. Krocko, M.; Canigova, M.; Duckova, V.; Artimova, A.; Bezekova, A. and Poston, J. (2011): Antibiotic resistance of Enterococcus species isolated from raw foods of animal origin in south west part of Slovakia. Czech J. Food Sci. Vol. 29(6): 654–659.
33. Ligozzi, M.; Bernini, C.; Bonora, M. G.; Fatima, M.; Zuliani, J. and Fontana, R. (2002): Evaluation of the Vitek 2 system for identification and antimicrobial susceptibility testing of medically relevant Gram-positive cocci. J. Clin. Microbiol, 40(5): 1681–1686.
34. Mannu, L.; Paba, A.; Daga, E.; Comunian, R.; Zanetti, S.; Dupre, I. and Sechi, L. A. (2003): Comparison of the incidence of virulence determinants and antibiotic resistance between Enterococcus faecium strains of dairy, animal and clinical origin. International Journal of Food Microbiology, 88: 291– 304.
35. Mathews, S.; Ngoma, L.; Gashe, B. and Mpuchan, S. (2013): General microbiological quality of ice cream and ice pop sold in Gaborone, Botswana. Ethno Med, 7(3):217-226.
36. McAuley, C. M.; Britz, M. L.; Gobius, K. S. and Craven, H. M. (2015): Prevalence, seasonality and growth of enterococci in raw and pasteurized milk in Victoria, Australia. Journal of Dairy Science, 98 (12): 8348-8358.
37. McGowan, L. L.; Jackson, C. R.; Barrett, J. B.; Hiott, L. M. and Fedorka-Cray, P. J. (2006): Prevalence and antimicrobial resistance of Enterococci isolated from retail fruits, vegetables, and meats. J. Food Prot., 69: 2976-2982.
38. Moraes, P. M.; Perin, L. M.; Todorov, S. D.; Silva Jr, A.; Franco, B. D. G. M. and Nero, L. A. (2012): Bacteriocinogenic and virulence potential of Enterococcus isolates obtained from raw milk and cheese. Journal of Applied Microbiology 113: 318-328.
39. Morandi, S.; Brasca, M.; Andrighett, C.; Lombardi, A. and Lodi, R. (2006): Technological and molecular characterization of enterococci isolated from north–west Italian dairy products. International Dairy Journal, 16: 867–875.
40. Morvan, A.; Moubareck, C.; Leclercq, A.; Hervé-Bazin, M.; Bremont, S.; Lecuit, M.; Courvalin, P. and Le Monnier, A. (2010): Antimicrobial resistance of *Listeria monocytogenes* strains isolated from Humans in France. Antimicrobial Agents and Chemotherpy, 54(6): 2728–2731.
41. Nam, H. M.; Lim, S. K.; Moon, J. S.; Kang, H. M.; Kim, J. M.; Jang, K. C.; Kang, M. I.; Joo, Y. S. and Jung, S. C. (2010): Antimicrobial resistance of Enterococci isolated from mastitic

bovine milk samples in Korea. Zoonoses and Public Health, 57(7-8): E59-E64.
42. Oguntoyinbo, F. A. and Okueso, O. (2013): Prevalence, distribution and antibiotic resistance pattern among enterococci species in two traditional fermented dairy foods. J. Ann. Microbiol., 63:755–761.
43. Olawale, A. K.; Onasanya, A.; Oyelakin, O. O.; David, O. M. and Famurewa, O. (2014): Enterococcus faecalis isolates of food origin and detection of their virulence determinant factors and genes in Osun State, Nigeria. J. Microbiol. Res. Int., 2(2): 2354-2128.
44. Patel, R.; Uhl, J. R.; Kohner, P.; Hopkins, M. K. and Cockerill, F. R. (1997): Multiplex PCR detection of vanA, vanB, vanC-1, and vanC-2/3 genes in Enterococci. J. of Clinical, Microbiology, 35(3): 703–707.
45. Pereira, R. I.; Prichula, J.; Santestevan, N. A.; Azevedo, P. A.; Souza Motta, A. and Frazzon, A. P. G. (2017): Virulence profiles in *Enterococcus* spp. isolated from raw buffalo's milk in South Brazil. Res. J. Microbiol., 12(4): 248-254.
46. Pesavento, G.; Calonico, C.; Ducci, B.; Magnanini, A. and Lo Nostro, A. (2014): Prevalence and antibiotic resistance of Enterococcus spp. isolated from retail cheese, ready-to-eat salads, ham, and raw meat. J. Food Microbiology (41):1-7.
47. Platero, A. M. M.; Valdivia, E.; Maqueda, M. and Bueno, M. M. (2009): Characterization and safety evaluation of enterococci isolated from Spanish goats' milk cheeses. International Journal of Food Microbiology, 132: 24– 32.
48. Qadri, S. M.; Nichols, C. W. and Qadri, S. G. (1978): Rapid sodium chloride tolerance test for presumptive identification of enterococci. J. Clin. Microbiol., 7(2): 238.
49. Sava, I. G.; Heikens, E. and Huebner, J. (2010): pathogenesis and immunity in enterococcal infections. Clin. Microbiol. Infect., 16(6):533-540.
50. Schlegelova, J.; Vlkova, H.; Babak, V.; Holasova, M.; Jaglic, Z.; Stosova, T. and Sauer, P. (2008): Resistance to erythromycin of Staphylococcus spp. isolates from the food chain. Veterinarni Medicina, 53 (6): 307–314.
51. Slantez, L. W. and Bartley, C. H. (1957): Numbers of *enterococci* in water, sewage and faeces determined by the membrane filter technique with an improved medium. J. Bacteriol., 74(5): 591-595.
52. Templer, S. P. and Baumgartner, A. (2007): Enterococci from Appenzeller and Schabziger raw milk cheese: antibiotic resistance, virulence factors, and persistence of particular strains in the products. Journal of Food Protection, 70 (2): 450–455.
53. Thumu, S. C. R. and Halami, P. M. (2012): Presence of erythromycin and tetracycline resistance genes in lactic acid bacteria from fermented foods of Indian origin. Antonie van Leeuwenhoek, 102:541–551.
54. Trivedi, K.; Cupakova, S. and Karpiskova, R. (2011): Virulence factors and antibiotic resistance in enterococci isolated from food-stuffs. Veterinarni Medicina, 56 (7): 352-357.
55. Tuncer, B. O.; AY, Z. and Tuncer, Y. (2013): Occurrence of enterocin genes, virulence factors and antibiotic resistance in 3 bacteriocin-producer *Enterococcus faecium* strains isolated from Turkish tulum cheese. Turk. J. Biol., 37: 443-449.
56. Vankerckhoven, V.; Van Autgaerden, T.; Vael, C.; Lammens, C.; Chapelle, S.; Rossi, R.; Jabes, D.; Goossens, H. (2004): Development of Multiplex PCR for the detection of asa1, gelE, cylA, esp and hyl genes in Enterococci and survey for virulence determinants among European hospital isolates of Enterococcus faecium. Journal of Clinical Microbiology, 42: 4473–4479.
57. Wu, X.; Hou, S.; Zhang, Q.; MA, Y.; Zhang, Y.; Kan, W. and Zhao, X. (2016): Prevalence of virulence and resistance to antibiotics in pathogenic enterococci isolated from mastitic cows. J. Vet. Med. Sci. 78(11): 1663–1668.
58. Zdolec, N.; Dobranic, V.; Butkovic, I.; Koturic, A.; Filipovic, I. and Medvid, V. (2016): Antimicrobial susceptibility of milk bacteria from healthy and drug-treated cow udder. J. Vet. Arhiv86 (2): 163-172.
59. Zoletti, G. O.; Siqueira, J. F. and Santos, K. R. N. (2006): Identification of Enterococcus faecalis in root-filled Teeth With or Without Periradicular Lesions by Culture dependent and Independent Approaches. J. Endod 32(8): 722-726.

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