ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 9, Issue, 10, pp.59597-59602, October, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ESP GENE AND MULTIPLE ANTIBIOTICS RESISTANCE OF *E.FEACALIS* RECOVERED FROM MASTITIC COW'S MILK IN EGYPT

[1]Gamal A.M.Younis, *[,1]Rasha M.Elkenany and [2]Amany M.Saleh

[1]Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt
[2]Head of Preventive Medicine Department in Talkha Veterinary Administration, Mansoura 35516, Egypt

ARTICLE INFO

Article History:
Received 29th July, 2017
Received in revised form
16th August, 2017
Accepted 09th September, 2017
Published online 31st October, 2017

Key words:

Enterococcus spp,
E.feacalis, Antibiotic resistance,
Multiplex PCR,
Esp gene, cylA gene.

ABSTRACT

Enterococcus spp are one of the environmental mastitis pathogens with public health hazard and have different harmful effects on milkand dairy farms. The aim of this study was to determine the prevalence of *Enterococcus* spp in mastitic cow's milk in Egypt, detection of some virulence genes as *esp* and *cyl A* of *E.feacalis* and their resistance to different classes of antibiotics. 130 (71.82 %) out of 181 milk samples were identified as *Enterococcus* spp particularly *E.feacalis* (33.34 %). Multiplex PCR was applied for detection of some virulence genes of *E.feacalis* as *Enterococcus* surface proteins *(esp)* and cytolysin *(cylA)*. *ESP* was the predominant gene in all tested *E.feacalis* isolates, whereas *cylA*was completely absent. In addition, antibiotic sensitivity tests noticed that *Enterococcus* spp isolates had multidrug resistance to different classes of antibiotics as 69.77% streptomycin, 53.49% gentamicin and 38.37% ampicillin. Also, higher resistances of *E.feacalis* isolates were observed to streptomycin (47.73%), ampicillin and gentamicin (45.46%, each). On the other hand, the *Enterococcus* spp especially *E.feacalis* were susceptible to ciprofloxacin. In conclusion, mastitc cow's milk is considered as potential reservoirs of virulent and antibiotic resistance *Enterococci* with public health hazard when milk is consumed without any thermal treatment.

Citation: Gamal A.M.Younis, Rasha M.Elkenany and Amany M.Saleh, 2017. "*ESP* gene and multiple antibiotics resistance of *E.feacalis* recovered from Mastitic cow's milk in Egypt", *International Journal of Current Research*, 9, (10), 59597-59602.

INTRODUCTION

Bovine mastitis is one of the most common diseases affecting dairy cattle in Egypt causing deleterious economic losses as decrease milk production, increase health care costs, culling and death rates. The main *Enterococcus* spp cause mastitic infections are *E.faecalis* and *E.feacium* (Pitkala *et al*., 2001; Bradley *et al*., 2007). *Enterococcus* spp are gram-positive commensal bacteria that inhabit in the oral cavity and gastrointestinal tract of humans, animals, reptiles, amphibians, birds, and insects (Aarestrup *et al*., 2002). They are facultative anaerobic, catalase negative, oxidase negative, non spore forming and seem to be single or diplococci or in chains (Fisher and Phillips, 2009). They have ability to survive under unsuitable environmental conditions due to their resistance for freezing, low pH and moderate heat treatment (Elhadidy and Elsayyad, 2013; Van Tyne and Gilmore, 2014). They posses rapid dissemination of antibiotic resistance that made treatment as complicated process (Sillanpaa *et al*., 2004; Gilmore *et al*., 2013). They have ability to be opportunistic invaders to the udder due to their presence in organic bedding materials (Elhadidy and Elsayyad, 2013). They act as nosocomial pathogen with large number of community acquired and health care associated bacterial infections. They have zoonotic importance with a high mortality rate up to 61% (Lopes *et al*., 2005; Rosellini *et al*., 2010). Recently, *Enterococcus* spp are widely used in food processing industry as probiotics (Dogru *et al*., 2010; Nueno-Palop and Narbad, 2011). So, some attention should be taken for prevention of spreading enterococcal infections. Several virulence genes were produced by *Enterococcus* spp, as *Enterococcus* surface protein (*esp*) promotes the adhesion, colonization and biofilm formation, gelatinase (*gelE*) hydrolyzes gelatin, collagen and promotes adhesion and biofilm formation, aggregation substance (AS) may promote adhesion, invasion and biofilm formation, hyaluronidase (*hyl*) promotes invasion to the host tissue, cytolysin (*cylA*) is a bacterial toxin which has the ability to lyse human, horse and rabbit erythrocytes, many prokaryotic cells and other eukaryotic cells, MSCRAMM (microbial surface component recognizing adhesive matrix molecule), capsular polysaccharide, cell wall carbohydrate, and extra-cellular superoxide (Fisher and Phillips, 2009, Chuang-Smith *et al*., 2010;Van Tyne and Gilmore, 2014). Therefore, the aim of the present work was directed to the incidence and antimicrobial resistance of *Enterococcus* spp especially

***Corresponding author:* Rasha M.Elkenany,**
Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Mansoura University, Mansoura 35516, Egypt.

E.faecalis in mastitic cow‘s milk in Egypt as well as some of virulence (*cylA* and *esp*) genes of the isolated *E.faecalis* using multiplex polymerase chain reaction (mPCR).

MATERIALS AND METHODS

Sampling

A total of 218 milk samples were collected from lactating cows with clinical or subclinical mastitis of three large dairy herds distributed in Dakahlia government, Egypt during May 2016. The mastitic cases were classified with clinical observation and California mastitis test into clinical (48) and subclinical (133). All milk samples were analysed by microbiological examination.

Isolation and identification of *Enterococcus* spp

Mastitic milk samples were cultured by streaking on the surface of membrane *Enterococcus* agar (MEA) (Oxoid) and incubated at 37ºC for 24-48h. The isolated colonies were identified as *Enterococcus* spp by colony characters, Gram‘s staining and biochemical tests (oxidase test, catalase test, bile-aesculin test, and growth on 6.5% NaCL test). All suspected Enterococcalisolates were further tested for heat resistance, growth at pH 9.6, growth at 10ºC and 45ºC, gelatin liquefaction and tellurite tolerance test. All isolates were stored at -20ºC in brain heart infusion broth (Oxoid), containing 15% (v/v) glycerol for further examination.

Detection of virulence genes by mPCR

The *esp* and *cylA* genes of *E.feacalis* harboured virulent roles to the host were detected by Multiplex PCR. Genomic DNA was prepared using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) according to the manufacturer’s protocol with modifications. The DNA amplification was performed according to Vankerckhoven‘s protocol with primers Metabion (Germany) as illustrated in Table (1), the thermocycler was programmed as following: denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 45 sec, primer annealing at 50°C for 45 sec, DNA extension at 72°C for 45 sec, and the final DNA extension at 72°C for 10 min. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. A Gelpilot 100 bp plus Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a geldocumentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of isolated *Enterococcus* spp was performed using the disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI 2011) guidelines on Mueller-Hinton agar (Oxoid). The following discs with antibiotics (Oxoid) were evaluated: aminoglycosides [gentamicin (10 mg) and streptomycin (10 mg)], fluoroquinolone [ciprofloxacin (5 mg)], glycopeptides [vancomycin (30 mg)], penicillins [ampicillin (10 mg)], phenicols [chloramphenicol (30 mg)] and macrolids [nitrofurantoin (300µg)]. After incubation at 37ºC for 24h, the results were interpreted according to CLSI (2011) criteria.

RESULTS

Isolation and identification of *Enterococcus* spp

Suspected colonies of *Enterococcus* spp appeared as pinpoint light red to maroon color colony on (MEA). Microscopically, they were identified with gram‘s stain as gram-positive diplococci. Biochemically, they were catalaseand oxidase negative, therefore they were differentiated from Staphylococcus aureus, bile-esculin test was positive and survived in 6.5% NaCl, so they were differentiated from Streptococcus spp. Tellurite tolerance test was positive for differentiation of *E.feacalis* from other *Enterococcus* spp (Table 2 and Fig. 1,2). The mastitic cases were classified with clinical observation and California mastitis test into clinical (48) and subclinical (133) cases. The prevalence of *Enterococcus* spp, based on cultural and biochemical characteristics, was 71.82% which87.5% in clinical mastitic cases and 66.2% in subclinical mastitic cases. *E.faecalis* was observed as the most prevalent strains of *Enterococcus* spp isolates (44 /130 samples; 87.5 %), while other *Enterococcus* spp were (86 /130 samples; 66.15 %) (Table **3**).

Table 1. PCR primers and products used for detection of genes encoding for some virulence factors

Target gene	Primers	sequences 5’ → 3’	Amplified product (bp)	Reference
esp	ESP14F	AGATTTCATCTTTGATTCTTGG	510	Vankerckhoven *et al*., 2004
	ESP12R	AATTGATTCTTTAGCATCTGG		
cylA	CYTI	ACTCGGGGATTGATAGGC	688	
	CYTIIb	GCTGCTAAAGCTGCGCTT		

Table 2. Characteristic features of *E.feacalis* and *Enterococcus* spp

Items	Test	*E. faecalis* (44)	*Enterococcus spp* (86)
1)	Growth at:		
	4°C	−	+
	45°C	+	+
2)	pH 9.6	+	+
3)	Growth in:		
	6.5% NaCl	+	+
	0.04% Tellurite	+	−
4)	Survival at 60°C for:		
	15 min	+	+
	30 min	+	+
5)	Gelatin liquefaction	+	−
6)	Catalase	−	−
7)	Esculin hydrolysis	+	+
8)	Oxidase	-	-

Table 5. Antibiotic suscptability profile of *Enterococcus* isolates

Antibiotic discs	*E.feacalis* (n=44)			Other *Enterococcus spp* (n=86)			Total (n=130)		
	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)	S(%)	I(%)	R(%)
Ciprofloxacin (5 µg)	44(100%)	-	0(0%)	56(65.11%)	17 (19.77%)	13(15%)	100(76.92%)	17(13.08%)	13(10%)
Ampicillin (10 µg)	24(54.54%)	-	20 (45.46%)	53(61.63%)	-	33 (38.37%)	5759.23%	-	53(40.77%)
Gentamicin (10 µg)	24(54.54%)	-	20(45.46%)	28(32.56%)	12(13.95%)	46(53.49%)	52(40%)	12(9.23%)	66(50.77%)
Chloramphenicol (30 µg)	22(50%)	8(18.18%)	14 (31.82%)	47(54.65%)	14(16.28%)	25(29%)	69(53.08%)	22(16.92%)	39(30%)
Nitrofurantoin (300 µg)	21(47.72%)	10(22.72%)	13(29.55%)	45(52.33%)	11(12.79%)	30(34.88%)	66(50.77%)	21(16.15%)	43(33.08%)
Vancomycin (30 µg)	20(45.45%)	15(34.09%)	9 (20.45%)	65(75.58%)	8(9.30%)	13(15.11%)	85(65.38%)	23(17.69%)	22(16.92%)
Streptomycin (10 µg)	17(38.64%)	6(13.64%)	21(47.73%)	15(17.44%)	11(12.79%)	60(69.77%)	32(24.61%)	7(13.08%)	81(62.31%)

S = sensitive, I = intermediate, R = resistant

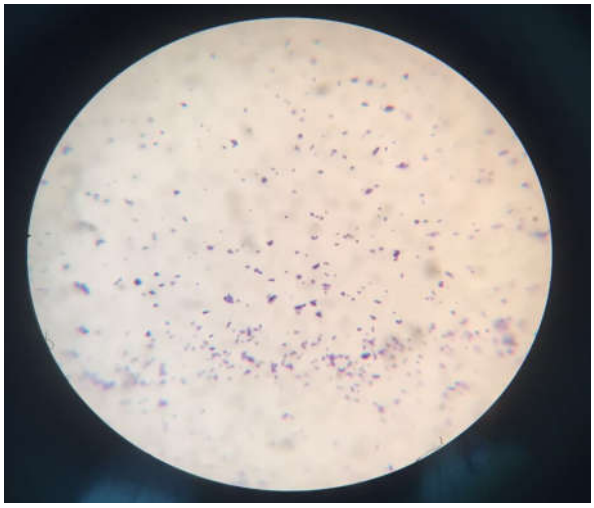

Figure 1. *Enterococcus* spp under microscope appear as cocci in single or pairs

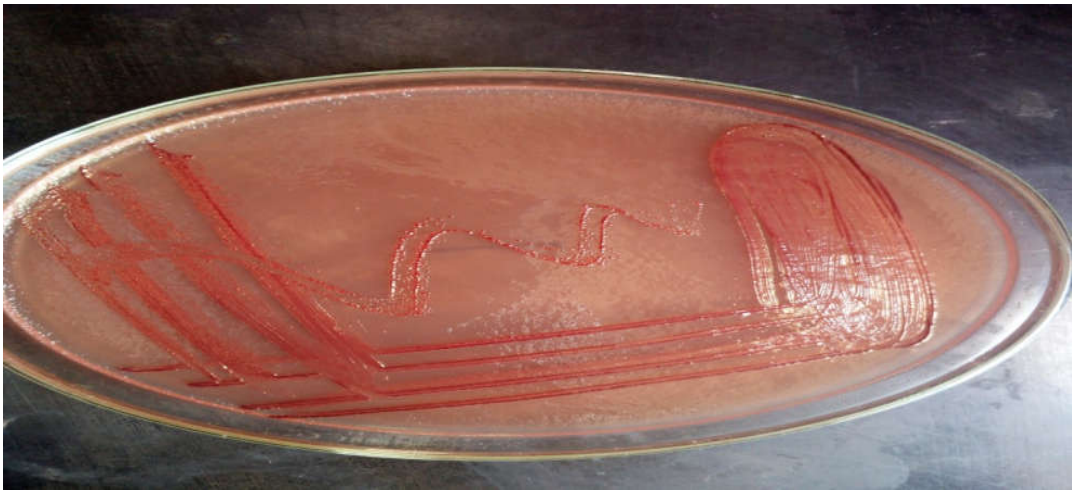

Figure 2. *Enterococcus* spp appear as pinpoint pink to dark red (maroon) color on m-*Enterococcus Agar*

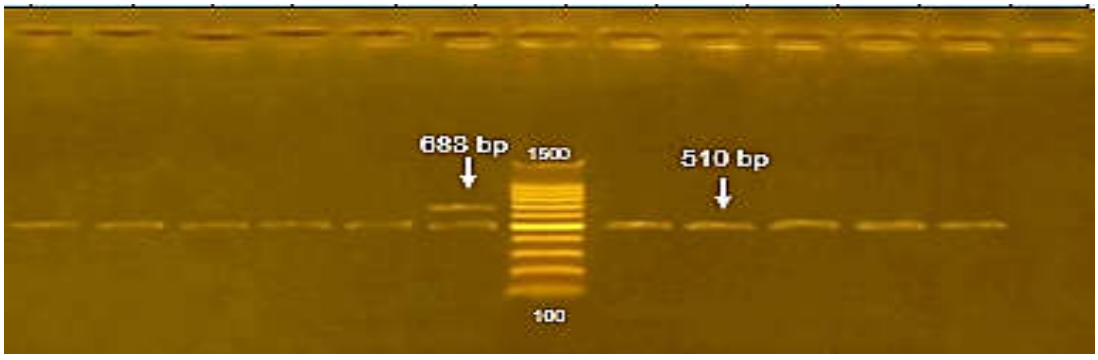

Figure 3. Agarose gel electrophoresis of multiplex PCR products showing amplification of CylA gene (510) and ESP (688bp) of *E.feacalis*; Lane L: DNA molecular weight marker (100 -1500bp), lane Pos: positive control, lane Neg : negative control, lanes 1-10: negative for CylA gene (510bp) and lanes 1-10 positive for ESP (688bp)

Detection of virulence factors by mPCR amplification

The presence of some virulence genes, as *esp* and *cylA* gene, was determined in *E.feacalis* isolates by using multiplex PCR. *Esp* gene was found in all examined isolates (10/10 samples; 100 %) and *cylA* was absent in all examined isolates (0/10 samples; 0 %) (Table **4** and Fig.3).

Antimicrobial susceptibility

Antibiotic resistance tests showed that *Enterococcus*spp were resistant to streptomycin (69.77%), gentamicin (53.49%), ampicillin (38.37%), nitrofurination (35%), cholaramphincol (29%), vancomycin (15%) and ciprofloxacin (15%). Meanwhile, *E.faecalis* isolates were resistant to streptomycin (47.73%), ampicillin (45.46%), gentamicin (45.46%), chloramphenicol (31.82%), nitrofurantoin (29.55%), and vancomycin (20.45%). It had been noticed that *E.faecalis*isolates were sensitive tociprofloxacin (100%) (Table **5**).

DISCUSSION

Bovine mastitis is caused by different virulent strains of bacteria. Recently, *Enterococcus* spp are considered as one of the main causes of environmental mastitis in cows in Egypt as breeding of cows is held under inappropriate conditions (Halasa *et al*., 2009). *Enterococcus* spp were identified with gram‘s stain, their feature on MEA and with other biochemical tests. These results were compatible in most of biochemical tests with other investigators (Cassenego *et al*., 2011; Nachtigal *et al*., 2013 and Prichula *et al*., 2013). In this study, The prevalence was higher in both clinical and subclinical mastitic cases due to several reasons as low hygienic measures in Egyptian dairy farms with faecal contamination of milk, their commensal characteristic feature and different modern rearing methods for dairy animals outside our country. Few researchers have been searched about this topic in Egypt. The proportions are extinct in other researchers as these results were disagreed with Ebrahimi *et al*. (2008) who detected the presence of *Enterococcus* spp in 4% of subclinical mastitis and absent in clinical mastitis. In addition, Olde *et al*. (2008) who noticed that *Enterococcus* spp were present in 4.7% of clinical mastitic cases in Canada and Kateete *et al*. (2013) who found *Enterococcus* spp were present in 28% of clinical mastitic cases in Uganda. *Enterococcus* spp especially *E.feacalis* have zoonotic importance and harmful effects on human and animal health due to their resistance to antibiotics and their commensal characters. Until now, little information is available about the molecular characteristics of *Enterococcus* spp and their resistance to different types of antibiotics isolated from mastitic cow‘s milk in Egypt. In this work, *E.faecalis* was observed as the most prevalent strains between *Enterococcus* spp isolates. These results were nearly coincided with Jackson *et al*. (2010) and Furlaneto-Maia *et al*. (2014) who found the presence of 40.7% and 27.77% as E. faecalis from isolated samples, respectively, and compatible with Katholm *et al*. (2012) who noticed that the prevalence of *Enterococcus* spp were high with 78% of examined 4258 Danish dairy herds.

When host gets infected with *Enterococcus* spp, different virulence factors have been produced to colonize and evade the immune system as enterococcaus surface protein (*esp*) and cytolysin (*cylA*). Espgenepromotes the adhesion, colonization and biofilm formation, while *cylA* gene is another one as a bacterial toxin. In the present study, the presence of some virulence genes as *esp* and *cylA* gene was determined in ten of *E.feacalis* isolates using multiplex PCR. *Esp* gene was detected in all tested *E.faecalis* isolates with prevalence of 100%. This result was consistent with previous studies that detected the higher prevalence of esp among clinical isolates (Hendrickx *et al*., 2008; Paganelli *et al*., 2012). However, Hussein (2013) recorded lower prevalence of esp gene (65%). Moreover, the absence of *cylA* was reported by other investigators (Zou *et al*., 2011; Iweriebor *et al*., 2015; El-Shahat *et al*., 2016). Recently, there is a new tendency in Egypt to use *Enterococcus* spp in dairy products. It shouldn’t be used in manufacture without molecular screening of a large set of virulence genes. As in this study, high incidence of virulence genes has been observed from mastitic milk which obtained from different dairy farms. The exaggerated and inappropriate usage of antimicrobial drugs in dairy farm animals in Egypt increase public health hazard with the production of antimicrobial resistance bacteria which able to transmit from animals to human with consumption of milk and dairy products leading to difficulty in treatment in both animals and human (Burgos *et al*., 2009).In the present study, antibiotic resistance tests were applied and the results were interrupted according to CLSI (2011) and showed that *Enterococcus* spp were resistant to streptomycin, gentamicin, ampicillin, nitrofurination, cholaramphincol, vancomycin and ciprofloxacin. These results were compatible with other investigators (Ebrahimi *et al*., 2008; Hammad *et al*., 2015; Raafat *et al*., 2016; Xiaohu *et al*., 2016). In contrast, other researchers detected lower rates of resistance to streptomycin and vancomycin (Hammad *et al*., 2015; Xiaohu *et al*., 2016). Concerning the resistance of *E.faecalis* isolates, higher resistance was observed to streptomycin, ampicillin, gentamicin, chloramphenicol, nitrofurantoin and vancomycin. Nearly similar observation of resistance to streptomycin was found by Oli *et al*. (2012). Moreover, the resistance of *E.faecalis* isolates to ampicillin and nitrofurantoin were parallel to that observed by Hussein (2013) but were conflicted with Castillo – Rojas *et al.* (2013) who found a higher resistance rate of *E.faecalis* to ampicillin (88.2%). Also, the resistance of *E.faecalis* to gentamicin was reported previously by Gajan *et al*. (2013) and Sadek *et al*. (2014) in percentage of 36.2% and 44.7%, respectively, but lower rate of gentamicin resistance was observed by Hussein (2013) in percentage of 20%. As well, its resistance to chloramphenicol was coincided with Pimentel *et al*. (2007) who found the resistance with 32%, while another study detected no resistance to chloramphenicol (Hussein 2013). In addition, its vancomycin resistance was comfortable with Furlaneto-Maia *et al*. (2014), but conflicted with Kürekci *et al*. (2016) who recorded lower resistance toward vancomycin with 5%.

However, all *E.faecalis* isolates were sensitive tociprofloxacin 44(100%) and this attributed to low usage of this antibiotic in treatment of cows in Dakahlia government. These results were coincided with Sadek *et al.* (2014) who detected 100% of *E.faecalis* isolates were sensitive to ciprofloxacin in Assuit, but were conflicted with Olawale *et al*. (2010) and Oli *et al*. (2012) who recorded 20% and14.10% resistance to ciprofloxacin, respectively. However, this study demonstrated the emergence of multiple antibiotic resistance *Enterococcus* strains that is considered alarming to the failure of enterococcal infection therapy. Unfortionatly, it is difficult to accurate estimation of the relation between human and animal usage of antibiotics on the spread of antibiotic-resistant bacteria in Africa and particularly in Egypt due to the surveillance systems are inadequate.

Conclusion

Enterococcus spp have harmful effects on farm animals because they posses different virulence genes. *E.feacalis* has been considered as indicator for fecal contamination and unhyaginic measures in dairy farms causing different health problems in human and animals. Raw milk from cows is considered as potential reservoirs of antibiotic resistance and virulent enterococci with public health hazard when milk is consumed without any thermal treatment. Further studies are required to assess the roles of *Enterococcus* spp and *E.feacalis* especially in mastitc cow‘s milk in Egypt to avoid unsafety consumption of fresh raw milk in dairy products.

Acknowledgement

Special thanks to Prof. Dr. Essam M. Mahmoud, Faculty of Veterinary Medicine, Mansoura University for assistance in collection of samples.

REFERENCES

Aarestrup, F.M., Hasman, H., Jensen, L.B., Moreno, M., Herrero, I.A., Dominguez, L. 2002.Antimicrobial resistance among enterococci from pigs in three European countries. *Appl. Environ. Microbiol*., 681 10: 4127-4129.

Bradley, A.J., Leach, K.A., Breen, J.E., Green, L.E., Green, M.J. 2007.Survey of the incidence and aetiology of mastitis on dairy farms in England and Wales. *Vet Rec*., 160: 253-257.

Burgos, M.J.G., Lopez, R.L., Abriouel, H., Omar, N.B., Galvez, A. 2009.Multilocus Sequence Typing of *Enterococcus* faecalis from Vegetable Foods Reveals Two New Sequence Types. *Food borne Pathogens and Disease*. 6(3): 321-327.

Cassenego, A.P.V., D’Azevedo, P.A., Ribeiro, A.M.L., Frazzon, J., Van Der Sand, S.T., Frazzon, A.P.G. 2011.Species distribution and antimicrobial susceptibility of Enterococci isolated from broilers infected experimentally with Eimeria spp. and fed with diets containing different supplements. *Braz J Microbiol.,* 42 (2): 480-488.

Castillo – Rojas, G., Mazar – Hiriart, M., Leon, S.P., Rosa, I., Agis-Juárez, R.A., Huebner, J., López-Vidal, Y. 2013. Comparison of *Enterococcus*faecium and *Enterococcus* faecalisstrains isolated from water and clinical samples, antimicrobial susceptibility and genetic relationships. PLOS ONE. 8 (4): 1-10.

Chuang-Smith, O.N., Wells, C.L., Henry-Stanley, M.J.,Dunny, G.M. 2010.Acceleration of *Enterococcus* faecalis biofilm formation by aggregation substance expression in an ex vivo model of cardiac valve colonization. PLOS ONE.5:15798.

CLSI (Clinical and Laboratory Standards Institute) 2011. Performance standards for antimicrobial susceptibility testing; twentieth informational supplement. In CLSI Document M100-S21, J.A. Pp. 84–87. Wayne, PA: Clinical and Laboratory Standards Institute.

Dogru AK, Gencay YE, Ayaz ND. 2010. Comparison of virulence gene profiles of *Enterococcus* faecium and *Enterococcus* faecalis chicken neck skin and faeces isolates. KafkasUnv. Vet. Fak.Derg., 16 (A): 129 -133.

Ebrahimi A, Nikookhah F, Nikpour S, Majiian F, Gholami M. 2008. Isolation of Streptococci from milk samples of normal, acute and subclinical mastitis cows and determination of their antibiotic susceptibility patterns. *Pak. J. Biol. Sci*., 11: 148-150.

Elhadidy M, Elsayyad A. 2013. Uncommitted role of enterococcal surface protein, Esp, and origin of isolates on biofilm production by *Enterococcus* faecalis isolated from bovine mastitis. *J MicrobiolImmunol Infect*, 46: 80-84.

El-Shahat A, Hussien H, Hussan Z, Abdella W. 2016. Genotyping and Virulence Genes of *Enterococcus faecalis* Isolated Form Kareish Cheese and Minced Meat in Egypt.Microbiology. 11: 133-138.

Fisher K, Phillips C. 2009. The ecology, epidemiology and virulence of *Enterococcus*. Microbiology. 155: 1749-1757.

Furlaneto-Maia L, Rocha KR, Henrique FC, Giazzi A, Furlaneto MC. 2014. Antimicrobial Resistance in *Enterococcus* spp Isolated from Soft Cheese in Southern Brazil. *Adv. Microbiol*., 4 (3):175-181.

Gajan EB, Shirmohammadi A, Aghazadeh M, Alizadeh M, Deljavan A, Ahmadpour F. 2013. Antibiotic resistance in *Enterococcus faecalis* isolated from hospitalized patients. *J Dental Res. Dental Clin. Dental Prospect*, 7(2):102-104.

Gilmore MS, Lebreton F, van Schaik W. 2013.Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. *Curr.Opin.Microbiol*., 16: 10-16.

Halasa T, Nielen M, De Roos AP, Van Hoorne R, de Jong G, Lam TJ, Van Werven T, Hogeveen H. 2009. Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. *J. Dairy Sci*., 92: 599-606.

Hammad AM, Hassan HA, Shimamoto T. 2015. Prevalence, antibiotic resistance and virulence of *Enterococcus*spp.In Egyptian fresh raw milk cheese. *Food Control*, 50: 815-820.

Hendrickx AP, Bonten MJ, van Luit-Asbroek M, Schapendonk CM, Kragten AH, Willems RJ. 2008. Expression of two distinct types of pili by a hospital-acquired *Enterococcus* faecium isolate. *Microbiology*, 154: 3212-3223.

Hussein N. 2013. Detection of some virulence factors of *Enterococcus faecalis* isolated from raw milk by Multiplex PCR. *J al-qadisiyah for Pure Science*, 18: 1-14.

Iweriebor BI, Obi LC, Okoh AI. 2015. Virulence and antimicrobial resistance factors of *Enterococcus* spp. Isolated from fecal samples from piggery farms in Eastern Cape, South Africa BMC. *Microbiology*, 15: 136.

Jackson CR, Lombard JE, Dargatz DA, Fedorka-Cray PJ. 2010. Prevalence, species distribution and antimicrobial resistance of enterococci isolated from US dairy cattle. *Letters in Applied Microbiology*, 52: 41-48.

Kateete DP, Kabugo U, Baluku H, Nyakarahuka L, Kyobe S, Okee M, Najjuka CF, Joloba ML. 2013. Prevalence and Antimicrobial Susceptibility Patterns of Bacteria from Milkmen and Cows with Clinical Mastitis in and around Kampala, Uganda. PLoS ONE. 8(5): e63413.

Katholm J, Bennedsgaard TW, Koskinen MT, Rattenborg E. 2012. Quality of bulk tank milk samples from Danish dairy herds based on real-time polymerase chain reaction identification of mastitis pathogens. *J Dairy Science*, 95: 5702-5708.

Kürekci C, Pehlivanlar SO, Yipel M, Aslantaş O, Gündoğdu A. 2016.Characterisation of Phenotypic and Genotypic Antibiotic Resistance Profile of Enterococci from Cheeses in Turkey Korean. *J Food SciAnimResour*., 36(3): 352-358.

Lopes M, Ribeiro T, Abrantes M, Figueiredo Marques J,Tenreiro R, Crespo MTB. 2005. Antimicrobial resistance profiles of dairy and clinical isolates and type strains of enterococci. *Int. Food Microbiol*., 103: 191-198.

Nachtigall G, Jesus AG, Zvoboda DA, Santestevan NA, Minotto E, Moura TM, d'Azevedo P, Frazzon J, Van Der Sand S, Frazzon APG. 2013. Diversida de e perfil de susceptibilida d antimicrobiana de*Enterococcus* spp. isolados das a´guas do ArroioDilu´vio-Porto Alegre, RS, Brasil. *R Bras Bioci*., 11 (2): 235-241.

Nueno-Palop C, Narbad A. 2011. Probiotic assessment of *Enterococcus faecalis* CP58 isolated from human gut. *Int. J. Food Microbiol*., 145: 390-394.

Olawale AK, Akintobi AO, Famurewa O. 2010. Prevalence of antibiotic resistant Enterococci in fast food outlets in Osun State Nigeria. *New York Sci. J*., 3 (1): 70-75.

Olde RG, Barkema HW, Kelton DF, Scholl D. 2008.Incidence rate of clinical mastitis on Canadian dairy farms. *J Dairy Sci*., 91: 1366-1377.

Oli AK, Rajeshwari H, Nagaveni S, Kelmani CR. 2012. Antimicrobial susceptibility pattern of *Enterococcus* species isolated from clinical samples in South India. *J. Recent Advances Applied Sci*., 27: 6-10.

Paganelli FL, Willems RJ, Leavis HL. 2012. Optimizing future treatment of enterococcal infections: attacking the biofilm?.*Trends Microbiol*., 20: 40-49.

Pimentel L, Semedo T, Rogério C, Teresa M, Pintado B, Manuela M, Malcata E, Xavier F. 2007. Assessment of safety of enterococci isolated throughout traditional terrinchocheese making: Virulence factors and antibiotic susceptibility. *Journal of Food Protection*, 70 (9): 2161-2167.

Pitkala A, Haveri M, Pyorala S, Myllys V, Honkanen-Buzalski T. 2001. Prevalence, distribution of bacteria, and antimicrobial resistance Bovine mastitis in Finland. *J Dairy Sci*., 87 (8):2433-2441.

Prichula J, Zvoboda DA, Pereira RI, Santestevan NA, Medeiros AW, Motta AS, D´Azevedo PA, Giordani AR, Frazzon APG. 2013. Perfil de suscetibilida de aosantimicrobianos e diversida de das espe´cies de enterococososisolados de leite cru de bu´falas no Sul do Brasil. *R Bras Ci Vet*., (2): 104-109.

Raafat SA, Abo-Elmagd EK, Awad RA, Hassan EM, Alrasheedy ZE. 2016. Prevalence of Vancomycin Resistant Enterococci in Different Food Samples. *Egyptian J Medical Microbiology*, 25 (4): 47-55.

Rosellini AJ, Lawrence AE, Meyer JF, Brown TA. 2010. The effects of extraverted temperament on agoraphobia in panic disorder. *J Abnormal Psychology*, 119: 420-426.

Sadek OA, Sayed SM, El Berbawy SM, Mansy MF, Hussien MF. 2014. Some antibiotic resistant bacteria of public health hazard isolated from raw milk sold in some Assiut City markets Ass. Univ. *Bull. Environ. Res*., 17 (1): 97-107.

Sillanpaa J, Xu Y, Nallapareddy SR, Murray BE, Hook M. 2004. A family of putative MSCRAMMs from *Enterococcus*faecalis.*Microbiology*, 150: 2069-2078.

Van Tyne D, Gilmore MS. 2014. Friend turned foe: evolution of enterococcal virulence and antibiotic resistance. *Annu Rev Microbiol*., 68: 337-356.

Vankerckhoven V, Van Autgaerden T, Vael C, Lammens C, Chapelle S, Rossi R, Jabes D, Goossens H. 2004. Development of a 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. *J Clinical Microbiology*, 42(10): 4473-4479.

Xiaohu WU, Shubao HOU, Quanwei Z, Youji MA, Yong Z, Wei KAN, Xingxu Z. 2016. Prevalence of virulence and resistance to antibiotics in pathogenic enterococci isolated from mastitic cows. *J Vet Med Sci*., 78 (11): 1663-1668.

Zou L, Wang H, Zeng B, Li JN, Li XT., Zhang AY, Zhou YS, Yang X, Xu CW, Xia QQ. 2011. Erythromycin resistance and virulence genes in *Enterococcus faecalis* from swine in china. *New Microbiol*., 34: 73- 80.

****