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Virulence potential of *Enterococcus gallinarum* strains isolated from selected Nigerian traditional fermented foods

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ABSTRACT

Five *Enterococcus* isolates from some Nigerian traditional fermented foods were identified as *Enterococcus gallinarum* by using phenotypic and genotypic tests. Safety properties such as antibiotic susceptibility, virulence gene detection, haemolysin, gelatinase and bacteriocin production were determined using standard methods. There was no resistance to clinically relevant antibiotics. Virulence gene for collagen binding antigen and aggregation substance were detected in 60% of the *E. gallinarum* strains; while surface adhesin was detected in 20%, but none of the strains had cytolysin activator and gelatinase. Phenotype characterizations of the *E. gallinarum* isolates indicated that none of the isolates produced haemolysin and gelatinase. *Enterococcus gallinarum* C103 and U82 had no antimicrobial activity against all the selected bacteria pathogens while *E. gallinarum* W184, T71 and W21 were active against some of the indicator bacteria pathogens. Only *E. gallinarum* T71 and W21 showed broad spectra of antimicrobial activity. Combination of virulence factors did not appear in these food isolates. Therefore, these strains particularly the two strains with high spectra of antimicrobial activity could be exploited as functional starters in foods.

Key words: Virulence genes, haemolysin, gelatinase, antibiotic susceptibility and antimicrobial activity, Enterococci

Introduction

Enterococci are normal human commensals of the gastrointestinal and urogenital systems of humans, several other mammals, and birds (Koch et al., 2004). Some enterococcal strains are used as probiotic agents and are thought to have beneficial effects on a number of gastrointestinal and systemic diseases (Ben-Omar et al., 2004). The incorporation of probiotic lactic acid bacteria into foods is gaining momentum, and the market for food products containing such microorganisms is growing rapidly (Stanton et al., 2001). However, the deliberate addition of Enterococci to foods has raised concerns, as a result of their relatively frequent association with human infections, particularly endocarditis, urinary tract infections, and nosocomial infections, and the increased frequency of resistance to antibiotics such as vancomycin observed among members of the genus (Aguirre & Collins, 1993; Adams & Marteau, 1995).

Enterococcus faecium and *Enterococcus faecalis* associated colonisation and infection in animals cause the vast majority of clinical enterococcal infections in humans, including important nosocomial infections, especially in immunocompromised patients (Vergis et al., 2002). Enterococci now rank among the top three nosocomial bacterial pathogens (Richards et al., 2000; Wisplinghoff et al., 2003), and strains resistant to currently available antibiotics pose real therapeutic difficulties (Hunt, 1998). Up to 90% of enterococcal infections in humans are caused by *Enterococcus faecalis*. The majority of the remainder is caused by *Enterococcus faecium*, and infections with the other species are quite rare (Jett et al., 1994). A number of enterococcal virulence factors have been described. Among them, gelatinase (GelE), aggregation substance (AS), and

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cytolysin have been studied most intensively (Jett et al., 1994; Elsner et al., 2000). In addition, enterococci are relatively 'promiscuous' microorganisms with many of the traits contributing to their virulence residing on conjugative plasmids, which may be readily transferred between microorganisms (Jett et al., 1994).

Furthermore, many food-related strains of Enterococci offer a means of improving food safety, as they have been shown to produce bacteriocins and when added to foods during manufacture these strains offer potential for the inhibition of food pathogens such as *Listeria monocytogenes* (Giraffa et al., 1997). However, it was suggested that Enterococci should only be used in foods if there are demonstrable benefits, given their association with human infection (van der Kamp, 1996). Therefore, in this study, the virulence potential of *Enterococcus gallinarum* strains isolated from some Nigerian fermented foods were evaluated in order to gain a better understanding of their usefulness in food application.

Materials and Methods

Sample collection

The samples of traditionally fermented vegetable condiments (ugba, ogiri, okpehe) and West African soft cheese (*wara*) were randomly purchased from local markets in Nigeria. The samples were separately packaged in sterile polythene bags and aseptically transported to the laboratory under cold conditions for analysis.

Bacterial strains and culture conditions

For isolation of presumptive *Enterococci*, 10 g of each sample were separately homogenized in 90 mL of sterile peptone water. Serial 10-fold dilutions were performed and aliquots were plated on Slanetz and Bartley Medium (Oxoid, Canada). After 48 h incubation at 37°C, typical small pinkish colonies of presumptive Enterococci were randomly picked from plates and subcultured in order to obtain pure isolates. Presumptive Enterococci isolates were cultured in Brain Heart Infusion broth (Oxoid, Canada) with incubation at 37°C for 18 h. Pure cultures were kept frozen at -20°C in BHI broth containing glycerol (50%).

Identification of strains

Strains identification was carried out according to physiological and biochemical characteristics, as described by Schleifer & Kilpper-Balz (1984). To confirm the identity of the isolates, total genomic DNA was extracted using the method described by Sambrook et al. (1989). Identification was carried out by sequencing of the 16S rRNA genes using the primers designated as FD1 (5'-AGAGTTTGATCCT GGCTCAG-3') forward and RD1 (5'-AAGGAGGTGATCC AGCC- 3') for reverse (Weisburg et al., 1991).

Determination of antibiotic susceptibility profile

The antibiotic susceptibility of the bacterial species isolated was performed on Müller-Hinton agar. Then, 0.1 mL of each bacterial isolates (10^5-10^6 cells/mL**)** was seeded into each of the Petri dishes containing sterile Müller-Hinton agar and were allowed to stand for 30 minutes to enable the inoculated organisms to pre-diffuse. The commercially available discs containing the following antibiotics: ampicillin (AMP – 10 µg), penicilin-G (P – 10 µg), vancomycin (VA – 30 µg), linezolid (LZ – 30 µg), gentamicin (HLG – 120 µg), tigecycline (TGC – 15 µg), erythromycin (E – 15 µg), pristinomycin (RP – 15 µg), ciprofloxacin (CIP – 5 µg), levofloxacin (LE – 5 µg), norfloxacin (NX – 5 µg), fosfomycin (200 µg), (Himedia, India) were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and were incubated at 37°C for 24 h. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters (NCCLS, 2001).

Determination of minimum inhibitory concentration (MIC) of vancomycin

The inocula were prepared by transferring pure colonies of isolate to 5 mL of Tryptone soya broth. It was then incubated at 37°C for 6 hours until light to moderate turbidity developed. The inocula turbidity was compared with that of the standard (prepared by mixing 0.5 mL of 1.175% barium chloride and 99.5 mL of 0.36 N sulphuric acid). The inocula were incubated further until a comparative turbidity was attained. The inocula were also standardized by taking the optical density reading, which should be between 0.08 and 0.13 at 620 nm (yields 10^5-10^6 cells/mL). A sterile nontoxic swab was dipped into the standardised inoculum; this swab was used to streak the entire surface of sterile Müller Hinton agar plates. The inocula were allowed to dry for 15 minutes with the lid in place, and then the vancomycin HiComb MIC strips were applied to agar surface with the aid of a sterile forceps, and incubated at 37°C for 24 h. The zone of inhibition was in the form of an ellipse. MIC value was the value at which the zone convened the comb-like projections of the strips (Hayes et al., 2004).

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Haemolysin activity

Blood agar plates were prepared using Nutrient agar base with about 6% of defibrinated human blood containing all four blood types. The blood agar plates were inoculated with the isolates and were incubated at 37°C for 24 h. Also the haemolytic patterns of the organisms were observed after 24 hours, dark green discolouration of the media indicated Alpha-haemolytic, while the transparency of the agar indicated Beta-haemolytic (Creti et al., 2004).

Gelatinase activity

Gelatinase production was detected by inoculating the Enterococci onto freshly prepared Nutrient agar containing 2% gelatin (20 g/L). Plates were incubated overnight at 37°C and then cooled to ambient temperature for 2 h. The appearance of a turbid halo or zone around the colonies was considered to be a positive indication of gelatinase production (Eaton & Gasson, 2001).

Virulence gene detection by PCR

For virulence gene detection by PCR, the method of Sedgley et al. (2005) was used with a few modifications. Specific primers targeting segments of the enterococcal virulence determinants genes were used for the amplification, for aggregation substance (asa); the forward primer was made up of asaF (5'-CCAGCCAACTATGGCGGAATC-3') and reverse primer asa R (5'-CCTGTCGCAAGATCGACTGTA-3'), the forward primer for surface adhesin (esp) was espF (5'-TTGCTAATGCTAGTCCACGACC-3') and the reverse espR (5'-GCGTCAACACTTGCATTGCCGA-3'). Also cytolysin activator (cylA) forward primer was cylAF (5'-GACTCGGGGATTGATAGGC-3') and the reverse cylAR (5'-GCTGCTAAAGCTGCGCTTAC-3'), forward primer for collagen binding antigen (ace) was aceF (5'-GGAATGACCGAGAACGATGGC-3') and the reverse aceR (5'-GCTTGATGTTGGCCTGCTTCCG-3') while the forward primer for gelatinase (gelE) was gelEF (5'-ACCCCGTATCATTGGTTT-3') and the reverse was gelER (5'-ACGCATTGCTTTTCCATC-3'). The PCR conditions were modified to accommodate a common annealing temperature profile of 58°C for all PCR reactions except for gelE (52°C), based on initial temperature gradient PCR amplifications of each target. Individual 25 µL reaction mixture containing 100–200 ng total DNA template along with master mix that included 1x PCR buffer, 10 mM dNTPs, 25mM $MgCl_2$, 1 unit of Taq DNA polymerase (Platinum Taq polymerase; Invitrogen, Carlsbad, California) and 100 pmol of each oligonucleotide primers was prepared. The PCR conditions were as follows: 5 min initial DNA denaturation step at 95°C followed by 35 consecutive cycles at 94°C for 20 sec; 58°C for 45 sec; 72°C for 60 sec. The amplified fragments were resolved by electrophoresis on a 1.5% agarose gel stained with ethidium bromide.

Detection of direct antimicrobial activity

The antimicrobial activity of bacterial colonies was assayed by using the toothpick method described by del Campo et al. (2001). The indicator organisms used included *Serratia marcescens* MTCC 06, *Micrococcus luteus* MTCC 106, *Proteus mirabilis* MTCC 425, *Proteus vulgaris* MTCC 426, *Bacillus cereus* MTCC 430, *Bacillus subtilis* MTCC441, *Klebsiella pneumoniae* MTCC 618, *Escherichia coli* MTCC 739, *Shigella flexneri* MTCC 1457, *Lactococcus lactis* MTCC 3038, *Enterobacter cloacae* MTCC 509, *Staphylococcus aureus* MTCC 737 and *Pseudomonas aeruginosa* MTCC 1934. Fifty microliter of an overnight culture of each indicator strain was added separately to 25 mL of molten Nutrient broth (Himedia, India) supplemented with 0.7% (wt/vol) agar, mixed and poured into sterile Petri dishes. A single colony of each isolate was transferred with a sterile toothpick to the agar plate previously seeded with the indicator microorganism. Then, the plates were incubated at 37°C for 24 h. Antimicrobial activity was detected by the presence of clear inhibition zones around the producer strains (only inhibition halos with diameters 3 mm and above were considered positive).

Results

The presumptive Enterococci strains were characterized as gram-positive, catalase negative, oxidase negative, non-spore forming cocci in singles and pairs with ability to grow in the presence of 6.5% NaCl at pH 9.6 and temperature 10 - 45°C. The strains were able to hydrolyze esculin, pyrrolidonyl-β-naphthylamide, arginine, but were unable to hydrolyze starch and were differentiated based on their sugar fermentation pattern. The result of the biochemical test was further confirmed at the gene level by performing PCR in PCR SPRINT Thermal cycler (Thermo Electron Corporation, Japan). PCR condition was standardized with a set of primers to amplify the 16S r RNA gene. Molecular sequencing of the five positive strains using both the forward and the reverse primers yielded DNA sequences about 1500 bp in length.

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Table 1. *Antibiotic susceptibility profile of the Enterococcus gallinarum strains.*

Isolates	Diameter of zones of inhibition (mm)											
	AMP	P	LZ	VA	HLG	TGC	E	RP	CIP	LE	NX	FO
E. gallinarum W21 1	21	19	19	16	16	14	20	20	14	18	17	-
E. gallinarum U82	16	18	14	-	15	16	16	18	10	16	15	10
E. gallinarum T71	17	18	20	16	17	15	14	17	21	19	16	18
E. gallinarum W184	18	20	16	16	13	17	16	19	15	16	14	14
E. gallinarum C103	18	11	12	19	-	15	-	13	10	11	17	19

Susceptibility: presence of a zone of inhibition (mm); Resistance (-): absence of a zone of inhibition (mm), Ampicillin (AMP), Peniciliin-G (P), Vancomycin (VA), Linezolid (LZ), Gentamicin (HLG), Tigecycline (TGC), Erythromycin (E), Pristinomycin (RP), Ciprofloxacin (CIP), Levofloxacin (LE), Norfloxacin (NX), Fosfomycin (FO).

Alignment of the sequences with their closest match from a BLAST search was then performed and analysis of data revealed that the sequences had their best match with *E. gallinarum*. The sequences were deposited in the GenBank database and accession numbers assigned to each strains; *Enterococcus gallinarum* W211 (JF915769), *Enterococcus gallinarum* T71 (JF774411), *Enterococcus gallinarum* W184 (JN020631), *Enterococcus gallinarum* U82 (JF774412) and *Enterococcus gallinarum* C103 (JF774410).

Antibiotic susceptibility profile of the strains was determined as shown in Table 1.

Enterococcus gallinarum T71 and W814 were sensitive to all selected antibiotics, while *E. gallinarum* W211 and U82 were sensitive to eleven out of the twelve antibiotics tested with varied diameter of zones of inhibition. *E. gallinarum* U82 was found to be resistant to vancomycin. This prompted the determination of the minimum inhibitory concentration of vancomycin as shown in Table 2. The minimum inhibitory concentration was observed to be 0.128 µg/mL which falls into the sensitive group since susceptibility (S), intermediate (I) and resistance (R) were defined according to MIC breakpoints from the National Committee for Clinical Laboratory Standards (Agar diffusion method). MICs: vancomycin (S:≤4 µg/mL; I=8-16 µg/mL and R:≥32 µg/mL). However, all of the strains were not resistant to clinically relevant antibiotics. Moreover, none of the strains was beta hemolytic, but *E. gallinarum* U82 was found to be positive for alpha hemolysis and they were all gelatinase negative (Table 3).

Furthermore, virulence gene detection by PCR showed that the gene for collagen binding antigen (Ace) was present in *E. gallinarum* T71, *E. gallinarum* W184 and *E. gallinarum* C103 (Figure 1).

Table 2. *Minimum inhibitory concentration of vancomycin.*

Antibiotic (µg/mL)	*E. gallinarum* U82	Antibiotic (µg/mL)	*E. gallinarum* U82
256.0	-	2.048	-
128.0	-	1.024	-
64.0	-	0.512	-
32.0	-	0.256	-
16.0	-	0.128	-
8.0	-	0.064	+
4.0	-	0.032	+
2.0	-	0.016	+

'+': indicates growth of bacteria (resistance to vancomycin),'-': indicates inhibition of growth of bacteria (sensitive to vancomycin)

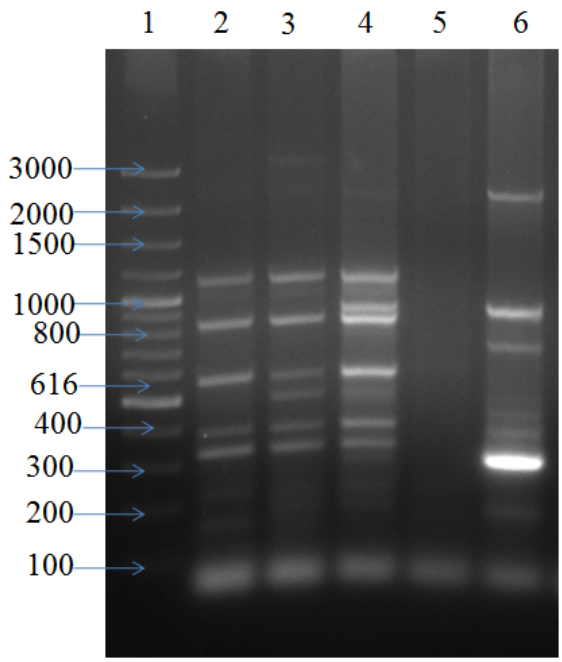

Figure 1. *Agarose gel electrophoresis of the collagen binding antigen gene (Ace) of the Enterococcus gallinarum strains. Lane 1: Marker Gene Ruler 100 bp plus (MBI Fermentas), Lanes 2-6: PCR amplified Ace (616 bp) gene of E. gallinarum. Lane 2: T71, Lane 3: C103, Lane 4: W184, Lane 5:U82, Lane 6: W211.*

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Table 3. *Haemolytic and gelatinolytic activity of the Enterococcus gallinarum strains.*

Isolates	α-Haemolytic	β-Haemolytic	γ- Haemolytic	Gelatinase
E. gallinarum W211	-	-	+	-
E. gallinarum U82	+	-	-	-
E. gallinarum T71	-	-	+	-
E. gallinarum W184	-	-	+	-
E. gallinarum CIO3	-	-	+	-

'+' : Detected, '-' : Not detected

Table 4. *Virulence gene detection by PCR in the Enterococcus gallinarum strains.*

Isolates	Virulence gene					
	Accession No	Ace	Asa	Cyl	Esp	Gel E
E. gallinarum W211	JF915769	-	+	-	-	-
E. gallinarum U82	JF774412	-	+	-	-	-
E. gallinarum T71	JF774411	+	+	-	+	-
E. gallinarum W184	JN020631	+	-	-	-	-
E. gallinarum C103	JF774410	+	-	-	-	-

'+' : Detected, '-' : Not detected

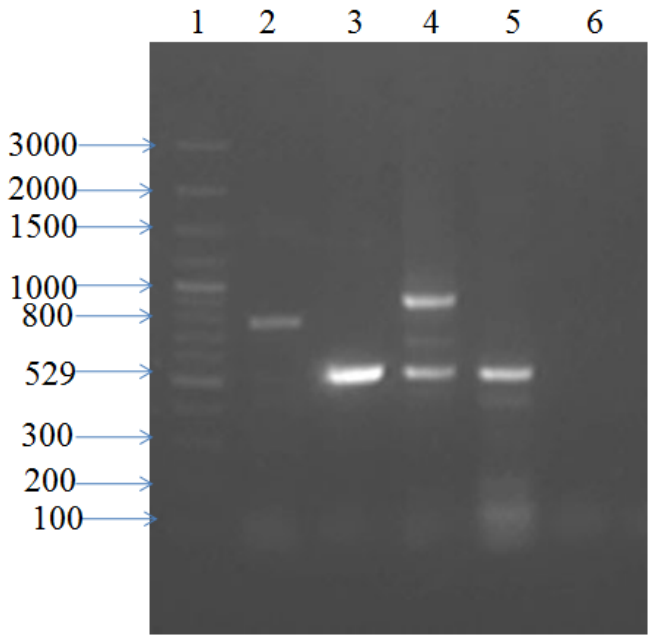

Figure 2. *Agarose gel electrophoresis of aggregation substance gene (Asa) of the Enterococcus gallinarum strains. Lane 1: Marker Gene Ruler 100 bp plus (MBI Fermentas), Lanes 2-6: PCR amplified Asa (529 bp) gene of Enterococcus gallinarum. Lane 2: W184, Lane 3: W211, Lane 4: U82, Lane 5: T71, Lane 6: C103.*

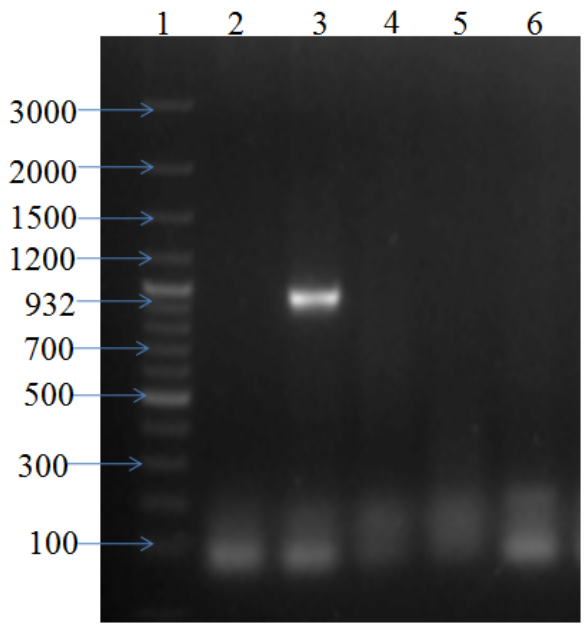

Figure 3. *Agarose gel electrophoresis of surface adhesion gene (Esp) of the Enterococcus gallinarum strains Lane 1: Marker Gene Ruler 100 bp plus (MBI Fermentas), Lanes 2-6: PCR amplified Esp (932 bp) gene of Enterococcus gallinarum. Lane 2: C103, Lane 3: T71, Lane 4: W211, Lane 5: W184, Lane 6: U82.*

Aggregation substance gene (Asa) was present in *E. gallinarum* W211, *E. gallinarum* U82 and *E. gallinarum* T71 (Figure 2). Surface adhesion gene (Esp) was detected in *E. gallinarum* T71 only (Figure 3), while none of these five strains of *E. gallinarum* had cytolysin activator (Cyl A) and gelatinase (Gel E) gene (Table 4).

Antimicrobial activity of the strains was determined against some selected indicator organisms as shown in Table 5. The *Enterococcus gallinarum* C103 and U82 had no antimicrobial activity against all selected pathogens.

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Table 5. *Antimicrobial activity of E. gallinarum strains against indicator organisms.*

Indicator organisms	Strains No	*E. gallinarum* W211	*E. gallinarum* U82	*E. gallinarum* T71	*E. gallinarum* W184	*E. gallinarum* C103
Serratia marcescens	MTCC 86	-	-	-	+	-
Micrococcus luteus	MTCC 106	-	-	+	-	-
Proteus mirabilis	MTCC 425	-	-	+	-	-
Proteus vulgaris	MTCC 426	+	-	+	-	-
Bacillus cereus	MTCC 430	+	-	+	-	-
Bacillus subtilis	MTCC441	-	-	-	-	-
Klebsiella pneumoniae	MTCC 618	-	-	+	-	-
Escherichia coli	MTCC 739	-	-	-	-	-
Shigella flexneri	MTCC 1457	-	-	+	-	-
Lactococcus lactis	MTCC 3038	-	-	-	-	-
Enterobacter cloacae	MTCC 509	+	-	-	-	-
Staphylococcus aureus	MTCC 737	+	-	+	-	-
Pseudomonas aeruginosa	MTCC 1934	+	-	-	-	-
Aeromonas hydrophilia	MTCC 646	+	-	+	-	-
Salmonella typhimurium	MTCC 98	+	-	+	-	-
Bacillus pumilus	MTCC 1607	-	-	+	-	-

'+' : Detected, '-' : Not detected

E. gallinarum W184, *E. gallinarum* T71 and *E. gallinarum* W211 had antimicrobial activity against the selected indicator bacteria. Only *E. gallinarum* T71 and *E. gallinarum* W211 exhibited broad spetra of antimicrobial activity.

Discussion

In the identification of the *Enterococcus gallinarum* strains, adequate phenotypic and genotypic characterization was employed as reported by Oladipo et al. (2013). An important clinical feature of *Enterococcus* species is the resistance to a wide range of antimicrobial agents as demonstrated in clinical, food and water isolates strains (Gold & Moellering, 1996; Giraffa et al., 1997; Eaton & Gasson, 2001). In addition, these bacteria are able to acquire resistance determinants through gene transference by plasmids and transposons.

The use of antimicrobials in animal feed as growth promoters has created large reservoirs of transferable antibiotic resistance genes in several ecosystems. Bacteria becomes resistant to antimicrobial agents by a number of mechanisms which are: production of enzymes, which inactivate or modify antibiotics, changes in the bacterial cell membrane, preventing the uptake of antibiotics and development of metabolic pathways by bacteria, which enable the site of an antimicrobial action to be by passed (Abbar & Kaddar, 1991).

Levy (1992) reported that antimicrobial resistance in bacterial pathogens is a major impediment to successful therapy, and in several instances, bacterial strains have arisen that are refractory to most available antimicrobial treatments. The public health consequences of antimicrobial resistance to many antibiotics have been debated. However, until recently clear evidence of health risk was not available. Multiple drug resistance is an extremely serious public health problem and it has been found to be associated with the outbreak of major epidemics throughout the world (Levy, 2001; Canton et al., 2003). A wide range of measures is needed to ensure that currently available antibiotics remain effective as long as possible. This can be achieved primarily by means of greater awareness among the public, health care professionals and the food- and agriculture sector regarding the importance of rational use of these medicines as well as ways to prevent infections and spread of antibiotic resistant bacteria (Freire-Moran et al., 2011).

A specific cause for concern and a contributing factor to pathogenesis of Enterococci is their resistance to a wide

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variety of antibiotics (Murray, 1990; Landman & Quale, 1997; Leclercq, 1997). But as reported by Franz et al. (2003), antibiotic resistance alone cannot explain the virulence of Enterococci. None of these strains showed multidrug resistance: the five strains were 93.3% sensitive to clinically relevant antibiotics like ampicillin, penicillin, ciprofloxacin, vancomycin etc., while resistance (6.7%) to antibiotics was obtained among three strains with resistance only to 1-2 out of twelve antibiotics used.

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. From all strains tested, *E. gallinarum* U82 was found to be resistant to vancomycin. This prompted the determination of the minimum inhibitory concentration of vancomycin, since the vancomycin resistance genes are transferable among different enterococcal species or even among different genera (Woodford et al., 1995). The inability to detect Enterococci promptly may cause delays in reporting vancomycin resistant Enterococci (VRE). This situation may lead to complex and costly containment efforts to eliminate VRE colonization and infection. The minimum inhibitory concentration was found to be 0.128 µg/mL, which falls into the sensitive group according to MIC breakpoints from the National Committee for Clinical Laboratory Standards (Agar diffusion method).

In this study, four out of five strains were non-haemolytic, while only *E. gallinarum* U82 was found to be α-haemolytic but none of the strains were β-haemolytic. Franz et al. (2001) reported that haemolysin plays an important role in Enterococcal virulence, as it may increase severity of infection. All strains were found to be gelatinase negative. Gelatinase as putative virulence determinant is an extracellular zinc endopeptidase capable of hydrolyzing gelatin, collagen and other proteins / peptidase, which has also been linked with infection (Eaton & Gasson, 2001)

Usually, virulence genes are plasmid-encoded and Enterococci possess effective gene transfer mechanisms (Clewell, 1990; Simiee & Gill, 1997). Therefore, investigation and evaluation of the present virulence factors among food isolates are necessary, especially with regard to the introduction of novel and commercial cultures, due to the risk of acquiring virulence factors via conjugation with other bacteria in the food processing environment. A virulence factor is an effector molecule that enhances the bacterial ability to cause disease in a host (Mundy et al., 2000). The presence of at least one virulence gene was confirmed in the five strains of *E. gallinarum*, but the gene for cytolysin/hemolysin activator and gelatinase were not detected in any of the strains; it was also noted that there was no phenotypic expression of these genes by the strains. Shankar et al. (1999) reported that *Esp* gene is considered to be an infection- associated virulence factor. On the other hand, Creti (2004) reported that the gene was discovered in 20% of isolates from healthy individuals and in 50% of environmental strains. Also, that *cylA* gene was present in 23% of all isolates and was distributed among healthy individuals, aggregation substance gene was present in 63.5% almost all isolates derived from healthy individuals (Creti, 2004). Eaton & Gasson (2001) showed that the *gelE* genes may be silent and that the phenotype may be negative even though a *gelE* gene is present, but in this study, *gelE* was absent in the strains and the phenotypic expression was also negative. Also, bacteriocin production as evident in three of the strains may provide the producer strain with a selective advantage over other strains, especially those closely related to the bacteriocin-producing strain (Tomita et al., 1997).

Enteroocci need to express a combination of virulence traits to be pathogenic (Johnson, 1994), but combinations of virulence factors did not appear in these food isolates. Therefore, these strains, particularly the two strains with high spectrum of antimicrobial activity, could be exploited as functional starters in foods.

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