Original Article

Molecular characterization of *Enterococcus* spp. clinical isolates from Cairo, Egypt

YA Hashem, *AS Yassin, MA Amin

Abstract

Purpose: Enterococci are responsible for serious diseases such as bacteraemia, endocarditis and urinary tract infections. The ability of enterococci to cause such diseases is due to acquisition of certain virulence factors such as haemolysin, gelatinase and enterococcus surface protein. This study has been conducted to investigate the occurrence of virulence factors and resistance to various antibiotics with emphasis on vancomycin in the *Enterococcus* spp. **Materials and Methods:** Clinical specimens were collected and isolates were identified by proper microscopic, culture and biochemical tests. Susceptibility and degree of resistance of the isolates to various antibiotics were determined. Virulence factors were examined by phenotypic tests followed by molecular methods. Bioinformatics analysis was used to detect regions in the genomes that might have originated from horizontal gene transfer. **Result:** The presence or absence of virulence genes did not affect the pattern of antimicrobial resistance in *Enterococcus* isolates; consequently, no relationship was found between virulence factors and resistance to different antibiotics used. Bioinformatics analysis showed that the virulence genes were mainly transferred by transposons. **Conclusion:** Among the enterococci, environmental factors may interfere in the expression of virulence factors. Horizontal gene transfer plays an important role in the spread of resistance and virulence genes.

Key words: *Enterococcus, Egypt, resistance, urinary tract infections, virulence*

Introduction

Enterococci are Gram-positive commensal bacteria that make up an important part of the intestinal flora of man and animals.[1] For many years, enterococci were believed to be harmless to humans. Moreover, *Enterococcus* species have been used widely over the last decade as probiotics and in the food industry as starter cultures.[2]

Recently, enterococci have become one of the most common nosocomial pathogens, with patients having a high mortality rate.[3] Enterococci are capable of causing various serious diseases such as endocarditis, bacteraemia, urinary tract infections (UTIs), and central nervous system infections. Most of these clinical infections are attributed to either *Enterococcus faecalis (E. faecalis)* or *Enterococcus faecium (E. faecium)*.[4]

*Corresponding author (email: <aymen.yassin@pharma.cu.edu.eg>)
Department of Microbiology and Immunology (YAH), Faculty of Pharmacy, Modern Sciences and Arts University, Giza, Cairo University (ASY, MAA), Cairo, Egypt

Received: 27-10-2013
Accepted: 14-04-2014

Access this article online	
Quick Response Code:	Website: www.ijmm.org
	DOI: 10.4103/0255-0857.148836

Several virulence factors may contribute to the ability of enterococci to cause such diseases. Adherence of enterococci to host cell is the first step in the process of infection, one of the adhesion factors is enterococcal surface protein (Esp).[5] Enterococci also secrete cytolysin (Cyl), a bacterial toxin that shows haemolytic activity against erythrocytes of human, rabbit and horses.[6] Another secreted molecule is gelatinase (GelE) which has the ability to hydrolyze gelatin, collagen, casein, haemoglobin and other small biologically active peptides.[7] Aggregation substance (Agg) is a virulence factor that causes the bacterial cells to aggregate or clump and, hence, facilitate plasmid transfer; it also plays a role in enterococcal endocarditis and UTI by supporting the adherence of bacteria to cardiac vegetations and renal epithelial cells.[7]

The severity of enterococcal infections has increased due to emergence of strains with multiple antimicrobial resistances. Resistance can be either intrinsic, such as resistance to low level of aminoglycosides, cephalosporins and penicillin, or acquired, such as resistance to glycopeptides, e.g., vancomycin and teicoplanin.[8] Vancomycin-resistant *Enterococcus* (VRE) infections are serious because glycopeptides are considered the last treatment available for life-threatening infections; therefore, it may lead to an increase in mortality rate.[9] The resistance of *Enterococcus* to vancomycin is mediated via group of genes (*vanA*, *vanB*, *vanC*, *vanD* and *vanE*). In the presence of vancomycin, these genes are transcribed and cell wall precursors with low affinity to vancomycin are synthesized.[10]

UTIs encompass infections of the kidney, ureters, bladder or urethra and are amongst the most common

bacterial infections worldwide.[11] Enterococci are considered to be among the most common pathogens associated with uncomplicated UTIs.[12]

The aim of the present study is to investigate the patterns of UTIs caused by enterococci among Egyptian patients and to analyze their antibiotic resistance profiles as well as their virulence factors, both phenotypically and molecularly. In addition, we attempt to investigate the possibility of spread of virulence and resistance genes by horizontal gene transfer by applying bioinformatics analysis of the available enterococci genomes. Studying resistance and virulence patterns of pathogens among patients admitted to local hospitals in Egypt can be used as a measure for understanding mechanisms leading to the spread of resistance, as a large number of the population rely on these hospitals due to socioeconomic factors.

Materials and Methods

Statement of ethical approval

All experiments were performed in accordance and approval of the ethical committee at Cairo University, Cairo, Egypt. In addition, all personnel who contributed any samples did this according to their written informed consent.

Sample collection

Clinical urine samples were collected from 100 patients admitted to the outpatient clinics of 'Abou-Elreesh' and 'El-Demerdash Hospitals' (University hospitals, belonging to Cairo University and Ain Shames University, respectively, in Cairo, Egypt). Clean-catch midstream urine was collected in sterile tubes and streaked on cysteine lactose electrolyte deficient agar or (CLED agar) and Enterococcsel agar. Isolates belonging to *Enterococcus* spp. were identified and stored using agar slants at 4°C; glycerol stocks were made and stored at −70°C. Reference standard of *E. faecalis* ATCC 29212 was obtained from (Naval Medical Research Unit-3, Cairo, Egypt).

Microscopic examination

Differentiation of isolates was carried out via Gram stains and cell morphology. Isolates which appeared Gram-positive cocci or coccobacilli cells in pairs or short chains when viewed through a microscope were suspected to be *Enterococcus*.

Biochemical and cultural identification

Catalase-positive reaction was indicated by a continuous bubble formation when hydrogen peroxide was introduced in bacterial colonies. Identification of *Enterococcus* isolates was confirmed by growth on 6.5% NaCl broth salt. Culture media used were Enterococcosel Agar HiCrome, *E. faecium* Agar, MacConkey's no. 2 agar and CLED agar. All media were from (Oxoid, UK) and were prepared by following the instruction of the manufacturers.

Detection of cytolysin production and gelatinase activity

Brain-heart infusion agar (Oxoid) supplemented with 5% sheep blood was used for the detection of cytolysin activity. Pure isolates were cultivated on blood agar plates, and the plates were incubated at 37°C for 24 h. Cytolytic activity was observed as (β) haemolysis surrounding bacterial colonies (complete haemolysis appeared as clear zones).[13]

Gelatinase assay was carried out by adding an inoculum from a pure culture into tubes containing 12% gelatin in 0.8% Nutrient Broth. Tubes were incubated for 24-72 h at 37°C and then placed in the refrigerator for approximately 30 min. The liquefaction of gelatin was considered as a positive result.[14]

Determination of antibiotics susceptibility (by Disc diffusion method)

The susceptibility of *Enterococcus* isolates to different anti-microbial agents was measured *in vitro* by disc diffusion method. From a pure culture, 5-6 colonies of the organism were transferred to a test tube containing 0.9% saline, and the suspension was standardized by 0.5 McFarland Standard. The entire surfaces of Mueller-Hinton agar (MHA) plates were inoculated with the cultures evenly; antibiotic discs were applied to their surfaces and the plates were incubated at 37°C for 24 h. After incubation, the diameters of the zone of complete inhibition were measured in mm. Isolates were classified as susceptible, intermediate or resistant in accordance with the Clinical and Laboratory Standard Institute (CLSI, 2011).

Determination of minimum inhibitory concentration of Vancomycin

MIC was determined by micro-broth dilution test using sterile 96-well microtitre plates. Antibiotic stock solution was prepared by dissolving vancomycin powder in sterile distilled water, and the concentration was adjusted to 512 μg/ml. A 1:10 dilution of 0.5 McFarland Standard was used; 50 μl each of antibiotic dilutions and organism suspension were mixed and incubated at 37°C for 24 hrs. The highest dilution which inhibited growth was considered MIC. MIC ≥32 μg/ml was considered to be indicative of resistant isolates.

Polymerase chain reaction amplification of virulent and resistance genes

PCR was carried out in a final reaction volume of 25 μl. A master mix containing green buffer, 1.5 mM $MgCL_2$, 200 μM of each deoxyribonucleotide, 10 pmol of each primer and 0.5 U Taq polymerase for a minimum of 10 samples was prepared and aliquoted in 22.5 μl quantities in individual PCR tubes. An amount of 1μg sample of DNA was added in each tube, and the final volume was adjusted to 25 μl. A list of primers used is in Table 1.[15-17]

The thermocycling conditions were initial denaturation at 94°C for 2 min, then 30 cycles of denaturation at 94°C for 30 s, annealing (48°C-54°C), depending on the primer pairs, for 30 s and extension at 72°C for 30 s with final extension cycle for 2 min (Techne Gradient, UK). Products were detected by agarose gel electrophoresis using agarose 1.5% W/V gel in 1X TAE buffer. Products were purified by the aid of AxyPrep PCR Clean-up Kit (Axygen, USA) according to manufacturer's instructions in order to be sequenced. DNA sequencing was carried out on five *E. faecium* and five *E. faecalis* PCR-positive samples using the Big Dye Terminator v3.1 cycle sequencing kit and Centri-Sep™ spin columns (Applied Biosystems, CA, USA) for cycle sequencing and products purification according to the manufacturer's protocols. Sample electrophoresis was then performed using automated sequencer ABI PRISM 310 Genetic Analyzer, (Applied Biosystems, CA, USA), followed by sequencing analysis using Sequencher software (Genes Codes Inc., MI, USA).

The DNA sequences for *gelE, agg, yl, esp* and *vanA* genes were retrieved from the NCBI database (http://www.ncbi.nlm.nih.gov/). The sequenced PCR products were saved in FASTA format for various applications. Multiple sequence alignments were done by the aid of NCBI Blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Genome wide analysis for pathogenic islands in enterococci was done using IslandViewer[18] (http://www.pathogenomics.sfu.ca), detection of prophages in sequenced bacterial genomes was done by ACLAME prophinder tool[19] (http://aclame.ulb.ac.be/Tools/Prophinder) and cumulative GC skew analysis was done by Genometrics analysis[20] (http://www2.unil.ch/comparativegenometrics/).

Results

Screening and isolation of enterococcus isolates

Urine samples were collected from 100 patients admitted to Abu-El Reesh and El-Demerdash hospitals. Out of 100 isolates collected, 73 isolates were identified as *Enterococcus* sp. Preliminary identification of the suspected isolates was conducted by Gram staining; the isolates showed a Gram-positive cocci or coccobacilli arranged in pairs and short chains. The identification of positive *Enterococcus* isolates was confirmed by other biochemical tests including catalase test; all the isolates were catalase negative. The ability of the isolates to grow in nutrient broth containing 6.5% NaCl was also confirmed.

Isolates appeared as yellow colonies on CLED agar. Cultivation of the isolates on Enterococcosel agar showed brownish-black colonies surrounded by a black zone, while cultivation on MacConkey no. 2 showed small, intensely red colonies. Identification to species level was done by cultivation on HiCrome *E. faecium* agar. Twenty-six isolates were identified as *E. faecium,* producing green colonies along with yellow colouration to the medium and 47 isolates were identified as *E. faecalis,* producing blue colonies on the media.

Phenotypic identification of virulence factors

Cytolysin production was screened by cultivation on blood agar, nine isolates produced complete (β) haemolysis, eight isolates produced partial (α) haemolysis and 56 isolates did not produce haemolysis on blood agar (γ haemolysis). Gelatinase assay by gelatin hydrolysis test revealed that none of the isolates phenotypically expressed gelatinase gene as they could not liquefy gelatin media.

Antimicrobial susceptibility pattern and MIC of enterococcus isolates to various antibiotics

The *Enterococcus* isolates were tested for resistance to different classes of antibiotics by using disc diffusion method. The antibiotic resistance profiles of the entire isolates collection against 11 antimicrobial agents are summarized in [Figure 1]. MIC values were determined for sensitive and resistant *Enterococcus* isolates to vancomycin. MIC ranged from 0.25 μg/ml to 256 μg/ml. However, most of the isolates had MIC from 1 μg/ml to 2 μg/ml. MIC results revealed that that two isolates were highly resistant to vancomycin.

Amplification of virulence and resistance genes of enterococcus isolates by PCR

Genes of virulence and resistance including gelatinase (*gelE*), aggregation substance (*agg*), cytolysin (*cyl*), Enterococcal surface protein (*esp*) and vancomycin resistance (*vanA*) of 50 *Enterococcus* isolates were amplified by PCR. The presence of the virulence and resistance genes detected in 50 *Enterococcus* isolates is revealed in Figure 2. Two isolates carried the four virulence genes. Five isolates carried three virulence genes, 24 isolates carried two of the four virulence genes, 13 isolates

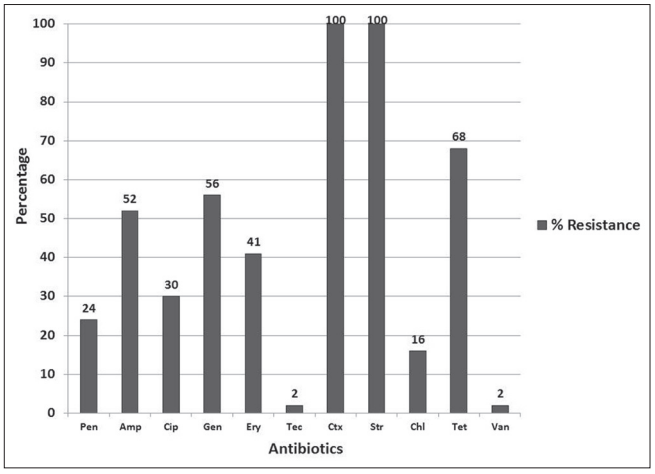

Figure 1: The antimicrobial resistance pattern of *Enterococcus* isolates expressed as percentage of the total number of collected isolates

Table 1: Primers sequences and predicted sizes of PCR products

Gene	Forward primer	Reverse primer	Size (bp)
gelE	5′-ACCCCGTATCATTGGTTT-3′	5′-ACGCATTGCTTTTCCATC-3′	419
agg	5′-AAGAAAAAGAAGTAGACCAAC-3′	5′-AAACGGCAAGACAAGTAAATA-3′	1553
cyl	5′-TGGCGGTATTTTTACTGGAG-3′	5′-TGAATCGCTCCATTTCTTC-3′	186
esp	5′-TTGCTAATGCTAGTCCACGACC-3′	5′-GCGTCAACACTTGCATTGCCGAA-3′	933
vanA	5′-GGGAAAACGACAATTGC-3′	5′-GTACAATGCGGCCGTTA-3′	732

PCR: Polymerase chain reaction

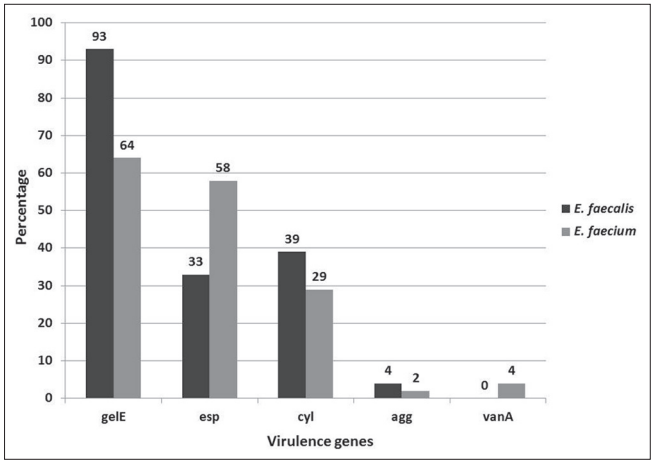

Figure 2: Presence of the virulence and resistance genes detected in *E. faecalis* and *E. faecium* separately as percentage of occurrence

carried one of the four virulence genes, two isolates carried the *VanA* gene and six isolates did not carry any of the virulence or resistance genes. From the 18 isolates that carried the *Cyl* gene, only nine isolates produced complete (β) haemolysis on blood agar. The gelatinase activity could not be monitored phenotypically in all isolates carrying the *gelE* gene. The occurrence of the *gelE*, *cyl* and *agg* genes was higher in *E. faecalis* isolates, while the *esp* gene was more prevalent in *E. faecium* isolates. The *VanA* gene was present only in *E. faecium* isolates.

Genometric characterization of horizontally transferred genes

Based on the concept that bacterial genomes tend to naturally have differences in sequence composition such as GC% and codon bias, regions were observed within a genome that has abnormal sequence composition as they could indicate that it had originated from another genome and was horizontally transferred.[18-20] The genomes of *Enterococcus* spp. can virtually carry virulence genes that were transferred by either transposon or phage through horizontal gene transfer. The genomes of the standard strains, *E. faecalis* V583 and *E. faecalis* 62, were used as model *Enterococcus* genomes for this purpose (as it was economically prohibitive to sequence the entire genomes of all our clinical isolates). The IslandViewer program was used to identify segments in the genomes that might have originated from horizontal gene transfer and revealed possible candidates as shown in Tables 2 and 3.

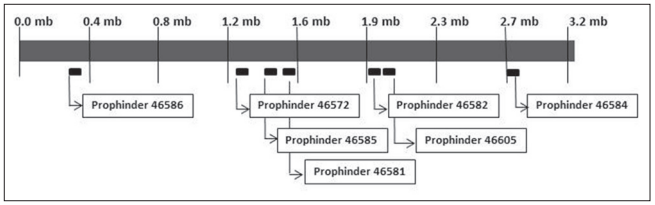

Figure 3: Prophages predicted in bacterial genome of *E. faecalis* V583. The boxes represent the location of this phage and phage ID is given to each identified phage

Other confirmatory tools used for detection of horizontally transferred genes

The ACLAME prophinder tool was able to identify seven phage or phage-like regions in *E. faecalis* V583 genome [Figure 3 and Table 4] which suggests that they originated from horizontal gene transfer. In silico analysis measuring GC skewness indicated four regions (grooves) in the genome (data not shown). These regions indicate different in GC content, suggesting that they originated from a horizontal gene transfer process.

Discussion

The increasing incidence of enterococcal infections in recent years suggests that the acquisition of certain virulence factors might play a role in increasing the pathogenesis of these organisms. The present study aimed to investigate the relation between the presence of virulence and resistance factors and the susceptibility of isolates to different antimicrobial agents as well as to explore the possibility of horizontal gene transfer for these genetic elements.

Genotypic detection of virulence genes was done by PCR. A total 36% of isolates were positive for the *cyl* gene giving a band appeared at 186 bp, although (β) haemolysis was observed in only 18% of the isolates. These findings compare favourably with previous studies where the presence of phenotypic characteristics, such as cytolysin, was lower than that expressed genotypically.[21] The occurrence of the *cyl* gene was higher in *E. faecalis* (39%) isolates than in *E. faecium* isolates (29%).

PCR detection of the *gelE* gene revealed that 84% of the isolates (highest percentage) were gelatinase-positive, yielding a band that appeared at 419 bp; however, none of

Table 2: Pathogenic islands of *Enterococcus faecalis* V583

GI range	Size	Product	Types
484860..485051	192	cylL-S protein	Transposon
146450..150367	3917	Aggregation substance	Transposon
2212353..2212961	608	D-alanyl-D-alanine dipeptidase	Unknown
2212967..2213995	1028	D-alanine--D-lactate ligase	Unknown
2214956..2215783	827	Vancomycin B-type resistance protein VanW	Unknown
2215801..2216607	806	D-alanyl-D-alanine carboxypeptidase	Unknown
2216783..2218126	1343	Sensor histidine kinase VanSB	Unknown
2218126..2218788	622	DNA-binding response regulator VanRB	Unknown

GI: Genomic island

Table 3: Pathogenic islands of *Enterococcus faecalis* 62

GI range	Size	Product	Types
895380..898400	3020	Enterococcal surface protein, *esp*	Transposon

GI: Genomic island

Table 4: Phage-like regions in *Enterococcus faecalis* V583 genome

Prophage	Coordinates range	CDS range
Prophinder: 46585	1398097-1413451	29375984-29376010
Prophinder: 46586	289354-326185	29374942-29374994
Prophinder: 46584	2703543-2734686	29377273-29377321
Prophinder: 46581	1416376-1441539	29376018-29376048
Prophinder: 46605	2005177-2048146	29376594-29376654
Prophinder: 46582	1925423-1962485	29376512-29376554
Prophinder: 46572	1245337-1259965	29375845-29375862

CDS: Coding sequences

the isolates expressed gelatinase phenotypically. Similar results were previously reported in which none of the *gelE* gene positive *Enterococcus* isolates were found to produce gelatinase phenotypically.[22] The occurrence of this gene was higher in *E. faecalis* isolates (93%) compared to *E. faecium* isolates (64%), as shown previously when *E. faecalis* isolates predominantly harboured the *gelE* gene (80%) while the gene was less predominant in *E. faecium* isolates (31.9%).[23]

The lack of phenotypic activity of the *cyl* and *gelE* genes may be explained by low levels or down regulation of gene expression or an inactive gene product. Environmental factors also are known to influence gene expression.[24] It was suggested by Creti *et al*., (2004)[25], that the presence of genes that are expressed only under *in vivo* conditions, to the presence of undetected gene mutations or to the fact that detection by PCR of a single gene inside an operon, as is the case of the *cylA* gene for cytolysin production, may overlook the absence of other genes that are necessary for phenotypic expression.

Genotypic detection of Agg and Esp revealed that the *agg* gene was present in 3% of the isolates yielding a band that appeared at 1553 bp while the *esp* gene was present in 42% of the isolates with band appeared at 933 bp. Although the role of the *esp* gene as virulence factor has been demonstrated,[26] all the isolates did not express the *esp* gene, in accordance with previous studies.[5]

The presence of the *gelE*, *cyl* and *agg* genes was higher in *E. faecalis* as previously reported,[23,27] where *E. faecalis* isolates were shown to harbour a broader spectrum of virulence determinants compared to *E. faecium* isolates. The *esp* gene was more prevalent in *E. faecium* than in *E. faecalis* isolates, as observed in previous studies.[28]

Antibiotic susceptibility tests revealed that 82% of the isolates had multiple antibiotics resistance with resistance to more than three of the antibiotics tested. Although *Enterococcus* strains harboured multiple antibiotic resistance, there was an elevated sensitivity rates to β-lactams and glycopeptides which deserves particular attention.[5] Two isolates were highly resistant to vancomycin with MIC equal to 256 μg/ml, while the rest of the isolates had MIC ranged from 1 to 2 μg/ml.

Genotypic detection for the *vanA* gene using PCR revealed that two isolates were positive for the gene, yielding a band at size 732 bp. The isolates that were positive for the *vanA* gene also showed a high level of resistance to vancomycin by MIC. This result indicates a low presence of vancomycin resistance in *Enterococcus* isolates. In a previous study conducted in Egypt,[29] a low presence of vancomycin resistance (4.2%) among enterococci was demonstrated. It was noticed that the isolates carrying the *vanA* gene were resistant to all antibiotics used; this result compares favourably with a study conducted in Italy,[17] in which vancomycin-resistant isolates were also multidrug-resistant. This is of concern, as vancomycin resistance may be transferred to more pathogenic microorganisms. In our study, the presence of the *vanA* gene was accompanied by the presence of the *cyl, esp* and *gelE* genes and absence of the *agg* gene; however, this is not always true as the *vanA* gene was previously linked with the presence of the *agg* gene.[30] The *VanA* gene was present only in *E. faecium* isolates but not in *E. faecalis* isolates, as observed in other studies.[10,31]

The presence or absence of virulence genes did not affect the pattern of anti-microbial resistance in *Enterococcus* isolates. Hence, no relation was found

between the presence of virulence genes and multiple antibiotics resistance used as previously mentioned.[32] Sequencing of virulence genes, including *cyl, agg, gelE and esp*, and resistance gene (*vanA*) of *Enterococcus* clinical isolates indicated no mutations in the sequenced fragments.

The data retrieved from sequence analysis revealed that the *vanA* gene that was present in *Enterococcus* spp. was also present in *Staphylococcus aureus* (*S. aureus*), as both possessed the same sequence. This result suggests that vancomycin resistance was transferred from enterococci to staphylococci since it was previously shown that enterococci transferred the *vanA* gene to vancomycin-resistant *S. aureus* (VRSA).[32]

Enterococci are noted for their capacity to exchange genetic information by conjugation,[13] and these processes are known to take place in the gastrointestinal tract.[33] Together with transmissible antibiotic resistance plasmids, virulence factors, such as cytolysin production, and the capacity for adhesion is known to be transmissible by highly efficient gene transfer mechanisms.[34]

We suggest that the shift of enterococci from a normal inhabitant of human gastrointestinal tract, that is considered medically unimportant to a pathogen causing serious diseases such as endocarditis, bacteraemia, UTIs and central nervous system infection, along with the increase in strains resistant to multiple antibiotics, especially glycopeptides, such as vancomycin, is a result of the fact that certain genes of virulence and resistance were transferred to enterococci. Consequently, we used bioinformatics tools and analyzed the genomes of standard model *Enterococcus* trains to investigate the possibility of gene transfer.

Bacterial genomes contain clusters of genes that are acquired by horizontal transfer called genomic islands (GIs) that are capable of integration into the chromosome of the host, excision and transfer to a new host by transformation, conjugation or transduction. A GI can code for many functions, can be involved in symbiosis or pathogenesis and may help an organism's adaptation. GI associated with pathogenesis is often called a pathogenicity island (PAI). These 'islands' are characterised by their large size (>10 Kb) and a different G + C content compared with the rest of the genome. Some GIs can excise themselves spontaneously from the chromosome and can be transferred to other suitable recipients.[15] Detection of GIs was aided by the use of Island Viewer software which showed that the virulence genes in our current study were mainly transferred by transposons.

The presence of phage or phage-like regions was aided by the use of ALCAME prophinder tool that detected seven phage or phage-like regions in *E. faecalis* V583 genome. By comparing the co-ordination ranges of phage or phage-like regions with those of virulence and resistance genes, we found that these genes were not transferred by prophages. The drawback of this tool is its inability to detect other mobile genetic elements.

GC skewness measurement of *E. faecalis* V583 genome showed four regions (grooves), indicating different GC content in these four regions thus suggesting horizontally transferred genes. Although this method is a fast tool for detection of horizontally transferred genes, the results it presents are not completely reliable since the skew pattern deviation is detected manually through observation of the grooves along the genome curve, and person-to-person variations may arise.

Conclusion

Virulence genes including Cyl, GelE, Agg and Esp were present in the majority of *Enterococcus* spp. isolates but only two isolates expressed the four virulence genes. Presence of genes was not usually associated with expression, however, other environmental factors may interfere. Antibiotic resistance was relatively high in clinical isolates with elevated sensitivity to vancomycin. No relation was found between the presence of virulence factors and resistance to different antibiotics used. Bioinformatics analysis of possible gene transfer revealed that transposons are the main elements responsible for horizontal gene transfer among enterococci.

References

1. Linden PK, Miller CB. Vancomycin-resistant enterococci: The clinical effect of a common nosocomial pathogen. Diagn Microbiol Infect Dis 1999;33:113-20.
2. Foulquie Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L. The role and application of enterococci in food and health. Int J Food Microbiol 2006;106:1-24.
3. Lopes Mde F, Simões AP, Tenreiro R, Marques JJ, Crespo MT. Activity and expression of a virulence factor, gelatinase, in dairy enterococci. Int J Food Microbiol 2006;112:208-14.
4. Franz CM, Holzapfel WH, Stiles ME. Enterococci at the crossroads of food safety? Int J Food Microbiol 1999;47:1-24.
5. Cosentino S, Podda GS, Corda A, Fadda ME, Deplano M, Pisano MB. Molecular detection of virulence factors and antibiotic resistance pattern in clinical *Enterococcus faecalis* strains in Sardinia. J Prev Med Hyg 2010;51:31-6.
6. Gulhan T, Aksakal A, Ekin IS, Savasan S, Boynukara B. Virulence factors of *Enterococcus faecium* and *Enterococcus faecalis* strains isolated from human and pets. Turk J Vet Anim Sci 2006;30:477-82.
7. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. Clin Microbiol Rev 1994;7:462-78.
8. Mundy LM, Sahm DF, Gilmore M. Relationships between enterococcal virulence and antimicrobial resistance. Clin Microbiol Rev 2000;13:513-22.
9. Kirschner C, Maquelin K, Pina P, NagoThil NA, Choo-Smith LP, Sockalingum CD, *et al*. Classification and identification of enterococci: A comparative phenotypic, genotypic, and vibrational spectroscopic study. J Med Microbiol 2001;39:1763-70.

10. Murrary BE. Diversity among the multidrug-resistant enterococci. Emerg Infect Dis 1998;4:37-47.
11. Barber AE, Norton JP, Spivak AM, Mulvey MA. Urinary tract infections: Current and emerging management strategies. Clin Infect Dis 2013;57:719-24.
12. Ronald A. The etiology of urinary tract infection: Traditional and emerging pathogens. Am J Med 2002;113 suppl 1A:14-9S.
13. Chow JW, Thal LA, Perri MB, Vazquez JA, Donabedian SM, Clewell DB, *et al*. Plasmid-associated hemolysin and aggregation substance production contribute to virulence in experimental enterococcal endocarditis. Antimicrob Agents Chemother 1993;37:2474-7.
14. Andrea M, Dib-Hajj F, Lamb L, Kaczmarek F, Shang W, Beckius G, *et al*. Enterococcal virulence determinants may be involved in resistance to clinical therapy. Diagn Microbiol Infect Dis 2007;58:59-65.
15. Gilmore MS, Segarra RA, Booth MC, Bogie CP, Hall LR, Clewell DB. Genetic structure of *Enterococcus faecalis* plasmid pAD1-encoded cytolytic toxin system and its relationship to lantibiotic determinants. J Bacteriol 1994;176:7335-44.
16. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol 1995;33:24-7.
17. Eaton TJ, Gasson MJ. Molecular screening of Enterococcus virulence determinants and potential for genetic exchange between food and medical isolates. Appl Environ Microbiol 2001;67:1628-35.
18. Langille MG, Brinkman FS. Island Viewer: An integrated interface for computational identification and visualization of genomic islands. Bioinformatics 2009;25:664-5.
19. Leplae R, Lima-Mendez G, Toussaint A. ACLAME: A CLAssification of Mobile genetic Elements, update 2010. Nucleic Acids Res 2010;38:D57-61.
20. Roten CA, Gamba P, Barblan JL, Karamata D. Comparative Genometrics (CG): A database dedicated to biometric comparisons of whole genomes. Nucleic Acids Res 2002;30:142-4.
21. Dahle'n G, Blomqvist S, Almstahl A, Carle'n A. Virulence factors and antibiotic susceptibility in enterococci isolated from oral mucosal and deep infections. J Oral Microbiol 2012;4.
22. Biavasco F, Foglia G, Paoletti C, Zandri G, Magi G, Guaglianone E, *et al*. Van A-type enterococci from humans, animals, and food: Species distribution, population structure, Tn1546 typing and location, and virulence determinants. Appl Environ Microbiol 2007;73:3307-19.
23. Rathnayake IU, Hargreaves M, Huygens F. Antibiotic resistance and virulence traits in clinical and environmental *Enterococcus faecalis* and *Enterococcus faecium* isolates. Syst Appl Microbiol 2012;35:326-33.
24. Finlay BB, Falkow S. Common themes in bacterial pathogenesis revisited. Microbiol Mol Biol Rev 1997;61:136-69.
25. Creti R, Imperi M, Berrtuccini L, Fabretti F, Orefici G, Di Rossa R, *et al*. Survey for virulence determinants among *Enterococcus faecalis* isolates from different sources. J Med Microbiol 2004;53:13-20.
26. Shankar N, Lockatell CV, Baghdayan AS, Drachenberg C, Gilmore MS, Johnson DE. Role of *Enterococcus faecalis* surface protein esp in the pathogenesis of ascending urinary tract infection. Infect Immun 2001;69:4366-72.
27. Dardir HA, Aba-Alkhail NA, Abdel-All AA. Safety evaluation of enterococcal strains isolated from dairy products and clinical samples using RT-PCR. World J Dairy Food Sci 2011;6:234-40.
28. Helmi H, AboulFadl L, Saad El-Din S, El-Defrawy I. Molecular characterization of antibiotic resistant enterococci. Res J Med Sci 2008;3:67-75.
29. Dupre I, Zanetti S, Schito AM, Fadda G, Sechi LA. Incidence of virulence determinants in clinical *Enterococcus faecium* and *Enterococcus faecalis* isolates collected in Sardinia (Italy). J Med Microbiol 2003;52:491-8.
30. Heaton MP, Discotto LF, Pucci MJ, Handwerger S. Mobilization of vancomycin resistance by transposon-mediated fusion of a VanA plasmid with an *Enterococcus faecium* sex pheromone-response plasmid. Gene 1996;171:9-17.
31. Sharifi Y, Hasani A, Ghotaslou R, Varshochi M, Hasani A, Aghazadeh M, *et al*. Survey of Virulence Determinants among Vancomycin Resistant *Enterococcus faecalis* and *Enterococcus faecium* Isolated from Clinical Specimens of Hospitalized Patients of North west of Iran. Open Microbiol J 2012;6:34-9.
32. Jankoska G, Trajkovska-Dokic E, Panovski N, Papovska-Jovanovska K, Petrovska M. Virulence factors and antibiotic resistance in *Enterococcus faecalis* isolated from urine samples. Sec Biol Med Sci 2008;1:57-66.
33. Huycke MM, Gilmore MS, Jett BD, Booth JL. Transfer of pheromone-inducible plasmids between *Enterococcus faecalis* in the Syrian hamster gastro-intestinal tract. Infect Dis 1992;166:1188-91.
34. Wirth R. The sex pheromone system of *Enterococcus faecalis*. More than just a plasmid-collection mechanism. Eur J Biochem 1994;222:235-46.

How to cite this article: Hashem YA, Yassin AS, Amin MA. Molecular characterization of *Enterococcus* spp. clinical isolates from Cairo, Egypt. Indian J Med Microbiol 2015;33:S80-6.

Source of Support: Nil, **Conflict of Interest:** None declared.