Journal of Medical Bacteriology

Diversity and Genetic Clonality of Vancomycin and Gentamicin Resistant Enterococci Isolated from Healthy Humans in Iran

Nasrin Noohi [1*], *Maliheh Talebi* [2]

[1] *Research Center for Conservation of Cultural Relics, Research Center of Iranian Cultural Heritage and Tourism Organization, Tehran, Iran.*

[2] *Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 11 Nov 2018
Revised: 18 Nov 2018
Accepted: 26 Nov 2018
Published: 20 Feb 2019

Keywords:
Antibiotic resistance, Enterococcus, PFGE, Normal flora, Vancomycin resistance genes.

ABSTRACT

Background: This study was done to determine the level of high level gentamicin resistant (HLGR) and vancomycin resistant enterococci (VRE) in the healthy normal human population living in Tehran, Iran.

Methods: A total of 700 fecal samples were isolated from the period of September 2007 to September 2008 and the species were identified by the biochemical tests and PCR. Antibiotic susceptibility test, gentamicin and vancomycin resistant genes and the conjugation tests were performed to evaluate the transferability of the drug resistant genes. The clonality of the isolates was also determined by PFGE.

Results: The results showed that the prevalence of HLGR and VRE in the healthy normal population were 16.6% (100) and 1% (6) for HLGR and VRE, respectively. Amongst the HLGR enterococci, the most frequent species were found to be *Enterococcus faecalis* in 63% of the isolates. This was followed by *E. faecium* (33%), *E. gallinarum* (3%) and *E. casseliflavus* (1%). The frequency of the gentamicin resistant genes were 99 (99%), 91 (91%), 3(3%) and 1(1%) for aac(6')-Ie-aph(2")-Ia, aph(3')-IIIa, ant(4')Ia and aph(2")-Id genes, respectively. Among the vancomycin resistant genes, vanA was found in 1% (6) of isolates only. PFGE revealed high heterogeneity among the 63 *E. faecalis* strains and 33 *E. faecium* with 31 and 28 different patterns, respectively. The 6 VR *E. faecium* revealed 6 different patterns.

***Conclusion*:** The prevalence and heterogenous populations of HLGR in the normal flora of the humans found here were higher than reports from many countries, may be suggestive of excessive antibiotic usage in Iranian.

- ***Please cite this paper as:*** Noohi N, Talebi M. Diversity and Genetic Clonality of Vancomycin and Gentamicin Resistant Enterococci Isolated from Healthy Humans in Iran. *J Med Bacteriol.* 2019; **8** (1, 2): pp.27-36.

*Corresponding Author: Nasrin Noohi, Research Center for Conservation of Cultural Relics, Research Center of Iranian Cultural Heritage and Tourism Organization, Tehran, Iran.
Tel: +98- 021-66736452, *E-mail:* Nasrinnoohi@gmail.com

Introduction

Enterococci are natural inhabitants of the gastrointestinal tract of humans and animals and they are common in environments affected by animal and human fecal material (1). They have become as one of the leading cause of surgical wound infection, nosocomial bacteremia and urinary tract infections (2). The main reason for the enterococcus to be capable to survive in the hospital is their intrinsic or acquired resistance to antibiotics (3). The emergence of high level of gentamicin resistant enterococci (HLGR) and vancomycin resistant enterococci (VRE) have had serious indications in the treatment of the patients with various enterococcal infections (4). The wide presence of enterococcus in animal feces, foods of animal origin and hospital sewage treatment plants have made these bacteria to be major medical bacteria which are under intensive examination (5).

Seven vancomycin and more than ten gentamicin resistant genes have so far been identified. *VanA* has been shown to cause resistant to vancomycin and teicoplanin (6, 7, 8). High-level gentamicin resistance (HLGR) (MIC ≥ 500 mg/ml) in enterococci is predominantly mediated by *aac(6')-Ie-aph(2'')-Ia*, which encodes the bifunctional aminoglycoside-modifying enzyme AAC(6′)-Ie-APH(2″)-Ia (8).

It has been shown that the resistant enterococcal strains are able to persist for a long time in healthy human (9). There are enormous reports of the frequency of the resistant genes and diversity of population of the enterococcal species in the environmental and hospital settings. However, to the best of our knowledge, this is the first report determining the level of the enterococcal diversity at the species and genetic level in the normal human populations in this region of the world. The findings reported here may allow us to identify the community source of the naturally acquiring HLGR and VRE resistant.

Materials and Methods

Sample collection

Fecal samples were taken from the 700 healthy, non-hospitalized persons in Tehran during the period from September 2007 to September 2008. A questionnaire was given to the healthy individuals and the exclusion criteria include no hospitalization (or hospital visit) or antibiotic exposure for the last 6 and 2 months, respectively.

The age of the selected individuals was from 15 months to 83 years with 54.5% male and 45.5% female.

Isolation and species identification

Stool specimens were cultured in BHI broth supplemented with 6 and 32 g/ml of vancomycin and gentamicin for isolation of VRE and HLGR enterococcal species, respectively, for 24 h of incubation at 45 ºC. They were then subcultured on m-Enterococcus agar (Becton Dickinson and co., Sparks, MD, USA) with the same amount of antibiotics at 37 ºC for 48h. Three enterococcal colonies were taken from each plate and sub-cultivated on blood agar. All isolated strains were identified by the conventional biochemical tests, including colony morphology, Gram staining, growth and blacken of bile-aesculin agar, growth in 6.5% NaCl, tolerance to 0.04% tellurite, catalase test, pyrolidonyl arylamidase test, deamination of arginine, motility, pigmentation and several carbohydrate fermentation tests (10). Identification of *Enterococcus* species was confirmed by PCR as described previously (11).

Susceptibility testing

The isolates were tested for resistance to tetracycline (30 µg), erythromycin (15 µg), chloramphenicol (30 µg), ampicillin (10 µg), ciprofloxacin (5 µg), gentamicin (120), chloramphenicol (30 µg), linezolid (30 µg), vancomycin (30 µg), synercid (15 µg) and teicoplanin (30 µg), from (BBL, Sensi Disk, USA), by the disk diffusion method as described

by CLSI (12). MIC of vancomycin was determined by E-test (AB Biodisk, Solna, Sweden) method on Mueller–Hinton agar according to the manufacturer's instructions. MIC results were interpreted according to Clinical and Laboratory Standards Institute guidelines. MIC of gentamicin (Sigma-Aldrich, USA) was determined by microbroth dilution in Mueller-Hinton-Broth (Becton Dickinson). Vancomycin was tested in the range 0.25-256 µg/ml and gentamicin was tested in range 16-2048 µg/ml.

DNA extraction

Enterococci were grown in BHI broth medium for 16-18 h. Cultures were then centrifuged at 10000 rpm for 10 min. The pellet was re-suspended in Tris EDTA sucrose (TES) buffer (Tris HCL, 10mM; EDTA, 1mM; sucrose 50%, pH 7.5) containing lysozyme (20mg/ml) and then incubated at 37 ºC for 20 min. After adding 12 µl of 10% SDS, this mixture was placed in ice for 10 min and harvested at 7500 rpm for 15 min. The supernatant was removed in another tube and 300 µl of phenol/chloroform was added and after gently mixes, centrifuged at 13000 rpm for 10 min. Precipitation of DNA followed by adding equal volume of cold ethanol and finally DNA was dissolved in 50 µl of TE buffer containing RNase (13).

PCR

Confirmation of species identification and determination of glycopeptides and aminoglycoside resistant genes were performed by PCR. The *vanA* and *vanB* genes were amplified with primers described by Kariyama et al. (14) PCR assay was performed in a total volume of 25 µl contain 10mM Tris-HCL (pH 8.3), 1.5 mM MgCl2, 0.2 mM each dNTP, 0.5U of Taq DNA polymerase (HT Biotechnology, Cambridge, United Kingdom) and each primer (40 pmol). PCR cycle was done follows; an initial denaturation at 94 ºC for 3 min, with 30 cycles of denaturation at 94 ºC for 1 min, annealing at 54ºC for 1 min and extension at 72 ºC for 1 min and a final extension at 72ºC for 7 min. *E. faecalis* V583 and *E. faecium* BM4147 were used as the positive controls for vanA and vanB, respectively. The *aac (6')-Ie-aph (2'')-Ia, aph(2'')-Ib, aph(2'')-Ic, aph(2'')-Id, aph(3')-IIIa and ant(4')-Ia* genes were amplified with primers described by Vakulenko et al (15). PCR experiments were performed in a volume of 25 µl with the following content; 2 µl of DNA template, 1.5mM MgCl2, 0.2mM each dNTP, 2.5 µl of 10X PCR buffer, 2.5U Taq DNA polymerase (HT Biotechnology, Cambridge, United Kingdom) and 2 pmol of each primer. PCR was performed with an initial lysing step of 3 min at 94 ºC, 35 cycles of 40s at 94 ºC, 40s at 55 ºC, and 40s at 72 ºC and final extension step of 2 min at 72 ºC.

Filter mating

Filter mating was performed with *E. faecalis* and *E. faecium* isolates by using the plasmid-free *E. faecalis* JH2-2 recipient strain. Filters were incubated for 24 h and transconjugants were selected on BHI agar containing 120 µg/ml of gentamicin or 20 µg/ml vancomycin, 20 µg/ml of rifampin (Sigma-Aldrich, USA) and 10 µg/ml of fusidic acid (Sigma-Aldrich, USA). Transfer frequency was expressed as the number of transconjugants divided by the number of recipients. *E. faecalis*14 and *E. faecalis* 4110 were used as reference donor strains for gentamicin and vancomycin resistance gene, respectively.

Pulsed-Field Gel Electrophoresis

The enterococci isolates were grown in BHI broth overnight with shaking at 37ºC. The cultures were centrifuged and cells were re-suspended in 5 ml of TE buffer (10 mM Tris, 0.1 mM EDTA [pH 8.0]), mixed with equal volume of 2% low melting agarose (Bio-Rad, Hercules, CA) in distilled water and poured into a plug mold. The plugs were treated with lysis buffer containing lysozyme (1 mg/ml) and RNase

(5mh/ml) to lysis buffer (6 mM Tris [pH 7.5], 100mM EDTA [pH7.5], 1M NaCl, 0.5% Brij-58, 0.2% sodium deoxycholate, and 1% sodium lauryl sarcosine) overnight at 37 ºC. This solution was replaced by ES solution (0.5 M EDTA [pH 9.5], 1% sarcosine) and then ESP solution (0.5 M EDTA [pH 9.5], 1% sarcosin, and 0.5 mg of proteinase K/ml) and were incubated 48h at 50ºC. The plugs were washed with TE buffer and stored at 4 ºC. After digestion with 20 U smaI (Roche, Manhiem, Germany), the plugs were placed in the wells of 1% agarose in 0.5% × TBE and electrophoreses with switch times ramped from5 s to 35 s at 6 V with a run time of 27h at 14 ºC in the Bio-Rad CHEF-DRIII system. DNA from Salmonella choleraeraesuis serotype Branderup H9812 (Pulsenet, www.cdc.gov/pulsenet) was used as the molecular size marker. The gels were stained with ethidium bromide and the restricted DNA was visualized with ultraviolet light (16). The banding patterns were analyzed by the UPGMA method using the software Gelcompare II version 4.0 (Applied Maths, Sint-Matens-latem, Belgium) and interpreted using the guidelines or differing by one to three bands were regarded as identical and were assigned the same type. The isolates that differ by more than three bands were not considered to be related and were regarded as different types (17).

Result

Out of 700 healthy and non-hospitalized persons living in the city of Tehran, 100 (14.3%) and 6 (0.85%) carried HLGR and VRE in their gastrointestinal tracts, respectively. Out of 100 HLGR enterococci isolated in this study, 63 (63%) were identified as *E. faecalis*, 33 (33%) *E. faecium*, 3 (3%) *E. gallinarum* and 1 (1%) *E. casseliflavus*. All of the VRE isolates were identified as *E. faecium*. The highest percent of HLGR enterococci 30.2% (26 isolates) were isolated from age group under 15 years (53.8% *E. faecalis*, 46.2% *E. faecium*). The percent of high level gentamicin resistance in enterococci isolated from age groups 15-30 and 30-45, 45-60 and above 60 years were 16.2% (71.5% *E. faecalis*, 28.5% *E. faecium*), 19.7% (82.3% *E. faecalis*, 17.6% *E. faecium*), 22.1% (52.6% *E. faecalis*, 31.6% *E. faecium*, 10.5% *E. gallinarum*, 5.2 *E. casseliflavus*), 11.1% (60% *E. faecalis*, 40% *E. faecium*), respectively. All 6 VRE strains isolated from age group 30-60 (2 strains isolated from age group 30-45 and 4 strains isolated from age group 45-60).

High level of resistance to tetracycline (94%) and erythromycin (93%) were also found among the HLGR isolates. The level of resistance to linezolid, vancomycin, teicoplanin, ciprofloxacin, chloramphenicol and ampicillin were 0, 6, 5, 38, 22 and 23%, respectively.

Contrary to *E. faecium* isolates (with resistance rate 69.7%), all *E. faecalis*, *E. gallinarum* and *E. casseliflavus* isolates were susceptible to ampicillin. All 6 VRE isolates were resistant to gentamicin, tetracycline, erythromycin and ampicillin. All of VR and HLGR *E. faecium* isolates were susceptible to quinopristin-dalfopristin antibiotic. Resistance patterns of *E. faecium* and *E. faecalis* isolates against the tested antibiotics are shown in table 1 and table 2, respectively.

Table 1. Detection of aminoglycoside resistance genes by PCR; lane M, marker; lane 1-5, product of *aac (6')-Ie-aph (2'')-Ia* positive strains (348bp) and lane 6-9, product of aph(3')-IIIa positive strains (523 bp).

Antibiotic	Number (%)
CIP,TE,E,AP	11 (33.3)
CIP,AP,E	2 (6.06)
TE,E,AP	8 (24.4)
CIP,TE,E,AP	3 (9)
CIP,TE,AP	1 (3)
TE,E,AP	5 (15.2)
TE,AP	1 (3)
TE	1 (3)
C	1 (3)

Table 2. Patterns of Antibiotic resistance of HLGR *E. faecalis* isolated from healthy humans.

Antibiotic	Number (%)
CIP,TE,E,C	6(9.5)
TE,E,C	12(19.6)
CIP,TE,E	9(14.28)
TE,E,C	31(49.2)
CIP,E	2(3.17)
TE	2(3.17)
C	1(1.58)

Out of 100 HLGR isolates, 4 (4%) were resistant to one, 3 (3%) were resistant to 2 and 92 (92%) were resistant to three or more antibiotics. In all 15 different common antibiotic resistance patterns were observed.

Evaluation of gentamicin resistant genes among the HLGR isolates showed 99, 91, 3% and 1% contained aac (6')-Ie-aph (2")-Ia, aph(3')-IIIa , ant(4')-Ia and aph(2")-Id genes (Fig 1,Table 3). The aph(2")-Ib and aph(2")-Ic genes were not detected in any of the isolates tested. All of the 6 VRE contained the *vanA* with no *vanB* gene. The results of conjugation test showed that average of transfer frequencies for gentamicin and vancomycin resistance genes were 10-6-10-9 and10-5-10-7, respectively.

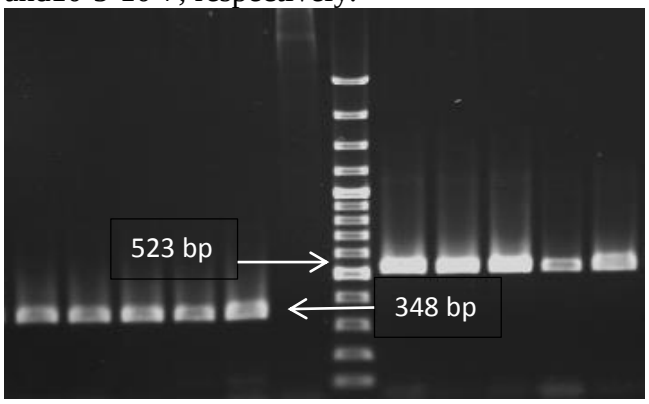

Figure 1. Detection of aminoglycoside resistance genes by PCR; lane M, marker; lane 1-5, product of aac (6')-Ie-aph (2")-Ia positive strains (348bp) and lane 6-9, product of aph(3')-IIIa positive strains (523 bp)**.**

The clonality of HLGR and VRE enterococci isolates were analyzed by PFGE. Thirty one PFGE patterns were found in HLGR *E. faecalis* isolates (Fig 2). Two predominant clonal groups, A1 and B1, were found in the HLGR *E. faecalis*, comprising of 15 isolates with 9 and 6 isolates. The remaining isolates were distributed in 29 different PFGE patterns, each one composed by one, two, three or four isolates. Three HLGR strains with ant(4')-Ia gene showed 3 different PFGE patterns.

The VRE isolates presented a high degree of genetic diversity and each of them had unique PFGE pattern (Fig 4). PFGE analysis of 33 strains of HLGR *E. faecium* reveals 28 different patterns (Fig 3).

Discussion

The rate of fecal carriage of VRE in our study was 1%, which could be considered relatively low (23, 9). In Europe, the rate of fecal carriage of VRE in the community has been reported from 2 to 28% (24).

Although no such comparison has been made in Iran because of the lack of any investigation in determining the level of VRE in animals, it is expected that the level of VRE in animals to be as low as seen in healthy humans. On the other hand, the heavy and indiscriminate use of other antibiotics such as gentamicin in humans has resulted up to 30% resistance in enterococcal populations.

Previous studies have reported the prevalence of VRE in sewage and hospital settings in Iran to be around 3 and 5.4%, respectively (11). Hasnnejad in 2015 reported that all Enterococcus strains isolated from healthy humans were sensitive to vancomycin (25). These venues could, therefore, be the sources for the transfer of VRE to the healthy individuals.

In some studies the level of HLGR enterococci isolates from healthy humans has been reported to be around 27 (Hong Kong) and 11% (Portugal), respectively (9, 26). The highest HLGR amongst different age groups in our study

Table 3. The distribution of aminoglycoside resistance genes in different species of HLGR enterococci**.**

Species / Genes	*E. faecalis*	*E. faecium*	*E. gallinarum*	*E. casseliflavus*	total
aac(6')-Ie-aph(2'')-Ia	62(98.4)	34(100)	3(100)	1(100)	99(99)
aph(3')-IIIa	60(95.2)	29(85.29)	2(66.6)	1(100)	91(91)
aph(2'')-Id	1(1.58)	0	0	0	1(1)
ant(4')-Ia	2(3.17)	1(2.94)	0	0	3(3)
aph(2'')-Ic	0	0	0	0	0
aph(2'')-Ib	0	0	0	0	0

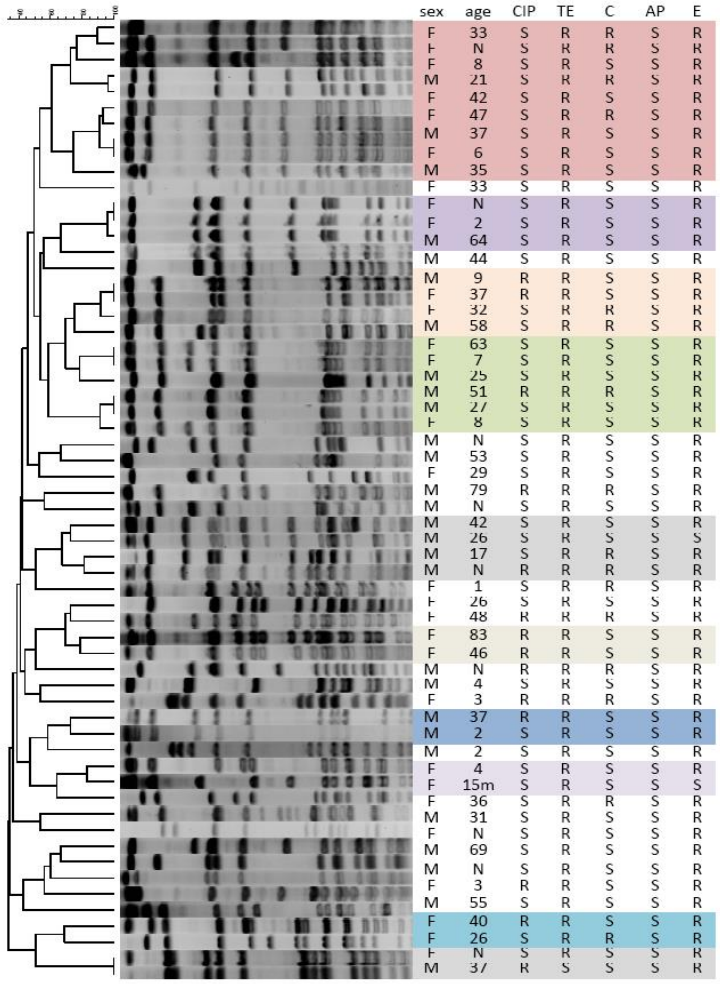

Figure 2. Dendrogram based on pulsed-field gel electrophoresis (PFGE) *SmaI* restriction pattern analysis of HLGR *E. faecalis* isolates obtained from healthy humans**.**

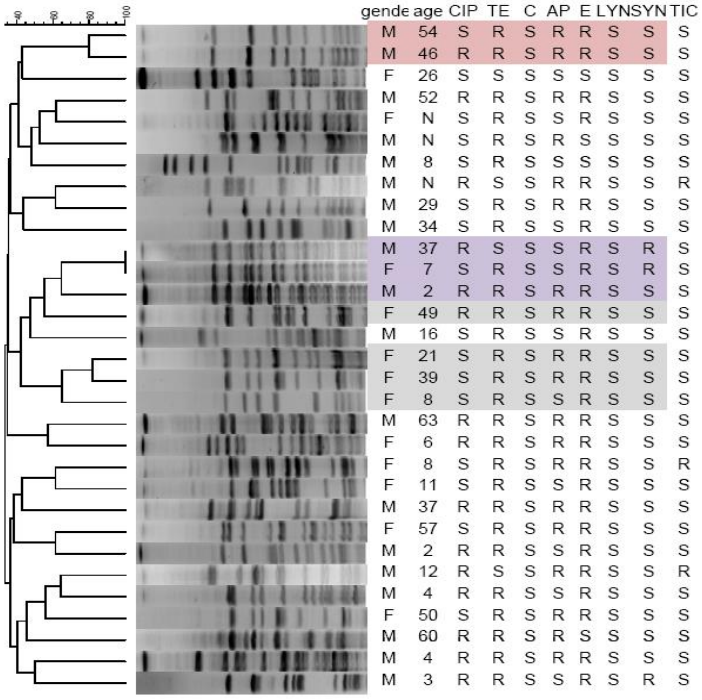

Figure 3. Dendrogram based on pulsed-field gel electrophoresis (PFGE) *SmaI* restriction pattern analysis of HLGR *E. faecium* isolates obtained from healthy humans.

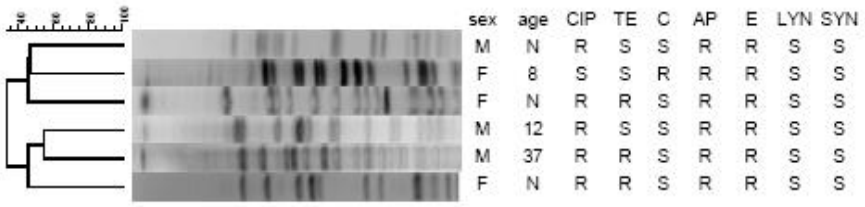

Figure 4. Dendrogram based on pulsed-field gel electrophoresis (PFGE) *SmaI* restriction pattern analysis of VRE isolates obtained from healthy humans**.**

was 30% HLGR. This is similar to the report in Hong Kong which may indicate that the HLGR in the Asian countries far exceed European countries. Previously, we showed that HLGR enterococci isolated from hospitalized patients in Iran to be about 59%, which twice as much as found in the normal populations (21). This could be due to common usage of aminoglycosides in Iran.

It was a common belief that *E. faecalis* to be the predominant enterococcal species accounting up to 90% of all clinical isolates and the rest to be *E. faecium* (1, 3). Here, our resulted indicated that 63 and 33% of the HLGR isolates were *E. faecalis* and E. faecium, respectively.

Previously, we found similar prevalence of HLGR enterococcal species isolated from hospitalized patients with UTI (64%) and municipal and hospital sewage (29%) (19, 27). Collectively, this information describe the fact that the HLGR enterococcal species to be uniformly distributed in any settings in Iran. The

aac (6')-Ie-aph (2")-Ia gene was the most prevalent gentamcin coding gene (99%) in this study. Choukhachian reported the prevalence of aac (6')-Ie-aph (2")-Ia gene in HLGR enterococci was 67.74% (28). Except for aac (6')-Ie-aph (2")-Ia, aph(3')-IIIa gene was frequently observed in our study and the combination of aac (6')-Ie-aph (2")-Ia + aph(3')-IIIa gene was observed in 90% of isolates. One isolate was shown to be resistant to gentamicin without detection of aac (6')-Ie-aph (2")-Ia, aph(2")-Ib, aph(2")-Ic and aph(2")-Id genes which may suggest the presence of other gentamcicin resistant gene that was not detected by our PCR.

Because synergy between the aminoglycosides and cell-wall–inhibiting antimicrobials has been previously described and is important clinically, the combinations of genes conferring resistance to aminoglycoside antimicrobials in enterococci in healthy humans are great concern. All VRE isolates had an acquired type of resistance (*vanA*). Moreover, VRE with *vanA* genotype that are susceptible to teicoplanin was also observed in this study.

Conjugation studies performed here showed that gentamicin and vancomycin resistance were transferable with high frequency to the recipient strain. In some cases even co-transfer of aph (3')-IIIa and aac (6')-Ie-aph (2")-Ia genes were detectable. The results may suggest high transferability of gentamicin resistance genes from our isolates. However, although aac (6')-Ie-aph (2")-Ia and vanA genes are frequently located in the gene clusters associated with specific plasmids, transposons or other widely distributed elements (9, 29), we could observe the transfer of *vanA* gene into our recipient.

Although an extensive genetic diversity among VRE isolates was observed by PFGE, two predominant clonal groups of HLGR *E. faecalis* were detected. Moreover, it was found that some of the isolates within the same clonal group were isolated at different time periods, suggesting genetically stable strains. On the other hand, different antimicrobial susceptibility patterns were also observed which could be due to transfer of the antibiotic resistance genes to the susceptible group of the same clonal type. In addition, the HLGR *E. faecium* isolates showed a higher degree of genetic diversity than HLGR *E. faecalis*, indicating capability of the *E. faecium* to disseminate and exposing more to the various environmental settings resulting in higher diversity.

Conclusion

In summary, the rate of vancomycin resistance was low in healthy population in Iran. On the contrary, the gentamycin resistance isolates were more prevalent in normal gut flora, which carry resistant genes to other antibiotics as well; maybe the source of community acquired enterococci antibiotic resistant infections.

Acknowledgment

We acknowledge Southern regional state Health bureau of Ethiopia for allowing access MDR-TB patient data. We are also grateful of MDR-TB patients who participated in this study.

Conflict of interest

No potential conflicts of interest were disclosed.

References

1. Huycke MM, Sahm DF, Gilmore MS. Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. *Emerg Infect Dis* 1998; **4**(2):239-49.
2. Hancock LE, Gilmore MS. Pathogenicity of enterococci. Gram-Positive Pathogens book 2000; **25**:251-8.
3. Marothi YA, Agnihotri H, Dubey D. Enterococcal resistance. *Indian J Med Microbiol* 2005; **23**(4):214-9.
4. Tansuphasiri U, Khaminthakul D, Pandii W. Antibiotic resistance of enterococci isolated from frozen foods and environmental water. *Southeast Asian J Trop Med Public Health*

2006; **37**(1):162-70.
5. McDonald LC, Kuehnert MJ, Tenover FC, *et al*. Vancomycin-resistant enterococci outside the health-care setting: prevalence, sources, and public health implications. *Emerg Infect Dis* 1997; **3**(3):311-7.
6. Pootoolal J, Neu J, Wright GD. Glycopeptide antibiotic resistance. *An Rev Tox* 2002; **42**:381-408.
7. Courvalin P. Genetics of glycopeptide resistance in gram positive pathogens. *Int J Med Microbiol* 2005; **294**(8):479-86.
8. Vakulenko SB, Mobashery S. Versatility of aminoglycosides and prospects for their future. *Clin Microbiol Rev* 2003; **16**(3):430-50.
9. Novais C, Coque TM, Sousa JC, et al. Antimicrobial resistance among fecal enterococci from healthy individuals in Portugal. *Clin Microbiol Infect* 2006; **12**(11):1131-4.
10. Facklam RR, Collins MD. Identification of Enterococcus species isolated from human infections by a conventional test Scheme. *J Clin Microbiol* 1989; **27**(4):731-4.
11. Talebi M, Rahimi F, Katouli M, et al. Prevalence and antimicrobial resistance of enterococcal species in sewage treatment plants in Iran. *Water Air Soil Pollut* 2007; **185**(111):9435-8.
12. National committee for clinical laboratory standards. Performance standards for antimicrobial susceptibility testing, 11th informational supplement 2001, vol21. National Committee for Clinical Laboratory Standards, Wayn, Pa.
13. The Enterococcus research site. Total DNA purification from Enterococcus. http://W3. ouhsc.edu/Enterococcus/DNA Purification from Enterococcus.
14. Kariyama R, Mitsuhata R, Chow JW, et al. Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant Enterococci. *J Clin. Microbiol* 2000; **38**(8):3092-5.
15. Vakulenko SB, Donabedian SM, Voskresenskiy AM, et al. Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. *Antimicrob Agents Chemother* 2003; **47**(4):1423-6.
16. Schwartz DC, Contor CR. Separation of yeast chromosome sized DNAs by pulsed field gradient gel electrophoresis. Cell 1984; 37(1):67-75.
17. Tenover FV, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by Pulsed Field Gel Electrophoresis: Criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**(9): 2233-9.
18. Carlet G. The gut is the epicenter of antibiotic resistance. *Antimicrob Resist Infect Control* 2012; **1**:39-45.
19. Saifi M, Pourshafie MR, Eshraghian MR, et al. Anti-microbial resistance of enterococci isolated from urinary tract infections in Iran. *Iran Biomed J* 2008; **12**(3):185-90.
20. Rahimi F, Taleb M, Saifi M, et al. Distribution of enterococcal species and detection of vancomycin resistance genes by multiplex PCR in Tehran sewage. *Iran Biomed J* 2007; **11**(3):161-7.
21. Feizabadi MM, Maleknejad P, Asgharzadeh A, et al. Prevalence of aminoglycoside-modifying enzymes genes among isolates of Enterococcus faecalis and *Enterococcus faecium* in Iran. *Microb Drug Resist* 2006; **12**(4):265-8.
22. Shokoohizadeh L, Ekrami A, Labibzadeh M, et al. Antimicrobial resistance patterns and virulence factors of enterococci isolates in hospitalized burn patients. *BMC Res Notes* 2018; **11**:1-5.
23. Balzereit-Scheuerlein F, Stephan R. Prevalence of colonization and resistance patterns of vancomycin-resistant enterococci in healthy, non-hospitalised persons in Switzerland. *Swiss Med Wkly* 2001; **131**(19):280-2.
24. Kolar M, Pantucek R, Bardon J, et al. Occurrence of vancomycin-resistant enterococci in humans and animals in the Czech Republic between 2002 and 2004. *J Med Microbiol* 2005; **54**(10):965-7.
25. Hasannejad Bibalan M, Eshaghi M, Sadeghi J, et al. Clonal Diversity in Multi Drug

Resistant (MDR) Enterococci Isolated from Fecal Normal Flora. *Int J Mol Cell Med* 2015; **4**(4):240-4.
26. Boost M, Lai L, Donoghue M. Drug resistance in fecal enterococci in Hong Kong. *J Infect Chemother* 2004; **10**(6):326-30.
27. Saifi M, Soltan Dallal MM, Pourshafei MR, et al. High level resistance of *Enterococcus faecium* and *E. faecalis* isolated from municipal sewage treatment plants to gentamicin. *Iranian J Publ Health* 2008; **37**(1):103-7.
28. Choukhachian M, Nahaei MR, Ahangarzadeh Rezaee M, et al. High-level Gentamicin Resistance and Detection of *aac (6')Ie-aph (2")Ia* Gene in Enterococci Isolated from Pediatric Hospital in Northwest of Iran. *Arch Clin Infect Dis* 2018; **13**(5): e62921.
29. Daikos GL, Bamias G, Kattamis C, et al. Structures, locations, and transfer frequencies of genetic elements conferring high-level gentamicin resistance in *Enterococcus faecalis* isolates in Greece. *Antimicrob Agents Chemother* 2003; **47**(12):3950-3.