Distribution of Gentamicin Resistant Genes of Nosocomial Enterococcus spp from Intensive Care Unit of Shahid Beheshty Hospital in Kashan, Iran

Mona Esmailzadeh¹, Mahmood Saffari¹, Rezvan Moniri¹, Hamid Reza Gilasi² and Marzieh Jabbary¹

¹Department of Microbiology and Immunology, School of Medicine, Kashan University of Medical Sciences, P.O. Box 8715988141, Kashan, Iran.
²Department of Biostatistics and Epidemiology, School of Health, Kashan University of Medical Sciences, Kashan- Iran. P.O. Box 8715988141, Kashan, Iran.

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The aim of this study was to determine, the rate and molecular characterization of aminoglycoside resistance genes (aac(6')-Ie-aph(2'')-Ia, aph(2'')-Ib, aph(23 )-Ic, and aph(23 )-Id) among high level gentamicin resistance (HLGR) enterococcus isolates in Kashan, Iran. A total of 180 enterococcus species were tested for high level gentamicin resistance by using disk diffusion method and minimum inhibitory concentration (MIC>500 ìg/mL) confirmatory test. High level gentamicin resistance strains were further assessed for aminoglycoside resistance genes. Antibiotic susceptibility pattern revealed that 43 isolates (23.9%) were high level gentamicin resistance (HLGR) (MIC>500 ìg/mL), 24 isolates (55.8%) of HLGR isolates were resistant to Chloramphenicol, 13 isolates (30.2%) to Quinupristin-dalfopristin, 7 isolates (16.3%) to Linezolid and 9 isolates (20.9%) of HLGR isolates were multi-drug resistant. The PCR method revealed that 76.7% of high level gentamicin resistance isolates carried aac(62 )Ie-aph(23 )Ia gene; but aph(23 )Ib, aph(23 )Ic, and aph(23 )Id genes were not detected among our isolates. The aac (6')-Ie-aph (2'')-Ia was detected in (71.9%) and (28.1%), of Enterococcus faecalis and E. faecium, respectively. These results point to that high level aminoglycoside resistance genes are extensively disseminated among ICU isolates of enterococci.

**Keywords:** Enterococcus, aminoglycoside, Intensive Care Unit.

Enterococcus is one of the most important causes of nosocomial infections among patients in intensive care unit (ICU). Due its importance it was recently ranked as second common agents of bacteremia in ICU¹. Although at first it was considered as an endogenous colonizer, by acquisition resistance genes with horizontal transformation mechanism and conjugation changed to hazardous pathogen²,³, by acquisition resistance genes with horizontal transformation mechanism and conjugation changed to hazardous pathogen²,³. The aac (62 ) le-aph (23 ) Ia is one of the main genes that carried by high-level gentamicin resistance (HLGR) enterococcus⁵. The importance of this gene is elimination of synergistic effects between penicillin and glycopeptide or aminoglycoside antibiotics by encoding a bi functional enzyme⁶,⁷. The aph (23 ) Ib, aph (23 ) Ic and aph (23 ) Id are the other genes that are coded by phosphotransferases which cause HLGR strains⁶. The abuse of antibiotics especially in patients with acute diseases at this ward poses enormous nosocomial infection with multi-drug resistant (MDR) bacteria¹. Multi-drug resistant enterococcus can be a serious problem for treating human and causes increased rates of failure treatment⁶. Since then, the high level aminoglycoside resistance has become a serious problem in most of hospitals; so identification of clinical isolates of HLGR enterococcus strains is

* To whom all correspondence should be addressed.
essential for an appropriate management for curing the infections. Little is known about the prevalence of aminoglycoside resistance genes in HLGR enterococcus strains recovered from rectal swabs of patients in ICU in Iran. So the aim of this study was determination of high level gentamicin resistance and distribution of aminoglycoside resistant genes of Enterococcus species from ICU. And also this study evaluates the rate of resistance to linezolid, Quinupristin-dalfopristin and Chloramphenicol among high level gentamicin resistance of enterococcus species. The other criterion which is determined is distribution of MDR isolates among high level gentamicin resistance of enterococcus species.

MATERIALS AND METHODS

Sample collection
A cross-sectional study was organized between October 1, 2013, and October 15, 2014. One hundred eighty non-repetitive enterococcus isolates were recovered from two hundred ten cotton rectal swabs of patients after 48 hours of their hospitalization at ICU (74 isolates of surgical ICU, 59 isolates of Neurosurgical ICU and 47 medical ICU) in Shahid Beheshti Hospital of Kashan, Iran. This is a general teaching hospital with different wards and 516 beds. There was no age and sex restriction for preparing samples. Any complication or underlying disease such as diabetes didn’t cause disturbance in this study. The study protocol was approved by the Ethics Committee of Kashan University of Medical Sciences. And also written informed consent was obtain from all study participants or their parents/guardian.

Bacterial isolates
A cotton rectal swab which was acquired from each patient at ICU immediately transferred to the 6.5% Nacl broth medium (Merk, catalogue number: 105459). It was incubated in 37°C within 2 hr and cultured on Bile-Esculin agar medium (Merk, catalogue number: 105459). Cultured plates were incubated at 37°C and were examined after overnight incubation. Phenotypical test for identification of enterococcus spp performed based on the conventional microbiological tests. Just one enterococcus isolate was analyzed from each patient\cite{9,10}.

Antimicrobial susceptibility test
The disk diffusion method and minimum inhibitory concentration (MIC) test were performed by using Mueller Hinton agar and Brain Heart Infusion agar for detection of HLGR isolates among 180 enterococcus species according to the Clinical and Laboratory Standards Institute (CLSI 2014) recommendation\cite{11}. And also susceptibility of HLGR strains was determined to Chloramphenicol (S: e”18mm, I: 13–17mm, R: d”12), Quinupristin-dalfopristin (S: e”19mm, I: 16–18mm, R: d”15mm), and Linezolid (S: e”23mm, I: 21–22mm, R: d”20mm) (MAST, UK). The reference strain E. faecalis ATCC 29212 and Staphylococcus aureus ATCC 25923 was used as a control. Results were clarified as susceptible, intermediate or resistant according to the criteria recommended by the CLSI and the manufacturer protocols (Mast, UK)\cite{11}.

DNA extraction
The crude DNA was extracted from 108 E. faecalis and 72 E. faecium by boiling method for confirming the species and identifies the genes of interest among HLGR isolates. The template DNA stored at “20°C until polymerase chain reaction (PCR) amplification was performed\cite{12}.

Genus identification of enterococcus by PCR
The identified genus of enterococcus species were confirmed by distinguishing ddl genes with PCR method using specific primers (Table 1). Amplification of ddl genes were performed under the conditions that were used in similar study\cite{13}. 25 5ØßL Final reaction mixtures was prepared with 10 pmol of each primer, 200mM of dNTP, 1 unit of Taq polymerase, 2.5 5ØßL of 10x reaction buffer, 1.5M MgCl2 in final concentration, and 100 ng DNA template. Amplification reactions were carried out in a thermocycler (Eppendorf master cycler, MA) under the following conditions: initial denaturation at 94°C for 3 min, followed by 30 cycles of amplification at 94°C for 1min, 54°C for 1 min and 72°C for 1 min with final extension at 72°C for 7 min\cite{13}. The amplified products were electrophoresed on 2% agarose gels. The gels were stained in ethidium bromide (0.5mg/mL) visualized in gel document system (Biorad, UK).

Characterization of aminoglycoside resistance genes among HLGR strains
Aminoglycoside modifying enzymes (AMEs) which includes aac(62 )Ie-aph(23 )Ia,
aph(23)Ib, aph(23)Ic and aph(23)Id genes identified by polymerase chain reaction. Amplification reactions were carried out in a thermocycler (Eppendorf master cycler, MA) under conditions that were experiment in similar studies\textsuperscript{14, 15, 16}. Amplification for aac(62)Ie-aph(23)Ia gene was performed under underneath conditions: denaturation at 94Ú°C for 3 min, followed by 32 cycles, annealing at 60Ú°C for 45 sec, extension at 72Ú°C for 1 min and final extension at 72Ú°C for 2 min \textsuperscript{14}. PCR conditions for aph(23)Ib and aph(23)Id were as follows: denaturation at 94Ú°C for 1 min, followed by 30 cycles, annealing at 55Ú°C for 1 min and extension at 72Ú°C for 2 min \textsuperscript{15}. And also the Amplification conditions that used for aph(23)Ic gene were as follows: denaturation at 94Ú°C for 40 sec, followed by 30 cycles, annealing at 56Ú°C for 30 sec, extension at 72Ú°C for 50 sec\textsuperscript{16}. A total volume of 50µl containing 100 ng genomic DNA from enterococcus species culture, 200 mM each of dNTP, 1× PCR buffer (20mM Tris-HCl, pH 8.4), 50mM KCl, 1.5mM MgCl\textsubscript{2}, 0.5 mM of each primer (Table 1) and 1.5U of Taq polymerase used for performing PCR. 10µl of Amplified samples were electrophoresed on 2% agarose gel in TBE buffer. The gel was stained with ethidium bromide 0.5 mg/ml. The amplified bands were observed under gel document system (Biorad, UK). As negative control reaction mixture without a DNA template was used. The positive amplicons were sequenced to confirm the result of PCR.

**DNA Sequencing and Sequence Analysis**

Sequencing performed for positive favorable gene (aac(62)Ie-aph(23)Ia) using the ABI Capillary System (Macrogen Research, Seoul, Republic of Korea). The sequence was analyzed using Chromas Pro version 1.7.5 Technelium (http://technelium.com.au/) and performed online by using the BLAST program of the National Center for Biotechnology Information server (http://www.ncbi.nlm.nih.gov/).

**Statistical analysis**

The statistical analysis of data was conducted using SPSS software version 15 (SPSS, Inc.). The Chi-square test or the Fisher’s exact test was used to compare proportions. \(P-Values < 0.05\) were considered statistically significant. Prevalence data is presented with 95% confidence intervals (CI).

**RESULTS**

180 isolates of enterococcus species obtained from 210 hospitalized patients in ICU. These isolates were collected from patients who had been hospitalized for two days or more in ICU of Shahid Beheshti Hospital of Kashan. The prevalence rate of enterococcus was 85.7% (108 \(E.\) faecalis and 72 \(E.\) faecium). The majority of patients who carried enterococcus isolates were males (67%). More than fifty seven percent of these patients who carried enterococcus species were \textgreater57\textsuperscript{e} years.

The prevalence of HLGR enterococcus was 23.9% (43/180) (MIC \textsuperscript{>500} ¼g/mL). Of these HLGR enterococcus isolates, 26 isolates (60.5%) were \(E.\) faecalis (Table 2). The majority of patients with HLGR strains were males (67%). More than fifty seven percent of these patients who carried enterococcus species were \textsuperscript{57\textsuperscript{e}} years.

The prevalence of HLGR enterococcus was 23.9% (43/180) (MIC >500 \(\mu\)g/mL). Of these HLGR enterococcus isolates, 26 isolates (60.5%) were \(E.\) faecalis (Table 2). The majority of patients with HLGR strains were males (74.4%). More than

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**Table 1. Primers used for polymerase chain reaction and sequencing**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequences (5’-3’)</th>
<th>PCR product (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>( ddl) ((E.) faecalis)</td>
<td>CACCTGAAGAAACAGGC</td>
<td>475</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>ATGGCTACTTCATTTCCAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( ddl) ((E.) faecium)</td>
<td>GAGTAAATCAGAGGCA</td>
<td>1091</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>CGCTGATGATCGATTGATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aac(62)Ie-aph(23)Ia</td>
<td>CCAAGAGCAATAGGGGCATA</td>
<td>220</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>CACTATCATAACCTACCCCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( aph(23)Ib)</td>
<td>ACTCCGTATTATTATCCTGCC</td>
<td>279</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>TCATCATATGCAAGGGCATC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( aph(23)Ic)</td>
<td>GAAGGCTTATGAAATCTAC</td>
<td>125</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>ACACAACCGACCAACAGAGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( aph(23)Id)</td>
<td>GGTGGTTTTTTTACAGGAATGTCCT</td>
<td>642</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>CCTCTCTCATACCAATCCATATAC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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sixty five percent of patients with HLGR strains were 60e” years. The mean duration of hospitalization was 12.56±13.927 day. In this study 20 isolates of HLGR strains (46.5%) were collected from surgery ICU (70% E. faecalis and 30% E. faecium), 5 isolates (11.6%) from neurosurgery ICU (60% E. faecalis and 40% E. faecium) and 18 isolates (41.9%) from medical ICU (72.2% E. faecalis and 27.8% E. faecium).

Antibiotic susceptibility pattern revealed that among 43 HLGR isolates, 55.8% (16 E. faecalis and 8 E. faecium) were resistant to Chloramphenicol, 30.2% (11 E. faecalis and 2 E. faecium) to Quinupristin-dalfopristin and 16.3% (6 E. faecalis and 1 E. faecium) to Linezolid (figure 1).

Among HLGR isolates of enterococcus, 20.9% (9/43) indicated multidrug-resistant (MDR) pattern. This pattern demonstrated resistant to at least one agent in three or more antimicrobial categories. Of isolates that were supposed HLGR bacteria by disk diffusion method and MIC test, 76.7% (33/43) comprising aac (62 ) le-aph (23 ) Ia. PCR assays and sequencing revealed that 60.6% (n=20) of E. faecalis and 39.4% (n=13) of E. faecium carried this gene. None of the aph (23 ) Ib, aph (23 ) Ic, and aph (23 ) Id genes were found at HLGR isolates. The nucleotide sequence of the PCR products of ddl genes and aminoglycoside resistant gene were identical to ddl (E. faecalis), ddl (E. faecium) and aac (62 ) le-aph (23 ) Ia in the GenBank nucleotide database (http://

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**Table 2.** The prevalence of 43 HLGR isolates of enterococcus species that confirmed with minimum inhibitory concentration (MIC) method in different ICU.

<table>
<thead>
<tr>
<th>Species</th>
<th>Surgery ICU NO. (%)</th>
<th>Neurosurgery ICU NO. (%)</th>
<th>Medical ICU NO. (%)</th>
<th>MIC&gt;500µg/mL NO. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecalis</td>
<td>14(70)</td>
<td>3(60)</td>
<td>13(72.2)</td>
<td>26(60.5)</td>
</tr>
<tr>
<td>E. faecium</td>
<td>6(30)</td>
<td>2(40)</td>
<td>5(27.8)</td>
<td>17(39.5)</td>
</tr>
<tr>
<td>Total</td>
<td>20(100)</td>
<td>5(100)</td>
<td>18(100)</td>
<td>43(100)</td>
</tr>
</tbody>
</table>

**Table 3.** Association between patient characterizations and the rate of high level gentamicin resistance enterococcus species that may carry aminoglycoside resistance genes.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>HLGR positive No. (%)</th>
<th>HLGR negative No. (%)</th>
<th>P- Value</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (28)</td>
<td>11 (39.3%)</td>
<td>17 (60.7%)</td>
<td>0.038</td>
<td>2.426 (1.03-5.69)</td>
</tr>
<tr>
<td>No (152)</td>
<td>32 (21.1%)</td>
<td>120 (78.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using ciprofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (56)</td>
<td>23 (41.1%)</td>
<td>33 (58.9%)</td>
<td>0.001</td>
<td>3.624 (1.771-7.415)</td>
</tr>
<tr>
<td>No (124)</td>
<td>20 (16.1%)</td>
<td>104 (83.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using meropenem</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (70)</td>
<td>24 (34.3%)</td>
<td>46 (65.7%)</td>
<td>0.009</td>
<td>2.499 (1.243-5.025)</td>
</tr>
<tr>
<td>No (110)</td>
<td>19 (17.3%)</td>
<td>91 (82.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Using amikacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (9)</td>
<td>6 (66.7%)</td>
<td>3 (33.3%)</td>
<td>0.002</td>
<td>7.243 (1.728-30.355)</td>
</tr>
<tr>
<td>No (171)</td>
<td>37 (21.6%)</td>
<td>134 (78.4%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In northwestern Iran the frequencies of HLGR E. faecalis among HLGR isolates, as well as the prevalence of E. faecalis 9.9% among fecal samples of patients. It could showed a low frequency of HLGR, approximately (23.9%) in ICU. Versus our result a study in turkey frequency of high-level gentamicin resistance in part of the world high level gentamicin resistance is forecast. In this study enterococcus species significantly associated with the presence of HLGR resistance (HLGR). High-level gentamicin used aminoglycosides against enterococcus, since its discovery in 1963. High-level gentamicin resistance (HLGR) has been widely investigated and different frequencies have been reported depending on various regions due to the variety in climate of hetero geographical regions and origin of isolates. So, the high prevalence of resistance to high level gentamicin is forecast. In this study the rate of E. faecalis and E. faecium were 60% and 40%, respectively, indicates high rates of E. faecalis which was identical to the other results of study conducted in Tehran, 64.4% E. faecalis and 35.6% E. faecium. E. faecalis was dominant in this study and similar to other studies from Iran, USA and UK and some of European countries and in contrast E. faecium is more prevalent in some countries such India and Japan. Our result showed high frequency of high-level gentamicin resistance (23.9%) in ICU. Versus our result a study in turkey showed a low frequency of HLGR, approximately 9.9% among fecal samples of patients. It could be due to inappropriate use of gentamicin among patients in Kashan. A higher frequency was mentioned in northwestern of Iran (60.4%), Kuwait (47%), China (64.15%) and Thailand (55.6%)20, 21, 6, 16. So according to the different studies in most part of the world high level gentamicin resistance in enterococcus species is increasing, however low frequency was noted in some countries such India (2%) and Saudi Arabia (20.9%)22, 23. At this study E. faecalis (60.5%) was the most prevalent species among HLGR isolates, as well as the prevalence of E. faecalis among non HLGR enterococcus species. In northwestern of Iran the frequencies of HLGR between E. faecalis and E. faecium were 59.4% and 40.6% and in northern Tehran was 61.3% and 33.9%, respectively, which is in agreement with our findings20, 17. And also in Sweden the prevalence of HLGR isolates between E. faecalis and E. faecium were 20% and 0%24. It sounds low administration of extended spectrum antibiotic in this country reduced the rate of HLGR isolates in this country. Although the prevalence of HLGR isolates in E. faecalis is more than E. faecium in our findings and some countries, these results are in contrast to studies conducted in China and turkey that shown 51.5% and 88% HLGR in E. faecium. To date, aac (62) Ie-aph (23) Ia gene is the most prevalent aminoglycoside-modifying enzymes genes. The present study demonstrated high prevalence (76.7%) of aac (62) Ie-aph (23) Ia gene among 43 (23.9%) HLGR isolates. This result is alarming due to the ability of enterococcus species for being reservoir and transporting antibiotic resistance genes among different bacteria. Although our result is more prevalent than Chile (14.8%), but a higher prevalence was shown in Iran (100%) and china (86.8%)26, 27, 6. In this study high frequency of this gene is more prevalent among E. faecalis 60.6% than E. faecium 39.4%. In addition, a study in Japan showed the frequency of this gene increased to 28% in E. faecium. The present study was shown a low frequency of this gene in gentamicin susceptible isolates that may be due to the presence of non-functional gene at these isolates16. The result of this study revealed that none of the isolates possess aph(23) Ib, aph(23) Ic and aph(23) Id genes which are comparable to the results of the studies in northwestern of Iran, China, Thailand, Kuwait and India20, 6, 16, 21, 29. The high rate of aac (62) Ie-aph (23) Ia among enterococcus species isolates in our region may be associated to clonal spread of a single clone, although further studies using molecular typing methods such as pulsed field gel electrophoresis (PFGE) are needed for approval this statement. Whereas a low frequency of aph(23) Ib gene in Cuba 5%, America 5% in clinical specimens and 3.4% in enterococcus blood strains in Cuba demonstrated. And also there were evidences in the presence of aph (23) Ic gene in Cuba 1.1% in enterococcus blood strains and 1.6% on clinical specimens and in America 2.5% in human specimens . In disagreement of our findings a low frequency of aph (23) Id gene in America 2.5% was demonstrated. However, the frequency
about 20.9% of multi-drug resistant (MDR) in our findings among 9 HLGR isolates (8 E. faecalis and 1 E. faecium) is comparably lower than the rate of MDR isolate in northern Tehran which was about 31.7%\(^2\). One of the reasons on this disagreement may due to the origin of the specimens. In our findings 5 E. faecalis MDR isolates are 100% resistant to Chloramphenicol, Linezolid, Quinupristin-dalfopristin and gentamicin. Two MDR isolates (1 E. faecalis and 1 E. faecium) are resistant to Linezolid, Chloramphenicol and gentamicin and also two E. faecalis that are MDR, are resistant to Quinupristin-dalfopristin, Chloramphenicol and gentamicin. There is warning because infection caused by such resistant isolates can be difficult to treat. The rate of Chloramphenicol resistant among HLGR isolates 55.8% in our findings (66.7% E. faecalis and 33.3% E. faecium) is considerably more than the rate of Chloramphenicol resistant among multi-drug resistant enterococcus (26.3% E. faecalis and 4.8% E. faecium) in China\(^7\). It reveals that E. faecalis isolates are more resistant to this antibiotic which is in agreement to the result of study in China\(^7\).

Although in China none of the isolates were resistant to linezolid but in our findings 16.3% of isolates were resistant to the linezolid\(^6\). An important step in controlling the dissemination of this microorganism is to identify the risk factors that associate with it. Finally, in term of relation between patients characterizations and acquisition HLGR enterococcus, we found that using extended-spectrum antibiotics such ciprofloxacin, meropenem, amikacin, and chronic disease such as diabetes in hospitalized patients in ICU tend them to acquisition HLGR enterococcus (p<0.05) (according table 3). In contrast, in study conducted in turkey with these risk factors no significant differences observed\(^33\).

CONCLUSION

All of these data indicates that aph (2") Ib, aph (2") Ic and aph (2") Id genes don’t play an important role in producing HLGR isolates in this region, but aac(6') le-aph(2") Ia gene which is more prevalent is a main gene that produces HLGR isolates by enzymatic mechanisms.

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REFERENCES


3. P. Ruiz-Garbajosa, R. del Campo, T. M. Coque et al., 'Longer intestinal persistence of Enterococcus faecalis compared to Enterococcus faecium clones in intensive-care-unit patients,' Journal of Clinical Microbiology, 2009; 47(2); pp. 345-351.

4. S. Pournaras, A. Tsakris, M. F. Palepou et al., 'Pheromone responses and high-level aminoglycoside resistance of conjugative plasmids of Enterococcus faecalis from Greece,' Journal of Antimicrobial Chemotherapy, 2000; 46(6); 1013-1016.


8. K. H. Kwon, S. Y. Hwang, B. Y. Moon et al., 'Occurrence of antimicrobial resistance and virulence genes, and distribution of enterococcal clonal complex 17 from animals and human beings in Korea,' Journal of Veterinary Diagnostic Investigation, 2012; 24(5); 924-931.


22. R. Sekar, R. Srivani, R. Vignesh, H. Kownhar, and E. M. Shankar. 'Low recovery rates of high-level aminoglycoside-resistant enterococci could be attributable to restricted usage of aminoglycosides in Indian settings,' Journal of Medical Microbiology, 2008; 57(3): 397-398.


28. S. Watanabe, N. Kobayashi, D. Quiñones, S. Nagashima, N. Uehara, and N. Watanabe. 'Genetic diversity of enterococci harboring the high-level gentamicin resistance gene aac (62) -Ie-aph (2 3 )-Ia or aph (2 3 )-Ie in a Japanese
hospital,” Microbial Drug Resistance, 2009; 15(3); 185-194.


32. S. M. Donabedian, L. A. Thal, and E. Hershberger et al., “Molecular characterization of gentamicin-resistant enterococci in the United States; evidence of spread from animals to humans through food,” Journal of Clinical Microbiology, 2003; 41(3); 1109-1113.