Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22, No. (1) : 2020 : 174-180

ISSN-0972-3005

PREVALENCE OF HIGH-LEVEL GENTAMICIN RESISTANCE AND DISTRIBUTION OF ITS GENES IN *ENTEROCOCCUS* SPECIES ISOLATED FROM PATIENTS OF URINARY TRACT INFECTION IN A TERTIARY CARE HOSPITAL IN BANGLADESH

TASNIM AHSAN[1], MUHAMMAD MANWAR MORSHED HEMEL[2] AND S.M. SHAMSUZZAMAN[1]

[1]Department of Microbiology, Dhaka Medical College, Bangladesh
[2]Programme for HIV and AIDS, Infectious Diseases Division, ICDDR,B Bangladesh

Received 8 November, 2019; accepted 12 December, 2019)

Keyword: *Urinary Tract Infections, Enteroccus, Gentamicin, Drug resistance, Bangladesh*

Abstract – Enzymatic modification causes high-level gentamicin resistance to *Enterococci* which eliminate the synergistic bactericidal effect of combined exposure to a cell wall-active agent and gentamicin. This study was conducted to determine the prevalence of high level gentamicin resistant *Enterococci* (HLGRE) and the distribution of high level gentamicin resistant genes in them. A total of 550 urine samples were taken from patients in a tertiary care hospital. Among them, a total of 46 *Enterococci* were isolated and subsequently analyzed. *Enterococci* were screened for HLGR by Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute guidelines. Minimum inhibitory concentration (MIC) of all isolates for gentamicin was determined by agar dilution method. Polymerase chain reaction (PCR) was carried out for HLGR *Enterococcus* isolates to identify aminoglycoside modifying enzymes genes responsible for resistance. Among the isolated *Enterococci*, 17 (36.97%) were resistant to high level gentamicin by disc diffusion method. Nineteen (41.3%) *Enterococci* were positive for HLGR by both MIC method (MIC> 500 µg/mL) and by PCR. Only *aac (6′)- Ie- aph (2″)-Ia* gene was found to responsible for HLGR and other genes such as *aph (2″)-Ib* and *aph (2″)-Ic* gene were not detected in this study. High frequency of HLGRE (41.3%) may be regarded as a warning to the community. In identifying HLGRE, along with disc diffusion method, the MIC method should also be adopted routinely.

INTRODUCTION

Enterococci are gram positive bacteria that are distributed widely in the environment and in the gastrointestinal tract of humans, animals and insect (Ali *et al.,* 2017). *Enterococci* were traditionally regarded as low-grade pathogens but have emerged as an increasingly important cause of nosocomial infections from 1990s (Shete *et al.,* 2017). Inherent ability to acquire resistance to antimicrobials and horizontally transfer antimicrobial resistance to other bacteria are attributable to these abilities (Iweriebor *et al.,* 2015). These organisms most commonly infect the urinary tract, bloodstream, endocardium, abdomen, biliary tract, burn wounds, indwelling foreign devices and less frequently the central nervous system, lung, soft tissue, paranasal sinuses, ear, eye and periodontal tissue (Jett and Huycke, 1994). *Enterococcus* species are considered the second most common pathogen across all health care associated infection types, third most common pathogen among catheter associated urinary tract infection and the single most common pathogen among central line–associated bloodstream infections (Weiner *et al.,* 2016). Nearly 50 species of *Enterococci* have been identified (Parte, 2013). The majority of human *Enterococcal* infections are caused by *E. faecalis* (80–90%), with *E. faecium* comprising most of the remainder (10–15%) (Fernandes and Dhanashree, 2013). Other *Enterococcal* species known to cause human infection include *E. avium*, *E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. raffinosus and E. mundtii* (Higuita and Huycke, 2014).

All *Enterococci* are naturally (intrinsically) resistant to many antimicrobial agents such as semisynthetic penicillins (e.g., oxacillin), cephalosporins of all classes, monobactams, polymyxins and low level aminoglycosides.

Combination of a cell wall active antibiotic such as penicillin, ampicillin or vancomycin and an aminoglycoside such as gentamicin is essential for severe enterococcal infection (Padmasini *et al.,* 2014). *Enterococci* have synergistic susceptibility when treated with a cell wall acting antibiotic and an aminoglycoside. However, some aminoglycosides are not susceptible to synergism (Aslangul *et al.,* 2005). Emergence of high level resistance to gentamicin (MIC of > 500 µg/mL) by some *Enterococci* has nullified the synergistic effect of combination therapy and most often associated with high level resistance to all other alternative aminoglycosides (Levine, 2006). High level gentamicin resistance in *Enterococci* is predominantly mediated by acquisition of genes which encodes the aminoglycoside modifying enzyme. There are three types of aminoglycoside modifying enzymes: (1) N-Acetyltransferases (AAC); (2) O-Adenylyltransferases (ANT); and (3) O-Phosphotransferases (APH) (Shete *et al.,* 2017). To evaluate the gene responsible for enzymatic modification among *Enterococci*, there had only one gene found predominantly responsible, the aac(6')-Ie-aph(2")-Ia, gene. Even though few studies reported newer genes such as aph(2")-Ib, aph(2")-Ic, and aph(2")-Id recently, however evidences are scarce regarding the prevalence of these genes globally (Vakulenko *et al.,* 2003). The transfer of nosocomial antibiotic resistance within bacterial strains can occur through the exchange of plasmid and transposon, that can lead to difficult to treat clinical infections (Abriouel *et al.,* 2008). Therefore, the recognition of such resistant strains can be beneficial in limiting serious nosocomial infections.

The study was conducted to measure prevalence of high level gentamicin resistant *Enterococci* (HLGRE) in patients of urinary tract infection in a tertiary care hospital in Bangladesh and distribution of high level gentamicin resistance genes [aac(6')-Ie-aph(2")-Ia, aph(2")-Ib and aph(2")-Ic] of *Enterococcus spp.* by PCR.

MATERIALS AND METHODS

Study design and sample

This was a cross sectional study where a total of 550 urine samples were collected from patients of Dhaka Medical College Hospital of Bangladesh, from January, 2017 to December, 2017. Urine samples were collected aseptically from clinically suspected urinary tract infected patients attending the inpatient and outpatient department of Dhaka Medical College Hospital.

Ethical obligations

Informed written consent was obtained from all the participants during the enrolment in the study. Data were entered in an anonymized and de-identified manner prior to analysis. Ethical approval for this study was taken from the Ethical Review Committee (ERC) of Dhaka Medical College.

Microbiological methods

The isolates of *Enterococci* were identified and speciated on the basis of colony morphology, gram stain, and various biochemical reactions such as catalase test, bile esculin test, growth in 6.5% NaCl, mannitol fermentation,arginine dihydrolase test, sorbitol fermentation, arabinose fermentation and growth in pyruvate. *Enterococcus faecium* and *Enterococcus faecalis* were further confirmed by PCR analysis using specific primers.

Antibiotic susceptibility testing

All *Enterococcal* isolates were tested for their susceptibility to various antibiotics active against *Enterococci* species by Kirby-Bauer method as per CLSI guideline 2016 (CLSI, 2016). Screening of HLGRE was done with 120 µg disc. *Staphylococcus aureus* (ATCC 25923) was used as control strains to assess the performance of the method. Minimum inhibitory concentration (MIC) of gentamicine and vancomycine were determined by agar dilution method. The highest dilution that inhibited the growth of the organism was taken as MIC of the test organism. Any *Enterococcus* showing a MIC of >500 µg/mL to gentamicin was considered as HLGRE.

Molecular Analysis of Aminoglycoside Modifying Genes by PCR

The genes analyzed in the present study were *aac(6')-Ie-aph(2")-Ia, aph(2")-Ib, aph(2")-Ic* responsible for high-level aminoglycoside resistance in *Enterococci* (Table 1).

DNA extraction

Three hundred microlitre distilled water was mixed with bacterial pellet and was vortexed until mixed well. The microcentrifuse tube was kept in block heater (DAIHA Scientific, Seoul, Korea) at 100 °C for 10 minutes for boiling. After boiling the tube was immediately kept on ice. Then the tube was centrifuged at 4 °C at 14000 x g for 6 minutes. Finally

Table 1. Following primers were used in this study

Gene	Oligonucleotide Sequence (5′ To 3′)	Product Size (bp)
aac(6′)-Ie-aph(2′′)- Ia	F:CAGGAATTTATCGAAAATGGTAGAAAAG	
	R:CACAATCGACTAAAGAGTACCAATC	369
aph(2″)- Ib	F:CTTGGACGCTGAGATATATGAGCACR	
	R:GTTTGTAGCAATTCAGAAACACCCTT	867
aph(2″)- Ic	F:CCACAATGATAATGACTCAGTTCCC	444
	R:CCACAGCTTCCGATAGCAAGAG	

supernatant was taken using micropipette and was used as template DNA for PCR. This DNA was kept at -20 °C for future use.

Mixing of master mix and primer with DNA template

Amplification was performed in a final reaction volume of 25 µL. Each PCR tube contained 2 mL of extracted DNA, 12.5 mL master mix-PCR buffer, dNTP, Taq polymerase enzyme, $MgCl_2$, and loaded dye (Promega Corporation, USA), 2µL extracted DNA from *Enterococcus* spp. was mixed in 12.5 µL master mix together with 4 µL primer (forward and reverse). Volume of the reaction mixture was adjusted by adding 6.5 µL filtered deionized water (nuclease free). After a brief vortex, the tubes were centrifuged in a micro centrifuge for few seconds.

Amplification through thermal cycler

PCR assays were performed in a DNA thermal cycler (Eppendorf AG, Master cycler gradient, Hamburg, Germany). Each PCR run was comprised of preheat at 94 °C for 10 minutes followed by 36 cycles of denaturation at 94 °C for 1 minute, annealing at 58 °C for 45 seconds, extension at 72 °C for 2 minutes with final extension at 72 °C for 10 minutes.

Gel electrophoresis and visualization

Amplified products were run on to horizontal gel electrophoresis in 1.5% agarose (Bethesda Research Laboratories) in 1X TBE buffer at room temperature at 100 volt (50 mA) for 30 minutes. Five µL amplified DNA mixed with tracking dye was then loaded into an individual well of the gel. One hundred bp DNA molecular size markers were loaded into well at the middle or at two sides of the gel for comparing with the base pair of identified band. DNA bands were detected by staining with ethidium bromide (0.5 µL/mL) for 30 minutes at room temperature and then destained with distilled water for 15 minutes. Photographs were taken using digital camera with UV trans-illuminator (Gel Doc, Major science, Taiwan).

RESULTS

In this study, 46 (14.47%) *Enterococcus spp*. were isolated from 550 urine samples. Among them, 33(71.74%) were *E. faecalis*, 11 (23.91%) were *E. faecium*, 2 (4.35%) were unidentified. Among the 33 isolated *E. faecalis*, 28 (84.85%) were resistant to ciprofloxacin, 26 (78.78%) to gentamicin (10 µg), 20 (60.61%) to amikacin and 5 (15.15%) to nitrofurantoin. All the *E. faecium* were resistant to

Table 2. Antimicrobial resistance pattern of *Enterococci* (n=46)

Antimicrobial agents	*E. faecalis* N=33 n(%)	*E. faecium* N=11 n(%)	Unidentified spp. N=2 n(%)	Total N=46 n (%)
Gentamicin (10µg)	26 (78.78)	11 (100.00)	2 (100.00)	39 (84.78)
Gentamicin (120µg)	14 (42.42)	3 (27.27)	0	17 (36.97)
Ampicillin	8 (24.24)	3 (27.27)	1 (50.00)	12 (26.09)
Amikacin	20 (60.61)	8 (72.73)	1 (50.00)	29 (63.04)
Ciprofloxacin	28 (84.85)	11 (100.00)	1 (50.00)	40 (86.96)
Imipenem	9 (27.27)	4 (36.36)	0	13 (28.26)
Doxycycline	8 (24.24)	3 (27.27)	0	11 (23.91)
Nitrofurantoin	5 (15.15)	2 (18.18)	0	7 (15.22)
Vancomycin	0	0	0	0
Teicoplanin	0	0	0	0
Linezolid	0	0	0	0

gentamicin (10 μg), ciprofloxacin and 8 (72.73%) to amikacin, 3 (27.27%) to doxycycline and ampicillin, 2 (18.18%) to nitrofurantoin. Fourteen (42.42%) *E. faecalis* and 3 (27.27%) *E. faecium* were resistant to high level gentamicin (120 μg). None of the isolated *Enterococci* were resistant to vancomycin, linezolid and teicoplanin. Out of 46 isolated *Enterococci*, 8 (17.39%) had MIC 1024 μg/mL and 11 (23.91%) had MIC 512 μg/ml to gentamicin. So, total 19 (41.3%) *Enterococci* were high level gentamicin resistant (Table 2, 3). The MIC of vancomycine range from 1-4 μg/mL.

Among the 46 *Enterococcus spp*. 19 (41.3%) were positive for aac (6′)- Ie- aph (2″-Ia gene and these 19 *Enterococcus spp*. were also positive for HLGR by agar dilution MIC method. None of the isolated *Enterococci* were found positive for both aph (2″)-Ib and aph (2″)-Ic genes. Among the 33 *E. faecalis*, 15 (45.45%) were positive for aac (6′)- Ie- aph (2″)-Ia gene and among the 11 *E. faecium*, 4 (36.36%) were positive for aac (6′)- Ie- aph (2″)-Ia gene (Table 4, 5).

Table 3. MIC of *Enterococcus* to gentamicin

MIC of gentamicin (μg/mL)	Number	Percentage
1024	8	17.39
512	11	23.91
256	1	2.17
128	3	6.52
64	9	19.57
32	4	8.7
16	3	6.52
8	4	8.7
≤ 4	3	6.52
Total	46	100.00

Note: CLSI break point of MIC of gentamicin for *Enterococci*
Sensitive = ≤ 4 μg/mL, Intermediate = 8 μg/mL, Resistant = ≥ 16 μg/mL, CLSI break point of MIC of high level gentamicin for *Enterococci*, Sensitive = ≤ 500 μg/mL, Resistant = >500 μg/mL

DISCUSSION

Urinary tract infection is one of the most common infectious conditions in clinical practice. Acquisition of extrinsic resistance to high level aminoglycoside antibiotics in *Enterococci,* eliminates the synergistic effects of penicillin-gentamicin combination therapy.

In the present study, prevalence of enterococcal urinary tract infection was 14.47% which was similar to a study conducted in India that found prevalence of 16.91% (Bharti *et al.,* 2016). In contrast to the current study findings, two more studies conducted in India reported a prevalence rate of 8.45% and 5.9%, which are lower than the present study (Chakraborty *et al.,* 2015; Purohit *et al.,* 2017). The reason of higher isolation rate for *Enterococci* in the present study might be due to the fact that patients included in this study were from tertiary care hospital and many of them were catheterized.

In this study, among 46 *Enterococci,* 33 (71.74%) were identified as *E. faecalis* and 11 (23.91%) as *E. faecium* which is similar to findings reported by a study conducted in Bangladesh that showed 71.42% were *E. faecalis* and 23.81% were *E. faecium* in their study (Suchi *et al.,* 2017). Whereas another study conducted in Iran reported 67.8% *E. faecalis* and 24% *E. faecium* (Nasaj *et al.,* 2016). The more prevalence

Table 4. Distribution of high level gentamicin resistance genes among isolated *Enterococci* by PCR (N= 46)

High level gentamicin resistance genes	Positive n (%)	Negative n (%)	Total n (%)
aac (6′)- Ie- aph (2″)-Ia	19 (41.3)	27 (58.7)	46 (100.00)
aph (2″)-Ib	0 (0.00)	46 (100.00)	46 (100.00)
aph (2″)-Ic	0 (0.00)	46 (100.00)	46 (100.00)

N= Total number of isolated *Enterococcus spp.,* n= number of high level gentamicin resistance genes

Table 5. Distribution of high level gentamicin resistance gene *{aac (6′)- Ie- aph (2″)-Ia }* among identified *Enterococcus* spp. (2″) (N= 46)

Enterococcus spp.	Positive n (%)	Negative n (%)	Total n (%)
E. faecalis	15 (45.45)	18 (54.55)	33 (100.00)
E. faecium	4 (36.36)	7 (63.64)	11 (100.00)
Unidentified *spp.*	0 (0.00)	2 (100.00)	2 (100.00)

N= Total number of isolated *Enterococcus spp.,* n= number of high level gentamicin resistant genes.

of *E. faecalis* over *E. faecium* in causing infection may be due to the predominance of *E. faecalis* in the endogenous flora of the body (Boby *et al.,* 2016).

In the present study, no *Enterococcus* was found resistant to linezolid and teicoplanin by disc diffusion method and no VRE was detected by both disc diffusion method and MIC method. In this study, the MIC range of vancomycin for *Enterococci* was 1-4μg/mL. Three other studies conducted in Bangladesh also did not find any VRE in their studies (Akhter *et al.,* 2014; Islam and Shamsuzzaman, 2015; Tamanna *et al.,* 2013). This is probably due to less use of vancomycin, linezolid and teicoplanin as these drugs are expensive and many of them need parenteral administration. Absence of vancomycin, linezolid and teicoplanin resistance among enterococcal strain in this study allows the use of these antibiotics to treat the patients. The resistant pattern of *Enterococci* to nitrofurantoin was only 15.22%. So nitrofurantoin may be considered as a good solution for treatment of *Enterococcal* UTI due to its lower resistance and availability.

Gentamicin is one of the most commonly used aminoglycosides against *Enterococci (Chow et al., 1997)*. High level aminoglycoside resistance is a real problem. In this study, 84.78% of *Enterococci* were resistant to low level gentamicin (10μg) and 36.97% of *Enterococci* were resistant to high level gentamicin (120μg) by disc diffusion method. One study in Bangladesh reported 38.75% of *Enterococci* were resistant to high level gentamicin (120μg) by disc diffusion method which coincides with the current study (Tamanna *et al.,* 2013). In this study, MICs of gentamicin ranged from 4 to 1024 μg/mL using agar dilution method; among them 19 (41.30%) isolates were HLGR. Two studies conducted in India found 42.7% and 41.5% of HLGR by agar dilution method in enterococcal urine samples which almost correlate with the present study (Mittal *et al.,* 2016; Padmasini *et al.,* 2014). Another study conducted in Iran reported around 60.45% HLGR strains in their region which is higher than present study (Hasani *et al.,* 2012). They suggested co-transfer of these resistance genes along with VRE for the higher percentage of HLGR in their study.

In this study, 17 (36.97%) HLGRE detected by disc diffusion method also showed high level resistance to gentamicin (MIC >500 μg/mL) by agar dilution method. But, out of 29 gentamicin sensitive isolates by disc diffusion method, 2 more isolates showed high level resistance to gentamicin by MIC method. For more confirmation, repeation of disc diffusion test of these 2 isolates was done but again they showed sensitive to 120μg gentamicin disc. Agar dilution method detected total of 19 (41.3%) HLGRE in the present study. So, the result obtained for HLGR strains using the disc diffusion method (36.97%) and agar dilution method (41.3%) had minor discrepancy, which indicated the possibility of false susceptibility being detected by disc diffusion test. A study in Bangladesh reported that about 12% of the gentamicin sensitive isolates detected by 120μg gentamicin disc diffusion test showed high level resistance to gentamicin by MIC method (Tamanna *et al.,* 2013). Although the disc diffusion test is easy to perform but for more reliable results MIC of gentamicin should be done periodically.

Enzymatic modification is the most common type of aminoglycoside resistance. Four genes such as aac(6′)-Ie-aph(2″)-Ia, aph(2″)-Ib, aph(2″)-Ic and aph(2″)-Id are responsible for high level gentamicin resistance in *Enterococci* by coding aminoglycoside modifying enzyme (AME) (Vakulenko *et al.,* 2003). In this study all 46 enterococcal isolates were analyzed for the presence of aac(6′)-Ie-aph(2″)-Ia, aph(2″)-Ib and aph(2″)-Ic gene. Nineteen (41.3%) out of 46 *Enterococci* isolates carried aac(6′)-Ie-aph(2″)-Ia gene in this study which is similar to studies conducted in India that reported 38.2% and 48% of aac(6′)-Ie-aph(2″)-Ia gene respectively (Padmasini *et al.,* 2014; Shete *et al.,* 2017). The frequency of HLGR gene was variable in different countries. It was found to be lower in a study conducted in Turkey (24%) and was found higher in studies done in Thailand (56%) and China (64.4%) (Dadfarma *et al.,* 2013). Because of differences in climate and bacterial prevalence, the frequencies of HLGR gene in *Enterococcus* differ between regions.

E. faecalis, the predominant isolates in this study was found to carry aac(6′)-Ie-aph(2″)-Ia gene in 45.46% isolates and *E. faecium* was found to carry aac(6′)-Ie-aph(2″)-Ia gene in 36.36% of isolates. Studies in Iran and India also reported that majority of the enterococcal isolates which carried aac(6′)-Ie-aph(2″)-Ia gene were *E. faecalis* and was 61.3% and 39.5% respectively (Dadfarma *et al.,* 2013; Padmasini *et al.,* 2014). In contrast, one study in India reported 77% *E. faecium* and 44% *E. faecalis* was HLGR. This may be due to the presence of high frequency of *E. faecium* in their study (Shete *et al.,* 2017).

Though another study conducted in Iran reported the presence of aac(6′)-Ie-aph(2″)-Ia,

aph(2″)-Ib and aph(2″)-Ic gene in HLGR *Enterococcus* species, the present study found that HLGR phenotypes are correlated with the expression of only aac(6′)-Ie-aph(2″)-Ia gene. Studies conducted in different settings also found only aac(6′)-Ie-aph(2″)-Ia gene in their study (Dadfarma *et al.*, 2013; Hasani *et al.*, 2012; Padmasini *et al.*, 2014; Shete *et al.*, 2017). This observation emphasizes the restricted gene distribution and transfer of resistant genes within a geographical region. Hence, surveillance studies should be conducted among *Enterococcus* isolates from different sources in any given geographical area to document the AME gene profile. This study might be the first to report high level gentamicin resistance gene analysis among the *Enterococcus* species in Bangladesh.

CONCLUSION

High frequency of HLGRE (41.3%) may be regarded as a warning to the community and only the bifunctional aminoglycoside modifying enzyme (AME) encoding gene aac(6′)-Ie-aph(2″)-Ia was found responsible for such resistance in this study. In identifying HLGRE, disc diffusion method may not be accurate and sensitive enough as like as MIC method. So it should be adopted routinely in microbiology laboratories to differentiate between low level gentamicin resistance and high level gentamicin resistance by both disc diffusion method and MIC method. Rational use of antibiotics in health care settings for *Enterococci* as well as high-dose aminoglycoside disc tests along with agar dilution method in the laboratory and if possible screening for the AME gene with molecular techniques may help to efficiently select an appropriate protocol for antibiotic therapy and confine dangerous infections.

REFERENCES

Abriouel, H., Omar, N. B., Molinos, A. C., López, R. L., Grande, M. J., Martínez-Viedma, P. and Galvez, A. 2008. Comparative analysis of genetic diversity and incidence of virulence factors and antibiotic resistance among enterococcal populations from raw fruit and vegetable foods, water and soil, and clinical samples. *International Journal of Food Microbiology.* 123(1-2) : 38-49.

Akhter, J., Ahmed, S. and Anwar, S. 2014. Antimicrobial Susceptibility Patterns of Enterococcus species Isolated from urinary tract infections. *Bangladesh Journal of Medical Microbiology. 8*(1) : 16-20.

Ali, L., Goraya, M., Arafat, Y., Ajmal, M., Chen, J.-L. and Yu, D. 2017. Molecular mechanism of quorum-sensing in Enterococcus faecalis: its role in virulence and therapeutic approaches. *International Journal of Molecular Sciences.* 18(5) : 960.

Aslangul, E., Ruimy, R., Chau, F., Garry, L., Andremont, A. and Fantin, B. 2005. Relationship between the level of acquired resistance to gentamicin and synergism with amoxicillin in Enterococcus faecalis. *Antimicrobial Agents and Chemotherapy.* 49(10) : 4144-4148.

Bharti, A. K., Farooq, U., Singh, S., Kaur, N., Ahmed, R., and Singh, K. 2016. Incidence of Enterococcal Urinary Tract Infection and it's Sensitivity Pattern among Patients Attending Teerthanker Mahaveer Medical College and Research Centre, Moradabad, India. *International Journal of Scientific Study.* 3(12) : 112-116.

Boby, F., Ahmed, S., Paul, S., Nasreen, S., Haque, N. and Roy, S. 2016. Antimicrobial susceptibility pattern of Enterococci isolated from clinical specimens at Mymensingh Medical College Hospital, Mymensingh. *Bangladesh. Journal of Bacteriology & Mycology Open Access, 3*.

Chakraborty, A., Pal, N. K., Sarkar, S. and Gupta, M. S. 2015. Antibiotic resistance pattern of Enterococci isolates from nosocomial infections in a tertiary care hospital in Eastern India. *Journal of Natural Science, Biology, and Medicine.* 6(2) : 394.

Chow, J. W., Zervos, M. J., Lerner, S. A., Thal, L. A., Donabedian, S. M., Jaworski, D. D. and Clewell, D. B. 1997. A novel gentamicin resistance gene in Enterococcus. *Antimicrobial Agents and Chemotherapy.* 41(3) : 511-514.

CLSI, C. 2016. Performance standards for antimicrobial susceptibility testing. *Clinical Lab Standards Institute*.

Dadfarma, N., Fooladi, A. A. I., Oskoui, M. and Hosseini, H.M. 2013. High level of gentamicin resistance (HLGR) among enterococcus strains isolated from clinical specimens. *Journal of Infection and Public Health.* 6(3) : 202-208.

Fernandes, S. C. and Dhanashree, B. 2013. Drug resistance & virulence determinants in clinical isolates of Enterococcus species. *The Indian Journal of Medical Research.* 137(5) : 981.

Hasani, A., Sharifi, Y., Ghotaslou, R., Naghili, B., Aghazadeh, M., Milani, M. and Bazmani, A. 2012. Molecular screening of virulence genes in high-level gentamicin-resistant Enterococcus faecalis and Enterococcus faecium isolated from clinical specimens in Northwest Iran. *Indian Journal of Medical Microbiology.* 30(2) : 175.

Higuita, N. I. A. and Huycke, M. M. 2014. Enterococcal disease, epidemiology, and implications for treatment *Enterococci: From commensals to leading causes of drug resistant infection [Internet]*: Massachusetts Eye and Ear Infirmary.

Islam, T. A. B. and Shamsuzzaman, S. 2015. Isolation and species identification of enterococci from clinical

specimen with their antimicrobial susceptibility pattern in a tertiary care hospital. *Bangladesh. J Coast Life Med.* 3(10) : 787-790.

Iweriebor, B. C., Obi, L. C. and Okoh, A. I. 2015. Virulence and antimicrobial resistance factors of Enterococcus spp. isolated from fecal samples from piggery farms in Eastern Cape, South Africa. *BMC Microbiology.* 15(1) : 136.

Jett, B. and Huycke, M. 1994. Gilmore MS. *Virulence of entero-cocci. Clin Microbiol Rev.* 7 : 462-478.

Levine, D. P. 2006. Vancomycin: a history. *Clin Infect Dis, 42 Suppl 1,* S5-12. doi: 10.1086/491709

Mittal, S., Singla, P., Deep, A., Bala, K., Sikka, R., Garg, M. and Chaudhary, U. 2016. Vancomycin and high level aminoglycoside resistance in Enterococcus spp. in a tertiary health care centre: a therapeutic concern. *Journal of Pathogens,* 2016.

Nasaj, M., Mousavi, S. M., Hosseini, S. M. and Arabestani, M.R. 2016. Prevalence of virulence factors and vancomycin-resistant genes among Enterococcus faecalis and E. faecium isolated from clinical specimens. *Iranian Journal of Public Health.* 45(6) : 806.

Padmasini, E., Padmaraj, R. and Ramesh, S.S. 2014. High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of Enterococcus species in Chennai, India. *The Scientific World Journal, 2014*.

Parte, A. C. 2013. LPSN—list of prokaryotic names with standing in nomenclature. *Nucleic Acids Research. 42*(D1) : D613-D616.

Purohit, G., Gaind, R., Dawar, R., Verma, P. K., Aggarwal, K. C., Sardana, R. and Deb, M. 2017. Characterization of Vancomycin Resistant Enterococci in Hospitalized Patients and Role of Gut Colonization. *J Clin Diagn Res.* 11(9) : Dc01-dc05. doi: 10.7860/jcdr/2017/25988.10548

Shete, V., Grover, N. and Kumar, M. 2017. Analysis of aminoglycoside modifying enzyme genes responsible for high-level aminoglycoside resistance among enterococcal isolates. *Journal of Pathogens.* 2017.

Suchi, S. E., Shamsuzzaman, S., Uddin, B. M. M. and Yusuf, M. A. 2017. Detection of virulence factors and antimicrobial resistance in enterococci isolated from urinary tract infection. *Bangladesh Journal of Infectious Diseases.* 4(2) : 30-34.

Tamanna, S., Barai, L., Ahmed, A. and Haq, J. A. 2013. High level gentamicin resistance and susceptibility to Vancomycin in enterococci in a tertiary care hospital of Dhaka City. *Ibrahim Medical College Journal.* 7(2) : 28-31.

Vakulenko, S. B., Donabedian, S. M., Voskresenskiy, A. M., Zervos, M. J., Lerner, S. A. and Chow, J. W. 2003. Multiplex PCR for detection of aminoglycoside resistance genes in enterococci. *Antimicrobial Agents and Chemotherapy.* 47(4) : 1423-1426.

Weiner, L. M., Webb, A. K., Limbago, B., Dudeck, M. A., Patel, J., Kallen, A. J. and Sievert, D. M. 2016. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. *Infection Control & Hospital Epidemiology.* 37(11) : 1288-1301.