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High Level Aminoglycoside Resistance (HLAR) in *Enterococcus* Species Isolated from Various Clinical Specimens

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A B S T R A C T

Enterococci are the most aerobic and facultative anaerobe, gram positive cocci. They constitute a part of normal intestinal flora. However they also occupy other sites such as oral cavity, skin etc. *Enterococci*, which earlier considered as low grade pathogens, has now emerged as one of the most important nosocomial pathogens worldwide and are associated with high mortality. Study was carried out in the Department of Microbiology, National Institute of Medical Sciences & Research Jaipur (India) between December 2016 and December 2017. The isolated Enetrococci are then tested for routine antibiotics sensitivity by disc diffusion method including High level Gentamicin and Streptomycin disc. Further confirmation of High level Gentamicin and Streptomycin resistance by E-test (MIC). We consider MIC test as gold slandered method. A total 110 *Enterococcus* isolates are obtained from various clinical specimens such as Blood, Urine, Pus. Among 110 *Enterococcus* species, 78 species are Enterococcus *faecalis* and 32 species are *Enterococcus faecium.* Out of 110 *Enterococcus* isolates 36 (32.7%) are resistant to High level Gentamicin and 24 (21.8%) are resistant to High level streptomycin by disc diffusion method and by E-test High level Gentamicin was 41 (37.2%) and High level streptomycin was 27 (24.5%) found. Prevalence of HLAR is high and could be a serious problem in hospital setup, screening for high level aminoglycoside resistance must be included in routine antibiotic susceptibility reporting for *Enterococcal* isolates.

Introduction

Enterococci are gram positive cocci, known only as intestinal commensals with little significance have evolved as deadly pathogens over last two decades. *Enterococcal* infections is always difficult to treat due to inherent resistance to many commonly used antibiotics like aminoglycosides, cephalosporins etc (Marothi *et al.*, 2014). *Enterococci*, initially considered as normal commensals of intestinal tract, have recently emerged as a medically important pathogen. Incidence of eneterococcal infections is significantly high in patient suffering from urinary tract infection, blood stream infection, and surgical site infections. Nosocomial eneterococcal infection is also common in organ transplant recipient's cancer patients and debilitated patients receiving broad spectrum antibiotics

(Sadar *et al.,* 1994). *Enterococci* have traditionally been treated with cell wall active agents in combination with an aminoglycoside. However resistance to low and high level aminoglycosides has been reported. Resistance to beta lactam antibiotics and vancomycin by some strains together with High level aminoglycoside resistance (Patterson *et al.,* 1998).

The present study was aimed to detecting the antimicrobial resistance pattern among enterococcus isolates obtained from clinical specimen in a tertiary care hospital in Jaipur with special emphasis on high level aminoglycosides and prevalence of enterococcus spp. in our hospital.

Materials and Methods

The study was carried out of one year December 2016 to December 2017 at the Department of microbiology, National Institute of Medical Sciences & Research, NIMS University Jaipur (Rajasthan). Various clinical specimens Urine, Pus, Blood, Body fluids were taken from patients attending NIMS hospital. Specimen were collected in a sterile, proper labelled container with aseptic precautions and processed as per standard microbiological procedures.

Organism identification by Henry *et al.,* (1998)

All samples were screened for the pus cells and organism. Specimen was culture on Blood agar and MacConkey and incubated for 37^{o}C for 24 hours. Growth was then processed for gram staining and catalase test. Gram positive cocci arranged in pairs showing catalase negative were considered as *streptococcus species*. Speciation of *Enterococcus* species done by grams staining, colony morphology, culture characteristics of the colonies and biochemical tests (Bile esculin hydrolysis test, Pyrrolidonyl Arylamidase, resistance to bacitracin and optochin, growth at 6.5% NaCl, growth at 37^{o}C and 45^{o}C, Hippurate hydrolysis test, sugar fermentation test). Grams stain smear shows gram positive cocci 1-1.5 x 0.5µm, oval shaped arranged in pairs and short chains.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried on Brain Heart Infusion (BHI) agar by modified Kirby-Bauer disc diffusion method (CLSI 28thed 2018). High level aminoglycoside resistance (HLAR) method was detected by following methods.

Disc diffusion method

Colonies of *Enetrococcus* was inoculated into broth and incubated at 37^{o}C for 4 hours. Growth was indicated by the appearance of turbidity in the medium. Turbidity of the medium was compared with 0.5 McFarland tube. Lawn culture was performed on BHI agar plate with the help of sterilized swab, Gentamicin and Streptomycin drugs was inoculated with a sterile - forceps and then incubated.

Minimum Inhibitory Concentration (MIC) method

Minimum Inhibitory Concentration of Gentamicin and Streptomycin was determined by E-test. The strains which were resistant by disc diffusion method were checked by MIC. The colonies were inoculated in BHI broth. Growth was indicated by the appearance of turbidity which was compared with 0.5 McFarland tube. Lawn culture was performed on BHI agar plate with sterile swab and E-strip was inoculated on BHI plate and incubated. All the result was interpreted according to CLSI guidelines 2018.

Results and Discussion

In our study a total of 110 *Enetrococcus* were isolated in a period of one year 2016 to December 2017. Among 110 *Enterococcus* species, 78 species are Enterococcus *faecalis* and 32 species are *Enterococcus faecium.*

Highest prevalence of *Enterococcus* was seen in females 74 (67.2%) followed by males 36 (32.7%). The maximum percentage of isolation was seen among the age group 40-60 years.

In our study maximum *Enterococcus* isolate from urine specimen 69 (62.7%), followed by Pus 21 (19%), Blood 12 (10.9%), Others 08 (7.2%).

Maximum isolation of Enterococcus isolates was isolated from urine specimen. It indicates that urinary tract infections are the most common infections caused by *Enterococci* in this set up (Table 1–2).

All the enterococcal isolates were subjected to test for High level Aminoglycoside resistance by two methods name Gentamicin and Streptomycin high concentration disc diffusion method and MIC method. We Consider E test is gold slandered method. The results of Gentamicin and Streptomycin by high concentration discs 120 µg and 300µg has shown in table 3 and 4.

Table.1 Showing sample distribution

Specimen	No. Of Isolates	Percentage (%)
Urine	69	62.7%
Pus	21	19%
Blood	12	10.9%
Others (Body fulids, sputum)	08	7.2%
Total	110	100%

Table.2 Showing *Enterococcus* species distribution

Entero.spp	No. Of Isolates	Percentage (%)
Enterococcus *faecalis*	78	70.9%
Enterococcus faecium	32	29.0%
Total	110	100%

Table.3 Showing high level aminoglycoside resistance in *Enterococcus* species by disc diffusion method

Antibiotics	Resistance (n-110)	Percentage
High level Gentamicin (120µg)	34	30.9%
High level Streptomycin (300µg)	17	15.4%

Table.4 Showing high level aminoglycoside resistance in *Enterococcus* species by MIC method

Antibiotics	Resistance (n-110)	Percentage
High level Gentamicin (0.064-1024 mcg/ml)	27	24.5%
High level Streptomycin (0.016-256 mcg/ml)	12	10.90

Table.5 Showing antibiotic sensitivity of *Enterococcus* species to other antibiotics

Antibiotic Used	Sensitivity	Resistant
Ampicillin (10µg)	39 (53.4%)	71 (64.5%)
Ciprofloxacin(5µg)	62 (56.3%)	48 (43.6%)
Norfloxacin*	18 (26.0%)	51 (73.9%)
Nitrofurantoin* (30µg)	28 (40.5%)	41 (59.4%)
Vancomycin (30µg)	110 (100%)	00
Linezolid (30µg)	110 (100%)	00

*Used for urine isolates only (69 samples)

In the present study maximum number of *Enterococci* were isolated from urine (62.7%) followed by Pus (19%). This is slightly lower than Ruoff *et al.,* (1990) who isolate maximum number of *Enterococci* from urine (68.2%). In another study of Talebi *et al.,* (2007) also reported maximum number of *Enterococci* from urine (85%) followed by Pus (15.5%). Karmarkar *et al.,* (2004) isolated 47.14% *Enterococci* from urine sample and described that urinary tract as commonest site of isolation of *Enterococci*. The maximal enterococcal urine isolation could be due to structural abnormalities in the urinary tract, indwelling catheter of following any instrumentation.

Antibiotic resistance among *Enterococci* is global problem. Antibiotic resistance in *Enterococci* is intrinsic or acquired. In our study the highest resistant is seen against Ampicillin 64.5%, another study of Salem Bekhit *et al.,* (2012) also report high resistance of ampicillin 70.4 % in *E. faecium*. In our study ciprofloxacin resistant is 43.6%, another study of Sarika Jain *et al.,* (2011) also reported high resistant of ciprofloxacin in enterococci 75%.

According to our study the highest sensitivity pattern is shown in vancomycin and linezolid for all samples and for urinary isolates nitrofurantoin shows 40.5% sensitive and norfloxacin shows 26% sensitive.

In our study High Level Gentamicin resistance by E test is 25.4% and high level Streptomicin resistance is 15.4%. In another study of Sanal C. Fernandes *et al.,* (2013) report 53 % resistance of High Level Gentamicin and 34.1 resistance in high level Streptomicin resistance. The presence of HLGR is predictive of the loss of the synergy between Gentamicin and a cell wall active agent such as ampicillin or vancomycin Murray *et al.,* (1998) (Table 5).

In conclusion *Enetrococcus* infections are mainly associated with nosocomial infection and increase incidence mainly due to indiscriminate use of broad spectrum of antibiotics. Proper infection control practices need to prevent nosocomial origin of *Enterococci*. This study emphasizes the need to screen for HLAR in patients suffering from eneterococcal infections as a routine screening for to detect HLGR and HLSR as

this will help to limit the spread of resistance and have a surveillance pattern.

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