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Prevalence of High Level Aminoglycoside Resistance of Enterococci in Various Clinical Specimens From A Tertiary Care Hospital of North Delhi.

Microbiology

KEYWORDS : Enterococci, high level aminoglycosides, high level streptomycin resistance, high level gentamicin resistance

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ABSTRACT

Enterococcus is the opportunistic pathogens, exhibits intrinsic resistance to a number of antimicrobial agents in addition to acquired multidrug resistance. The present study was conducted to determine enterococci exhibiting high-level aminoglycoside resistance in a tertiary care hospital. Enterococci were isolated from various clinical specimens and identified to the species level. High-level resistance (HLR) to gentamicin and streptomycin was determined by the clinical ATB ENTROC 5 strip, semi- automated system mini API method. Beta-lactamase production was detected using chromogenic method. Out of 60 enterococci isolates, 73.3% were found to have HLR to both streptomycin and gentamicin in which HLR to streptomycin was most common (95%). None of them produced beta-lactamase. Therefore, there is an urgent need for more minimal and restricted use of antimicrobials in to minimize the spread of such strains.

Introduction:

Enterococci have become increasingly important as nosocomial pathogen due to their ability to cause serious infections and also because of their increasing resistance to many antimicrobial agents. Serious infections caused by enterococci are often very difficult to treat and the mortality is high [1]. Genus enterococcus is intrinsically resistant to many antimicrobial agents, including low concentrations of aminoglycosides. Enterococcus is rapidly acquiring resistance to many antibiotics commonly used in hospitals. The rapid dissemination of high level aminoglycoside resistance in enterococci is the emerging problem (2). The combination of aminolgycosides with cell wall active agents showing the synergistic effect disappears in strains that show high level resiatance (HLR) to aminoglycosides (2). High level gentamicin resistance (HLGR) in E.faecalis was first reported in 1979 [1]. The aminoglycoside streptomycin was used clinically until 1970 when more than 50% of enterococci were found to be resistant to high level of this drug [1]. Considering these, the present study was designed with the aim of isolating and characterizing Enterococcus species from various clinical specimens and studying the prevalence of high level aminoglycoside resistance (HLAR) and β-lactamase production in tertiary care hospital.

Material and method:

A total of 60 isolates were recovered from various clinical specimens from a tertiary care Jaipur Golden hospital, Delhi, India over the period from September 2012 to April 2013. The specimen analyzed were blood, urine, pus swabs, ascitic fluid, catheter tip, drained pus, plueral fluid, and semen collected from in-patients and out-patients at hospital. The specimens were inoculated on blood and McConkey agar plates which were incubated at 37˚C for 24-48 hours. Enterococci were identified on the basis of their growth characteristics on blood agar, presence of gram positive cocci in pairs and short chains on gram staining, the catalase negative, ability to grow in 6.5% NaCl broth and and at pH 9.6, bile esculin hydrolysis and further identified to the species level by using Rapid ID 32 Strep, mini-API (bioMetrieux, Marcy l'Etoile, France). All strains were tested for susceptibility to penicillin (8µg/ml), ampicillin (8 µg/ml), erythromycin (.5-4 µg/ml), tetracycline (4-8 µg/ml), chloramphenicol (8-16 µg/ml), ciprofloxacin (1-2 µg/ml), levofloxacin (2-4 µg/ml), vancomycin (4-16 µg/ml), teicoplanin (8-16 µg/ml), nitrofurantoin (32-64 µg/ml), quinupristin- delfopristin (1-2 µg/ml), gentamicin (500 µg/ml), streptomycin (1000 µg/ml) antimicrobial agents by the clinical ATB ENTROC 5 strip, semi- automated system mini API. This strip was designed following NCCLS (CLSI) 2000 (1) committee recommendations. β-lactamase production was detected by iodometric method. Statistical analysis was carried out using the chi-squire test with a $p < 0.05$ set as a significance level. The study was approved by the Institutional ethical committee and the protocol was described to every volunteer and a written consent was obtained from volunteers of this study and the data has been maintained properly in the hospital record.

Result:

The 60 samples were tested, comprising of 32 urinary and 28 were non- urinary specimensurinar. Non- urinary samples included blood (16), pus (7), ascetic fluid (1), fluid (drained pus) (1), pleural fluid (1), semen (1) and catheter tip (1). Isolates obtained were E. faecalis (46.6%), E. faecium (35%), E. gallinarum (10%), E. avium (1.6%), E. hirae (1.6%), E. caecorum (1.6%), E. amnioenus (1.6%) and E. casseliflavus (1.6%) were obtained (Table 1). Urine showed the majority of isolates 32 (53.3%). Out of which 59.3% E. faecalis (19), 31.2% E. faecium (10), 9.3% E. gallinarum (3). Urinary isolates showed resistance to penicillin (62.5%), ampicillin (50%), ciprofloxacin (84.3%), vancomycin (9.4), teicoplanin (12.5%), nitrofurantoin (37.5%) high level gentamicin (75%) high level streptomycin (93.7%) while non-urinary isolates were resistant to penicillin (75%), ampicillin (57.1%), ciprofloxacin (71.4%), vancomicin (14.3%), teicoplanin (21.4%), nitrofurantoin (85.7%) high level gentamicin (75%) high level streptomycin (96.4%) (Table2).

For detection of high level aminoglycoside resistance in Enterococci high level gentamicin (500µg/ml) and high level streptomycin (1000µg/ml) were used. Out of 60 isolates 75% strains were resistant to high level gentamicin. Percentage distribution of resistant isolates were 40% E. faecalis, 44.4% E. faecium, 11.1% E. gallinarum, 2.2% E. avium and 2.2% E. caecorum where as out of 60 isolates 95 % were resistant to high level streptomycin in which E. faecalis (45.6%), E. faecium (36.8%), E. gallinarum (10.5%), E. avium (1.8%), E. caecorum (1.8%), E. hirae (1.8%) and E. casseliflavus (1.8%) (Table 3). Resistance to both high level streptomycin and high level gentamicin was seen in 73.3% isolates from which E. faecalis (36.6%), E. faecium (44.5%), E. gallinarum (11.4%) while E. avium (2.3%) and E.

caecorum (2.3%). (Table 3). A statistically significant difference was observed significant (P<0.002) among HLSR and HLGR isolated from various clinical specimens by the application of Chi squire test.

Table 1
PERCENTAGE DISTRIBUTION OF VARIOUS ENTEROCOCCUS SPECIES (n=60)

Species	No. of strains	% of strains
E. faecalis	28	46.6
E. faecium	21	35
E. gallinarum	6	10
E. avium	1	1.6
E. hirae	1	1.6
E. caecorum	1	1.6
E. amnioenus	1	1.6
E. casseliflavus	1	1.6
Total	60	100

Table 2
Antimicrobial resistance pattern in enterococcal isolates No. (%) of resistant strain

Antibiotics	Urinary isolates	Non-urinary isolates
Penicillin	62.5	75
Ampicillin	50	57.1
Ciprofloxacin	84.3	71.4
Vancomycin	9.4	14.3
Teicoplanin	12.5	21.4
Nitrofurantoin	37.5	85.7
Gentamicin	75	75
Streptomycin	93.7	96.4

Table 3
HLAR RESISTANCE PATTERN IN ENTEROCOCCUS SPECIES

NO. of isolates	HLSR (%)	HLGR (%)	HLAR (%)
E. faecalis (28)	26 (45.6)	18 (40)	17 (36.6)
E. faecium (21)	21 (36.8)	20 (44.4)	20 (45.5)
E. gallinarum (6)	6 (10.5)	5 (11.1)	5 (11.4)
E. avium (1)	1 (1.8)	1 (2.2)	1 (2.3)
E. hirae (1)	1 (1.8)	-	-
E. caecorum (1)	1 (1.8)	1 (2.2)	1 (2.3)
E. amnioenus (1)	-	-	-
E. casseliflavus (1)	1 (1.8)	-	-
Total (60)	57 (95%)	45 (75%)	44 (73.3%)

P = 0.002

Discussion:
Enterococci are commensals of the gastrointestinal tract of human beings but now they have become important nosocomial pathogens over the past 2 decades due to the ability to adhere to indwelling medical devices, and ability to adapt themselves into adverse environmental conditions (3).They are showing variation in resistance to anti microbial agents by particular enterococcal species, so correct species identification is needed (4). Antibiotic resistance in enterococci may be intrinsic or acquired. Intrinsic traits expressed by enterococci include resistance to semisynthetic penicillinase resistant penicillins, cephalosporins, low level of aminoglycosides and low level of clindamycin, whereas acquired resistance includes resistance to chloramphenicol, high level of clindamycin, tetracycline, high level of aminoglycosides, penicillin, β uroquinolones, erythromycin and vancomycin (1). In our study E. faecalis was the most common isolate (46.6%) followed by E. faecium (35%). According to Gary Cotter et al, E. faecalis is the predominant species isolated (89.61%) followed by E. faecium (10.4%) (5); according to Simonson et al., E. faecalis were 82.5% and E. faecium were 16.1% in their study (6), according to Mohammad Rahbar et al, 79.8% isolates were E. faecalis and 20.2% were E. faecium (7). In contrary Baragundi Mahesh et al., (8) found E. faecium as the predominant species (47.50%) in their study. In our study, Majority of the isolates were from urine, similar to other studies (9,10,11). This study showed E. faecium (62.5%) to be more resistant to ampicillin than E. faecalis (12.5%). This correlates with the study by Jyotsna Agarwal et al., E. faecium were 50% and E. faecalis were 7.14% resistant (12) and Steven Gordon et al., (13), Suresh at al (11).

Penicillin resistance also was more in E.faecium than E.faecalis. In contrast L.A Sechi et al., reported more E. faecalis isolates resistant to penicillin (14). Due to release of various amnoglycoside modifying enzymes, enterococci show HLAR. In this study high level gentamicin resistance was observed in both E. faecalis and E. faecium, consistent with other studies (15, 16). The E. faecalis were more resistant for streptomycin (45.6%) than gentamicin (40%), while according to Keddy E. faecalis (26.5%) were more resistant to gentamicin than E. faecium (20%) (16). Udo et al also found E. faecalis to be more resistant than E. faecium to both gentamicin and streptomycin (17). In the present study, 73.3% of the enterococci show HLAR and combined HLGR and HLSR was higher in E. faecium (45.5%) than E. faecalis (36.6%), according to Mendiratta et al 46% of the enterococci showed HLAR and combined HLGR and HLSR was significantly (p<0.05) higher in E. faecium

(59.1%) than E. faecalis (7.8%) (18). In our study, high HLSR were found in E. faecalis (45.6%) and high HLGR were found in E. faecium (44.4%) while according to Gordon at el high HLSR were found in E. faecium and HLGR were found in E. faecalis (13). Low affinity of the penicillin binding proteins is responsible for resistance to penicillin and ampicillin which is usually intrinsic (19) and it results in loss of synergistic effect between β lactams and aminoglycosides leading to treatment failures. Schouten et al (20) reported that as the prevalence of HLGR increases, β lactamase production in Enterococci may also increase. In our study, none of them produced β lactamase, as also reported by Jessudason et al., udo et al (21), (17). Increasing of resistance due to β lactamase production to the intrinsic resistance already seen in enterococci is becoming a major problem (22). This study revealed the prevalence of multidrug resistant HLAR strains of E. faecalis and E. faecium in this hospital.

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