ORIGINAL ARTICLE

Emergence of Vancomycin Resistant *Enterococci* in Paediatric Patients

ASIF SHAHEEN, NAIMA MEHDI, AIZZA ZAFAR, *MUHAMMAD ZUBAIR, HUMERA JAVED, SABA KABEER, SABA ABBAS, HASAN EJAZ*

ABSTRACT

Aim: To determine the frequency and antimicrobial susceptibility profile of vancomycin resistant *Enterococci* in paediatric patients.

Methods: This cross sectional study was conducted at The Children's Hospital and Institute of Child Health, Lahore, Pakistan from June 2012 to December 2012. A total number of 2,980 urine samples were collected from suspected cases of urinary tract infections and processed for microbiological analysis. The organisms were identified by routine microbiological techniques and biochemical tests. Antimicrobial susceptibility profile was determined by Kirby-Bauer disc diffusion method.

Results: From 2,980 urine samples, 830(27.8%) samples were positive for bacterial growth. *Enterococcus* species were isolated in 93(11.2%) cases. Out of 93 *Enterococci,* 15(16.2%) were vancomycin resistant *Enterococci* (VRE). All of the VRE were susceptible to linezolid. The sensitivity to nitrofurantoin and amikacin were 66.7% and 6.7%, respectively. All of these isolates were resistant to amoxicillin-clavulanic acid, ampicillin, penicillin, ciprofloxacin, norfloxacin and pipemidic acid. The highest cases of VRE were isolated from medical ward 7(46.6%) followed by urology ward 3(20%). There were 4(26.6%) VRE cases isolated from male patients and 11(73.4%) from female patients.

Conclusion: Isolation of VRE among the paediatric patients is an alarming situation rendering fewer antibiotics as a choice of treatment for paediatric population.

Keywords: Vancomycin resistant *Enterococci,* urinary tract infections, paediatric patients.

INTRODUCTION

Vancomycin resistant *Enterococci* (VRE) are bacterial strains of the genus *Enterococcus* which are resistant to antibiotic vancomycin[1]. They exist as normal flora of human and animals but may be responsible for serious infections.[2] There are 19 *Enterococcus* species, 2 of them are particularly pathogenic to man: *Enterococcus faecalis* which causes 80-90% infections while *Enterococcus faecium* causes 5-15%[3]. *Enterococci* are reported as the third and the second most frequent agents recovered from community and nosocomial acquired urinary tract infections (UTI) respectively[4]. They also cause intra-abdominal and pelvic sepsis, surgical wound infections, bacteremia, endocarditis and meningitis[5].

Enterococci show intrinsic resistance to cephalosporins, lincosamides, many β-lactams and low levels of aminoglycosides[6]. *Enterococci* acquire resistance to vancomycin by a change in peptide component of peptidoglycan from d-alanyl-d-alanine, which is normal binding site of vancomycin, to d-alanyl-d-lactate, to which drug has reduced ability to bind[7].

Department of Microbiology, The Children's Hospital and Institute of Child Health, Lahore, Pakistan
Correspondence to Dr. Hasan Ejaz, E-mail: hasanmicro@gmail.com

Eight types of acquired vancomycin resistant genes have been reported in *Enterococci* (vanA, vanB, vanC, vanD, VanE, vanG, vanL and vanM)[8]. The mechanism of resistance has been best characterized for the vanA cluster of seven genes. These genes are translated into enzymes, which make cell wall precursors ending in d-alanyl-d-lactate[9]. VanA resistant phenotype is most commonly encountered and confers high level resistance to vancomycin and teicoplanin[10]. VanB resistance in *Enterococci* is mediated by an abnormal ligase which favours the production of the pentadepsipeptide terminating in d-alanyl-d-lactate. It is only induced by vancomycin and not by teicoplanin. VanC produce low level resistance to vancomycin through the production of a pentapeptide terminating in d-alanyl–d-serine. VanD cause moderate resistance to vancomycin and teicoplanin due to constitutive production of peptidoglycan precursors ending in d-alanyl-d-lactate and the vanE phenotype corresponds to low level resistance to vancomycin and susceptibility to teicoplanin due to synthesis of peptidoglycan precursors terminating in d-alanyl–d-serine[11].

Risk factors for VRE infections include heavy use of antimicrobial drugs especially vancomycin, third generation cephalosporins, aztreonam, prolonged hospital stay, older age, proximity to case,

neutropenia, severity of illness, haematological malignancies, patient care by a health care worker with VRE colonization and immunosuppression.[12] The objective of the present study was to determine the frequency and antimicrobial susceptibility profile of VRE in paediatric patients.

METHODOLOGY

This cross sectional study was conducted in Microbiology Department of The Children's Hospital and Institute of Child Health, Lahore, Pakistan, over a time period from June 2012 to December 2012. By using convenient sampling technique, 2,980 urine samples were collected in sterile containers or sterile paediatric urine collection bags. The urine samples were collected from suspected cases of urinary tract infections (UTI's) between the ages of 1 day to 16 years. Urine samples were inoculated within one hour of their collection on CLED medium with the help of 2µl calibrated wire loop. After 24 hours incubation at 37^oC cultural characteristics and colony count of *Enterococcus* was performed. Bacterial growth with colony count of more than 10^5 was processed for the confirmation of *Enterococcus* by Gram's staining, catalase test, litmus milk reduction test, aesculine hydrolysis test and Lancefield grouping.

Isolated organisms were tested against various antibiotics *in vitro* by Kirby-Bauer disc diffusion method. Bacterial suspension was prepared and compared with McFarland 0.5 turbidity standard and a Blood agar plate was streaked with swab evenly in three directions by rotating the plate approximately 60^o to ensure even distribution. VRE screening was performed by placing 30µg disc of vancomycin. Discs of penicillin, ampicillin, amoxicillin-clavulanic acid, amikacin, ciprofloxacin, norfloxacin, pipemidic acid, nitrofurantoin and linezolid were also tested against *Enterococcus.* Interpretation of zone sizes of each isolate with each antimicrobial disc was reported as sensitive, resistant or intermediate sensitive using interpretation chart of zone sizes according to the CLSI guidelines[13].

RESULTS

A total number of 2,980 urine samples were analyzed. Out of which 830(27.8%) urine samples were positive for bacterial growth. Among them 93(11.2%) were *Enterococcus* species and 737(88.8%) were other than *Enterococcus* species. From a total of 93 positive cultures for *Enterococci* urology ward accounted for highest proportion 33 (35.4%) followed by medical ward 32(34.4%). The proportion of *Enterococci* from other wards were as follows: neurosurgery 4(4.3%) cardiac ward 4(4.3%) developmental 3(3.2%) plastic surgery 2(2.1%) haemotology/oncology 2(2.1%) gastroenterology 2 (2.1%) surgery 1(1.07%) neonatal unit 1(1.07%) and outpatient department 9(9.6%). In this study 38 samples from male patients and 55 from female patients were positive for *Enterococci* thus giving a ratio of 1:1.4. Out of 93 *Enterococci* 15(16.2%) were vancomycin resistant (Table 1) and their distribution among different wards was as follows: medical 7(46.6%), urology 3(20%), neurosurgery 2(13.3%), haemotology/oncology 2(13.3%), and outpatient department 1(6.6%). Vancomycin resistant cases among male patients were 4 (26.6%) while 11s (73.4%) cases were in females. Vancomycin resistant *Enterococci* showed 100% sensitivity to linezolid. The sensitivity to nitrofurantoin and amikacin was 66.7% and 6.7%, respectively. Complete level of resistance was observed with the following antibiotics: amoxicillin-clavulanic acid, ampicillin, penicillin, ciprofloxacin, norfloxacin, & pipemidic acid (Table 1).

Table 1: Antimicrobial susceptibility of *Enterococcus* species (n=93)

Antibiotic	Sensitive	Intermediate Sensitive	Resistant
Amikacin	16(17.2%)	1(1.1%)	76(81.7%)
Amoxicillin-clavulanic acid	37(39.8%)	0(0%)	56(60.2%)
Ampicillin	37(39.8%)	0(0%)	56(60.2%)
Ciprofloxacin	21(22.6%)	1(1.1%)	71(76.3%)
Linezolid	93 (100)	0(0%)	0 (0%)
Nitrofurantoin	67 (72%)	10(10.8%)	16(17.2%)
Norfloxacin	17(18.3%)	2(2.2%)	74(79.6%)
Penicillin	33(35.5%)	0 (0%)	60(64.5%)
Pipemidic acid	2(2.2%)	0 (0%)	91(97.8%)
Vancomycin	78(83.9%)	0 (0%)	15(16.1%)

Enterococci other than VRE were also found highly sensitive to linezolid (100%). The sensitivity to other antimicrobial discs were as follows: nitrofurantoin (72%) amoxicillin-clavulanic acid (39.8%), ampicillin (39.8%), penicillin (35.5%), ciprofloxacin (22.6%), norfloxacin (18.3%), amikacin (17.2%) and pipemidic acid (2.2%) (Table 2).

Table 2: Antimicrobial susceptibility of vancomycin resistant *Enterococci* (n=15)

Antibiotic	Sensitive	Resistant
Amikacin	1 (6.7%)	14 (93.3%)
Amoxicillin-clavulanic acid	0 (0%)	15 (100%)
Ampicillin	0 (0%)	15 (100%)
Ciprofloxacin	0 (0%)	15 (100%)
Linezolid	15 (100%)	0 (0%)
Nitrofurantoin	10 (66.7%)	5 (33.3%)
Norfloxacin	0 (0%)	15 (100%)
Penicillin	0 (0%)	15 (100%)
Pipemidic acid	0 (0%)	15 (100%)

DISCUSSION

Vancomycin resistant *Enterococci* (VRE) are a major problem in various health care settings. Increase of vancomycin resistance possess several challenges, including firstly the sole availability of expensive new antimicrobials for treatment of VRE associated infections, and secondly the possibility that vancomycin resistant genes may be transferred to other Gram positive microorganism[14,15].

According to the present study 830 urine samples were positive for bacterial growth. Among them 93 (11.2%) were *Enterococcus* species. From this 93 *Enterococcus,* 15 (16.2%) were vancomycin resistant *Enterococci.* These results are close to previous studies conducted at University Teaching Hospital Tabriz and Orumieh, Iran, and a study in a Brazilian tertiary hospital in which 20.5% and 15.5% VRE were detected, respectively.[16,17] Another study conducted in Ladoke Akintola University of Technology, Osogbo, Nigeria, reported 12.1% *Enterococcal* infection rate and 42.9 % VRE[18].

Isolation of *Enterococci* was 8.7% and VRE constitute 12% of the strains in a tertiary care center from north India.[19] In another study conducted in Institute de Veille Sanitaire, France, 10.5 % *Enterococcus* were resistant to vancomycin.[20] According to the study performed in Nanjing, China, *Enterococcal* infection rate was 14.6% which is in accordance to current study and vancomycin resistant *Enterococcal* infection rate was 5.3% which contradicts study.[21] Our study also contradicts to a study conducted in Prof. Edgard Santos Teaching Hospital of the Federal University of Bahia, Brazil. In which *Enterococci* rates 6.2% as a causative agent of urinary tract infections[22].

A prospective study from 32 hospitals in Colombia, Ecuador, Peru, and Venezuela from South America declared 6% VRE[23] VRE accounted for 3.9% of the isolate according to the study in Riyadh Hospital Saudi Arabia.[24] This Low prevalence of VRE in contrast to present study may be due to the proper control measures such as judicious use of antibiotics, less use of catheterization and early detection and isolation of VRE positive patients.

In present study Vancomycin resistant *Enterococci* showed (100%) sensitivity to linezolid. The sensitivity to nitrofurantoin and amikacin were 66.7% and 6.7%, respectively. A study conducted by in Rajavithi Hospital, Thailand reported all the isolates of VRE susceptible to linezolid and resistant to ampicillin, ciprofloxacin and norfloxacin.[25] A similar study performed for annual Canadian national surveillance to check antimicrobial susceptibilities of urinary pathogens reported 97% sensitivity to nitrofurantoin and 40% to ciprofloxacin for *Enterococcus* species[26].

In present study from a total of 93 positive culture for *Enterococcus* urology ward accounts for highest proportion 33(35.4%) followed by medical ward 32(34.4%). According to a study performed at Hospital Kuala Lumpur, Malaysia, the patients of *Enterococcal* infections were more prevalent in nephrology ward (39%) and medical wards (23%)[27]. According to a study performed in Rajavithi Hospital, Thailand, vancomycin resistance was found in medical (33.7%) and in surgical wards (15.1%). These results are similar to the results of our study in which medical ward accounts for highest proportion (46.6%) of VRE[26].

In this study 38 (40.8%) samples from male patients and 55(59.1%) from female patients were positive for *Enterococcus* thus giving the ration of 1:1.4. Vancomycin resistance among male patients was 4 (26.6%) while in females 11 (73.33%). A study reported 69.9% isolates of VRE from females and 30.1% from male patients in 28 US and 10 Canada medical centers, which is similar to present study. [28] This high ratio of VRE in female patients may be due to the colonization and proximity of anus to vagina. Isolation of VRE among the paediatric patients is an alarming situation rendering fewer antibiotics as a choice of treatment for paediatric population. Linezolid found to be the only choice of antibiotics among the VRE cases.

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