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 vancomycin1. 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%. Enterococcus are reported as the third and the second most frequent agents recovered from community and nosocomial acquired urinary tract infections (UTI) respectively. They also cause intra-abdominal and pelvic sepsis, surgical wound infections, bacteremia, endocarditis and meningitis.3

Enterococci show intrinsic resistance to cephalosporins, lincosamides, many β-lactams and low levels of aminoglycosides6. 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

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.9 VanB resistance in Enterococci is mediated by an abnormal ligase which favours the production of the pentapeptide 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.10

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. 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 (UTIs) 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°C cultural characteristics and colony count of Enterococcus was performed. Bacterial growth with colony count of more than 10⁵ 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° to ensure even distribution. VRE screening was performed by placing 30μg disc of vancomycin. Discs of penicillin, ampicillin, amoxicillin-clavulanic acid, amikacin, ciprofloxacin, norfloxacin, pipemicid 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.

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%) haematology/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%), haematology/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, & pipemicid acid (Table 1).

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

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

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 pipemicid acid (2.2%) (Table 2).

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

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>1 (6.7%)</td>
<td>14 (93.3%)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>0 (0%)</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0 (0%)</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0 (0%)</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>15 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>10 (66.7%)</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>0 (0%)</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0 (0%)</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Pipemidic acid</td>
<td>0 (0%)</td>
<td>15 (100%)</td>
</tr>
</tbody>
</table>
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 microorganisms.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 % VRE18.

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% VRE23 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 VRE28.

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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