ORIGINAL ARTICLE

Vancomycin and Ampicillin Resistance in Enterococcus: A Six Month Study of Urine Cultures at Sheikh Zayed Hospital, Lahore

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ABSTRACT

Objective : To study the frequency of Vancomycin and Ampicillin resistance enterococci isolated from urine culture specimens alongwith their antimicrobial susceptibility patterns.
Study Design : Retrospective study
Place and duration of Study : Department of Microbiology, Sheikh Zayed Hospital, Lahore from 1 June to 30 November 2013.
Material and Methods : All samples received for urine culture, from which vancomycin- and ampicillin-resistant Enterococci were isolated, were included in the study and an analysis was made of their antimicrobial susceptibility patterns.
Results : Out of the 4852 urine samples received for culture, Enterococci were isolated from 172 cases (3.5%). Out of these 172 samples, 30 (17.4 %) were vancomycin- and ampicillin-resistant. All of these 30 strains were sensitive to Linezolid (100%) while nitrofurantoin was sensitive in 16 strains (53.3 %).
Conclusion : Enterococci are inherently resistant to several antimicrobials and vancomycin and ampicillin resistance greatly limits the treatment options for such strains. An overview of their susceptibility patterns may help in devising treatment strategies for multi-drug resistant infections.

Keywords : *Enterococcus*. Vancomycin. Ampicillin. Antimicrobial susceptibility.

INTRODUCTION

Vancomycin and ampicillin resistance among enterococci has shown an alarming increase in the past several years. Enterococci are intrinsically resistant to cephalosporins, clindamycin, cotrimoxazole and aminoglycosides.[1] Most enterococcal infections are caused by *Enterococcus faecalis*. Rest are caused by *Enterococcus faecium*, a species which is more likely to be resistant to antimicrobials than *Enterococcus faecalis*. Urinary Tract Infection (UTI) is the most common infection caused by enterococci.[2] This study was designed to assess the prevalence of vancomycin and ampicillin-resistant enterococci in urine cultures during a six month period at a tertiary care hospital, alongwith an overview of their antimicrobial susceptibility results.

MATERIALS AND METHODS

All the urine samples received for culture in the Microbiology Laboratory from 1 June 2013 to 30 November 2013 were included in the study. The samples were subcultured on CLED (Cysteine Lactose Electrolyte Deficient) agar and incubated at 37°C for 24 hours. Enterococci were identified by Gram staining, catalase negativity, bile aesculin hydrolysis and serological agglutination with Lancefield Group D antiserum. Antimicrobial susceptibility was perfomed by the Kirby-bauer disk-diffusion method using Clinical Laboratory Standards Institute (2013) breakpoints and American Type Culture Collection (ATCC) *Enterococcus faecalis*-29212 as control strain. Results were analysed to include vancomycin and ampicillin resistant strains in this study, alongwith an overview of their susceptibility patterns to other antimicrobials.

RESULTS

A total of 4852 urine samples were received for culture during the above mentioned period. Enterococci were isolated from 172 samples (3.5%). Out of these 172 samples, 30 (17.4 %) were resistant to vancomycin and ampicillin. All of these 30 strains were sensitive to Linezolid (100%) while nitrofurantoin was sensitive in 16/30 (53.3 %) of these strains. Most of these strains were

received from Nephrology (16/30) while 4 were received from Urology, 3 from Paediatrics, 2 from General Medicine and 1 each from General Surgery, Gynaecology, Orthopaedics, Adult Intensive care and Coronary care units.

DISCUSSION

Enterococci constitute part of the normal flora of the human colon. Although >40 enterococcus species have been identified, nosocomial (hospital acquired) infections are mostly caused by *Enterococcus faecalis* and *E. faecium*.[3] Enterococci are the third most common cause of nosocomial infection in intensive care units, and multidrug-resistant enterococci are associated with higher healthcare costs and a higher number of fatalities. [4,5]

Ampicillin is the treatment of choice for enterococcal infections. Increased ampicillin resistance in enterococci is due to either beta-lactamase production or changes in structure or expression of Penicillin Binding Protein (PBP) 5 on the bacterial cell wall. It is seen more frequently in *E. faecium*. These subtypes were first seen in the US in late 1980's and were due to decreased affinity of PBP 5 for ampicillin[6]. Ampicillin resistance due to beta-lactamase production has been rarely reported. It cannot be reliably detected with disk diffusion or dilution methods, but can be detected by a nitrocefin-based beta-lactamase test. [7]

As far back as 2002, an American study found 85.8 % resistance to ampicillin among Vancomycin resistant enterococci.[2] A very recent study in Pakistan (January 2014) reported 86.36 % of VRE isolates as ampicillin resistant. [8]

Resistance to ampicillin also confers resistance to amoxicillin-clavulanic acid, ampicillin-sulbactam, piperacillin and piperacillin-tazobactam in non-beta-lactamase producing enterococci. [7]

The late 80's saw increased usage of vancomycin as standard therapy for Methicillin-resistant Staphylococci as well as for treatment of antibiotic associated diarrhea and pseudomembranous colitis due to *Clostridium difficile.* Vancomycin resistance was attributed to use of oral vancomycin for treatment of antibiotic associated diarrhea in healthcare settings. Vancomycin resistant enterococci (VRE) were first reported in 1980 in the USA and in Europe in 1986. The first case in Pakistan at Aga Khan University hospital, was published in 2002. [8]

Molecular analysis of VRE has documented at least six acquired phenotypes – VanA, VanB, VanD, VanE, VanG and VanL of which VanA and VanB are most frequently isolated.[1]

Risk factors for VRE colonization and infection include prolonged hospitalization particularly in intensive care units; injudicious antibiotic use, particularly extended spectrum cephalosporins and antibiotics used to treat anaerobic infections; indwelling urinary catheters; surgery; neutropenic and transplant patients. Person to person transmission is important in spread of VRE in the hospitals. Clinicians should differentiate between confirmed symptomatic UTI, asymptomatic bacteriuria and colonization with VRE before initiating chemotherapy[9]. Aggressive antibiotic treatment may be unwarranted in most cases. Diagnosis of UTI must involve urinalysis with quantitative urine cultures in patients with suggestive signs and symptoms. VRE isolated from urine in asymptomatic patients without pyuria may represent colonization only and does not require therapy.

Treatment options for VRE with concurrent ampicillin resistance include nitrofurantoin, linezolid, daptomycin, quinupristin–dalfopristin, doxycycline and tigecycline.

Nitrofurantoin has been used for several years as a favourable treatment option for uncomplicated urinary tract infections. Given its safety profile and susceptibility results, it is a cost-effective antimicrobial for urinary tract infections due to both gram positive and gram negative organisms. A study in 2006 found 97.3 % susceptibility to Nitrofurantoin in *E. faecalis* isolates. [10]

In this study, 53.3% susceptibility to nitrofurantoin was seen. Therefore, it can be an effective and cheap antimicrobial for treatment of ampicillin-resistant VRE.

Although more expensive than vancomycin, linezolid does not require testing for adequate serum drug concentrations or dosing adjustment for renal or hepatic insufficiency, and it has been regarded by some healthcare providers as more effective than vancomycin in treating nosocomial pneumonia and MRSA skin and soft tissue infections. Other advantages are 100 % bioavailability and an oral formulation in addition to the parenteral. A very recent study in Pakistan also reported 100 % susceptibility to linezolid in Vancomycin resistant enterococci.[8] In our study also, all the VRE isolates tested sensitive to linezolid (100%). Most reports of linezolid-resistant

enterococci (LRE) have been individual cases or small case series.[11-14]

An American study highlighted the use of daptomycin in Urinary Tract infections due to VRE. It is a lipopeptide antibiotic with bactericidal activity against Gram-positive pathogens. Daptomycin acts by binding to bacterial cell membrane which through a series of steps inhibits DNA, RNA and protein synthesis, and ultimately leads to cell lysis. Its bactericidal effect is a prized feature compared to the bacteriostatic effect of Linezolid. Daptomycin resistance among VRE strains remains rare.[15]

CONCLUSION

Vancomycin and ampicillin resistance, alongwith intrinsic resistance to cephalosporins and high level aminoglycoside resistance in enterococci greatly reduces treatment options for infections caused by such strains. Plasmid mediated transfer of antimicrobial resistance to *Staphylococcus aureus* is a known and dreaded consequence of enterococcal infections[16]. With the bacteria fighting back harder than ever, newer antimicrobials with novel mechanisms of activity are being developed and used blindly at substantial cost to the healthcare systems and pharmaceutical industry. Cross infection in hospitals must be controlled by barrier nursing, patient isolation and frequent handwashing by the attending staff and physicians. Particularly important (and rarely practiced) is the judicious and responsible use of antimicrobials; in some cases, often wrongly and/or unnecessarily prescribed. Surveillance is a key component of any infection control program to monitor antimicrobial resistance trends and to devise newer treatment strategies. Effective use of resources must be made by identification and control of risk factors where possible.

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