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Research Article

ANTIMICROBIAL RESISTANCE PATTERNS AND IN VITRO ACTIVITY OF DAPTOMYCIN IN URINARY ENTEROCOCCAL ISOLATES

***Aroma Oberoi and Vipin Sam Alexander**
Department of Microbiology, Christian Medical College, Ludhiana
**Author for Correspondence*

ABSTRACT
Vancomycin Resistant Enterococci (VRE) is an important cause of hospital acquired urinary tract infection. The aim of the study was to determine the extent of drug-resistance among urinary isolates of enterococci and also to evaluate the activity of daptomycin in VRE isolates. It is a retrospective study conducted over a period of one year in the Department of Microbiology of a tertiary care hospital in northern India. One hundred and ninety five isolates of enterococci obtained from midstream urine samples of patients were subjected to antimicrobial susceptibility with the following antibiotics: penicillin, ampicillin, vancomycin, gentamicin, teicoplanin, ciprofloxacin, nitrofurantoin and linezolid, by employing the disk diffusion method. Minimum inhibitory concentration values of vancomycin and daptomycin were determined by E-test. Of the 195 isolates of enterococci, 83.59 % were resistant to penicillin, 52.30% to ampicillin, and 66.15% to gentamicin, 5.7% to nitrofurantoin, 11.28% to teicoplanin and 2.05% to linezolid. Vancomycin resistance was detected in 39 (20%) isolates. All the vancomycin resistant strains were susceptible to daptomycin. The study indicates that the prevalence of VRE causing urinary tract infections is on the rise and alternative therapeutic options like daptomycin should be considered for its treatment.

Keywords: *Daptomycin, Vancomycin Resistant Enterococci, Minimum Inhibitory Concentration, Urinary Tract Infection, India.*

INTRODUCTION
Enterococcus is normal flora inhabiting the oral cavity, intestinal tract and vagina. Though considered organisms of low virulence, they are important causes of nosocomial infections as well as community acquired infections, mainly causing urinary tract infections, infective endocarditis, pelvic infections and neonatal infections.

The treatment of Enterococcal infections is difficult due to the ability of this organism to show inherent as well as acquired resistance too many antibiotics. Vancomycin Resistant Enterococci (VRE) are rampant in hospitals in USA and Europe, but its prevalence in India has been found to be comparatively low (Hidron *et al*., 2008) Limited options are available for chemotherapy of VRE infections with very few drugs like daptomycin, linezolid, quinupristin-dalfopristin and tigecycline in the pipeline.

Daptomycin, a cyclic lipopeptide derived from *Streptomyces roseosporus* is a bactericidal agent causing cell death as a result of impairment of synthesis of proteins, DNA and RNA. It exerts it activity by calcium-dependent binding to the cytoplasmic membrane of Gram positive bacteria, resulting in efflux of potassium (Carpenter *et al*., 2004). It is approved by the United States Food and Drug administration (US-FDA) for the treatment of complicated skin and soft tissue infections caused by Gram-positive bacteria, including *Staphylococcus aureus* (methicillin-susceptible and methicillin-resistant strains) and *Enterococcus faecalis* (vancomycin susceptible strains only). The rapid concentration-dependent bactericidal activity against a variety of Gram-positive organisms, including *S. aureus* (both methicillin sensitive and resistant-MSSA and MRSA), *E. faecalis* (both vancomycin susceptible and resistant) has been described for daptomycin. In several studies from across the world, daptomycin exhibits excellent in vitro activity against vancomycin resistant enterococci isolated from various types of infection with

percentage susceptibility rates ranging from 98% to 100% (Jones *et al*., 2007; Pfaller *et al*., 2007; Sader *et al*., 2009; Zhanel *et al*., 2008).

Few publications have reported in vitro activity of daptomycin in clinical isolates of enterococci from India, with limited information available on in vitro activity of daptomycin against urinary isolates of enterococci. Hence, this study was conducted with the aim of determining the antimicrobial susceptibility patterns of urinary enterococcal isolates with special reference to their susceptibility to daptomycin.

MATERIALS AND METHODS

The study was conducted in the Microbiology Department of a tertiary care teaching hospital in North India. A retrospective analysis was conducted over a period of one year from 1st July, 2012 to 30th June, 2013 in which enterococci isolated in significant counts (>10^5 cfu/ml) in pure culture from midstream urine samples were included in the study. Blood agar and MacConkey agar plates were used as primary plating media. The identification of the enterococcal isolates was done by standard microbiological techniques (Facklam *et al*., 1989)

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method for the following antibiotics (HiMedia Laboratories, Mumbai, India)): penicillin (10 µg), ampicillin (10 µg), vancomycin (30 µg), gentamicin (120 µg), teicoplanin (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg) and linezolid (30 µg). The antimicrobial susceptibility pattern was interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI). The zone diameters of vancomycin disks were measured in transmitted light after 24 hours of incubation at 37ºC.

Minimum inhibitory concentration

Enterococcal isolates resistant or intermediately susceptible to vancomycin were tested for minimum inhibitory concentration (MIC) by epsilometer test (E-test, Biomerieux, France). Enterococci which had MIC ≥32 µg/mL were considered resistant; MIC of 8-16 µg/mL, as intermediately resistant; and MIC of 4 µg/mL, as susceptible to vancomycin. MIC of daptomycin was determined by E-test (Biomerieux, France) for vancomycin resistant isolates. The daptomycin E-test strip contained daptomycin in the concentration range of 0.016 to 256 g/ml with a constant level of calcium (40 µg/ml) throughout the strip. Susceptibility breakpoint for daptomycin was considered as <4 µg/ml as per CLSI guidelines (CLSI, 2010). MIC values were read as per manufacturer's instruction and interpreted using the CLSI guidelines (CLSI, 2010).

RESULTS AND DISCUSSION

Results

A total of 195 enterococci (1.8%) were isolated from 10152 urine samples received during the study period. Among the 195 isolates, 163 (83.59 %) isolates were resistant to penicillin, 102 isolates (52.30 %) to ampicillin, 129 (66.15%) isolates to gentamicin, 11 (5.7%) to nitrofurantoin, 22 (11.28%) to teicoplanin and 4 (2.05%) to teicoplanin. Vancomycin resistance was detected in 39 (20 %) isolates by E-test method. Resistance to both vancomycin and teicoplanin was detected in 23 (11.79%) isolates. All the 39 vancomycin resistant strains were susceptible to daptomycin with MIC in the range of 1.5 to 4 µg/ml.

Discussion

In this study, rate of resistance of the enterococcal isolates to ampicillin (52.3%) is in agreement with findings of Chitnis *et al* (Chitnis *et al*., 2013). However, other studies have reported higher rates of ampicillin resistance. Resistance to penicillin was detected in very high percentage of isolates (83.59%), which is similar to findings in a study conducted by Rahangdale et al (Rahangdale *et al*., 2008). High level resistance to gentamicin was detected in very high proportion of the isolates (66.15%), which is in concurrence with other studies done in India.

The prevalence of vancomycin resistance in enterococci in India varies between zero and 30 percent across various studies. The prevalence of vancomycin resistance in our study was found to be 20 per cent

which is similar to the findings of Deshpande *et al* and Karmarkar *et al* (Deshpande *et al.,* 2013; Karmarkar *et al*., 2004). Prevalence of vancomycin resistance in urinary isolates are reported to be much lower, zero per cent in one study in west India and 5 per cent in another study from north India (Miskeen *et al*., 2002; Taneja *et al., 2004)*

Concomitant resistance to vancomycin and ampicillin was detected in 18.97 per cent (37/195) of the isolates. Ampicillin is considered the drug of choice for urinary tract infections caused by ampicillin susceptible strains of enterococci, including VRE. For treatment of VRE UTI concomitantly resistant to ampicillin, other therapeutic options are nitrofurantoin, fosfomycin, doxycycline, linezolid and daptomycin (Heintz *et al*., 2010)**.** In this study, resistance to daptomycin, nitrofurantoin and linezolid was found to be lowest among the vancomycin resistant strains

In the present study, all the vancomycin resistant strains were 100 per cent susceptible to daptomycin with MIC in the range of 1.5 to 4 µg/ml. This finding is echoed in other studies done in the subcontinent where it was found that daptomycin shows very good activity against VRE from various clinical samples (Dhawan *et al*., 2009; Mathai *et al*., 2009) The rapid bactericidal activity, predominant excretion in urine for 24 hours following intravenous administration and safety profile of daptomycin makes it an excellent chemotherapeutic option in VRE UTI (Heintz *et al*., 2010).

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