Vancomycin Resistant Enterococcus Causing Bloodstream Infection
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ABSTRACT
Enterococci have remained important pathogens causing bacteremia especially in hospital settings. Drug resistance among enterococcal isolates from blood is associated with increased mortality. We report a case of vancomycin resistant Enterococcus causing bloodstream infection. The E. faecalis strain carried vanA genotype.

INTRODUCTION
Isolation of enterococci resistant to multiple antibiotics has become increasingly common in the hospital setting. Enterococci can survive for long periods on environmental surfaces, contributing to their transmission. Vancomycin resistant Enterococcus (VRE) have been isolated from all objects and sites in health care facilities. Data suggest that environmental sources are more important in VRE bacteraemia. Endogenous sources, particularly gastrointestinal tract, play a pivotal role in Vancomycin sensitive Enterococcus (VSE) bacteraemia. The following factors were most strongly associated with VRE bacteraemia:

1. Central venous catheter, neutropaenia, secondary infection, VRE colonisation, exposure to antimicrobial therapy in the preceding thirty days including vancomycin, meropenem, cefepime, ciprofloxacin, piperacillin-tazobactam and metronidazole, intensive care unit (ICU) admission, total parenteral nutrition (TPN), indwelling urinary catheter (IDC), allogeneic bone marrow transplantation, and hypoalbuminaemia.

2. The following factors were associated with VSE bacteraemia:
   - Age, prior gastrointestinal disease, ICU admission, abdominal surgery, hypoalbuminaemia, secondary infection, IDC, exposure to piperacillin-tazobactam, metronidazole, and chemotherapeutic agents. Enterococcal bacteraemia is associated with significant patient morbidity; vancomycin resistance has been shown to be an independent predictor of mortality (Vergis et al., 2001, Diazgranados et al., 2005)

CASE REPORT
52 year male admitted in ICU for OP poisoning. Patient was intubated and on day three, as BP was falling down, central line was used. On day four, patient developed signs and symptoms of ventilator associated pneumonia. Cefazidime and amikacin were started empirically. After 3 days, blood culture was sent which was sterile after 48 hours of incubation. Vancomycin and meropenem were started. After persistent fever of one week after vancomycin therapy, patient was started on cefoperazone-sulbactam. Three blood cultures which were sent within first week of admission in ICU were sterile. Tracheal aspirates sent for bacterial culture and sensitivity on several occasions grew multidrug resistant Acinetobacter baumanii along with other hospital acquired multidrug resistant gram negative bacteria. Two weeks after ICU admission, in tracheal aspirate sent for culture and sensitivity along with multidrug resistant Acinetobacter baumanii, vancomycin resistant Enterococcus faecalis was isolated which was resistant to teicoplanin and sensitive to linezolid. At the same time, blood culture also grew Enterococcus faecalis resistant to vancomycin and teicoplanin and sensitive to linezolid. But the patient succumbed to sepsis.

Antimicrobial susceptibility testing

Disk diffusion method: Kirby Bauer disk diffusion method was used for determining the presumptive susceptibility of the isolate to vancomycin (30µg), ampicillin (10 µg), tetracycline (30 µg), ciprofloxacin (5µg) and high level gentamicin (120µg) and found to be resistant to all. Later checked with teicoplanin (30µg) found to be resistant and sensitive to linezolid (30µg).

Agar screening method: For vancomycin screening agar BHI base was used. The quality control strains used for vancomycin screening were Staphylococcus aureus ATCC 25923, and a known vancomycin resistant strain of Enterococcus faecalis (confirmed by MIC testing)

Minimum Inhibitory Concentration testing (Agar Dilution Method): The range of MICs tested was from 2µg/ml to 128µg/ml for vancomycin. MHA plates supplemented with the respective antibiotic from the corresponding stock solution were used. A 1 µl loop was
Case Report

Figure 1. Electrophoresis gel showing picture of ddl gene (941 bp) specific for *E. faecalis*. *Abbrev*: M-Molecular ladder, PC-Positive control, NC-Negative control

Figure 2. Electrophoresis gel showing presence of vanA gene (732 bp) in the test strain. *Abbrev*: PC-Positive control, NC-Negative control, M-Molecular ladder (100bp)
used to spot inoculate the strain onto the medium. Plates were incubated at 37°C for 24 hours.

Genotypic characterization of vancomycin resistance genes: The presence of genes encoding for products responsible for vancomycin resistance in enterococci was studied using polymerase chain reaction. DNA was extracted using the commercial kit. The primers used for the genotypic characterization of vancomycin resistant strains were the ones used by Dutka-Malen et al., 1995. The PCR products were resolved by electrophoresis on a 1% agarose TBE gel with 0.5µg of ethidium bromide in an electrophoresis unit. The TBE gel was visualized under UV light using a gel documentation system. PCR products amplified with primers specific for E. faecalis showed the presence of ddl gene (941 bp) and those with primers specific for vanA gene showed 732bp amplified product.

DISCUSSION
To date, six varieties of enterococcal glycopeptide resistance have been described (corresponding to vanA through vanE and vanG) (Perichan et al., 1997; Mckessar et al., 2000). Of these, the most clinically important are vanA and vanB. Van A strains show high level inducible resistance to both vancomycin (MIC≥64µg/ml) and teicoplanin. Risk factors for acquisition of VRE infections includes longer period of hospital stay or ICU stay, previous antimicrobial therapy (especially multiple antibiotics used at the same time), severity of illness, use of parenteral or oral vancomycin and use of third generation cephalosporins with or without use of vancomycin (Karanfil et al., 1992; Carmeli et al., 2002; Dalms and Lee 1998). A study done on Enterococcal blood isolates at a Tertiary care centre in North India where out of 170 isolates of E. faecalis from blood, three were resistant to vancomycin and teicoplanin and showed vanA phenotype (Mathur et al., 2003). A study on antimicrobial resistance of enterococcal blood isolates at a pediatric care hospital in India where out of 50 isolates from bacteremic children, four strains showed raised MIC to vancomycin (Kapoor et al., 2005). Glycopeptide resistance among enterococci is due to increased use of vancomycin in hospital settings. This emphasizes the importance of strict enforcement of antibiotic policies to prevent emergence and spread of antibiotic resistant bacteria.

REFERENCES