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Case Report

Microbiology Section

Clinico-Microbiological Investigation of Catheter Associated Urinary Tract Infection by *Enterococcus faecalis*: *vanA* Genotype

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ABSTRACT

Prolonged hospitalization and exposure to third generation cephalosporins are reported to facilitate the acquisition and colonization of Vancomycin Resistant *Enterococci* (VRE). Though VRE is not uncommon in India, urinary tract infection with a *vanA* genotype is a cause of serious concern as VRE co-exhibit resistance to aminoglycosides. In India, majority of the VRE isolates recovered from hospitalized patients include *Enterococcus faecium.* We report a case of catheter associated urinary tract infection by an endogenous, multidrug resistant *E. faecalis* of *vanA* genotype following prolonged hospitalization, ICU stay, catheterisation and exposure to 3G cephalosporin and metronidazole. The patient responded to linezolid therapy.

Keywords: High level aminoglycoside resistance (HLAR), Multidrug resistance, Vancomycin resistant *Enterococci* (VRE)

CASE REPORT

A 23-year-old male, a resident of Kanchipuram district (Tamil Nadu, India) presented to the Emergency Department of SRM Medical College Hospital, Katankulathur, a tertiary care centre with clinical signs of degloving injury of the left lower limb, over thigh, left aspect of knee, lateral aspect of leg and anterolateral aspect of ankle following a road traffic accident. Wound debridement was done under spinal anesthesia on the day of admission. Routine laboratory investigations were performed. Results from haemotological tests revealed the followimg values: Haemoglobin- 9.1g dl^{-1}, Total Count – 5600 cells/µl, Differential Count: N-87%, L-9%, E-3%, M-4%, Random blood glucose- 120 mg/dl, Urea-14mg/dl, Creatinine-0.7 mg/dl. Serum electrolytes: Na-134 $mEqL^{-1}$, K-9.8 $mEqL^{-1}$, Cl-106 $mEqL^{-1}$, HCO_3-20 $mEqL^{-1}$.

On the 11th day of admission, flap cover with superficial skin graft was performed and the patient was shifted to surgical ICU. Post surgery, the patient received cefaperazone-sulbactam-1.5g IV, gentamicin -80 +mg IV-BD, metronidazole 100 mg-TD, Dynaper IM – BD and was catheterised with closed bag system. During his stay in SICU, on hospital day 15, the patient developed clinical signs of UTI and urine culture was performed. Direct microscopy revealed gram positive cocci in chains, pus cells 7-8/hpf, epithelial cells 4-5/hpf. After incubation at 37°C for 18 hours, the urine culture showed significant bacteriuria (>10,0000 cfu/ml), magenta pink coloured colonies on MacConkey agar and non-haemolytic pin point colonies on 5% human blood agar plates. On further bacteriological examination using standard tests, the isolate was identified as *Enterococcus faecalis*.

In vitro antibiotic susceptibility testing performed by agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2013) guidelines revealed that the strain was resistant to penicillin, ampicillin, erythromycin, tetracycline, nitrofurantoin, amikacin, gentamicin, imipenem, meropenem, vancomycin and teicoplanin but susceptible to linezolid and tigecycline. Screening for high level aminoglycoside resistance (HLAR) revealed that the strain was resistant to gentamicin (120µg) (Microexpress, Tulip Diagnostics Pvt Ltd, India) and streptomycin (300 µg) (Hi-media Laboratories Pvt Ltd, India). Minimum Inhibitory Concentration (MIC) of gentamicin, and streptomycin was found to be ≥ 512 µg/ml by agar dilution method. Resistance to vancomycin was ensured based on the growth on vancomycin agar screen plate (6µg/ml) and the MIC of vancomycin was recorded to be ≥ 256 µg/ml by E-test (Himedia Laboratories Pvt Ltd, India).

Genomic DNA was extracted by boiling lysis method. PCR was performed to detect the presence of acquired vancomycin resistance gene (*van A*) using a previously described primer pair [1]. The isolate was found to harbour *vanA* gene cluster that encodes high level resistance to vancomycin and teicoplanin.

The isolate exhibited high level resistance to gentamicin and hence, genotyping was performed by multiplex PCR. Five common aminoglycoside resistance genes *aac(6')-Ie-aph(2")-Ia, aph(2")-Ib, aph(2")-Ic, aph(2")-Id* and *aph(3')-IIIa* were analyzed using previously described primers [2]. Analysis of the PCR product revealed that the isolate harboured *aac(6')-Ie-aph(2")-Ia* and *aph(3')-IIIa.* The high level gentamicin resistance (MIC ≥ 512 µg/ml) and the dual presence of AGMEs - *aac(6')-Ie-aph(2")-Ia* and *aph(3')-IIIa* revealed that the isolate was resistant to synergism with the cell wall active agents.

Biofilm forming ability of the enterococcal strain was assessed by the tissue culture plate method. Safranine (0.1%) was used to stain the adherent bacterial cells. The presence of *Esp* gene encoding Enterococcal surface protein (Esp) was assessed by PCR using previously described primers [3]. The current isolate was found to exhibit moderate *in-vitro* biofilm formation nevertheless, *Esp* was not detected. According to the antibiogram, the patient was treated with linezolid 600mg IV. The patient responded to therapy, 2 days later patient was shifted from the ICU to plastic ward and the closed bag system was removed.

DISCUSSION

The patient is likely to have acquired VRE during his stay at SICU, concurrently the other patients hospitalised at the SICU were not infected with VRE. Previous studies document that prolonged -hospitalization, ICU stay, catheterisation, exposure to antibiotics especially, the third generation cephalosporins, fluoroquinolones, anti-anaerobes are the potential risk factors for nosocomial colonisation and infection with VRE [4-6]. In this case, the selection pressure exerted by the broad spectrum antimicrobial especially the third generation cephalosporin is likely to have promoted the emergence of VRE and the antianaerobic drug; metronidazole is likely to have facilitated the colonization and persistence of VRE.

Recent Indian studies have reported that the majority of the VRE isolates recovered from hospitalized patients were found to be *E. faecium* [7–9]. However, we report a case of vancomycin resistant *E. faecalis* CAUTI. Also, catheterization had promoted the formation of biofilm and the concomitant entry of the endogenous VRE into the urinary tract resulting in catheter associated UTI (CAUTI).

vanA and *vanB* genotypes are reported to be the predominant among the glycopeptide-resistant genotypes in *Enterococci* [10]. *vanA* positive isolates are inducibly resistant with high MICs (> 64 µg/ml) of vancomycin and teicoplanin while, *vanB* isolates exhibit inducible resistance to vancomycin with an MIC of (32-64 µg/ml) but are susceptible to teicoplanin. The *vanA* operon can easily be transferred through acquired resistance. *E. faecalis* isolated from this patient exhibited glycopeptide resistance of the *vanA* phenotype.

Aminoglycosides are the most frequently prescribed antibiotics in clinical practice as they possess good pharmacokinetics and exhibit synergism with beta-lactams and glycopeptides. Production of aminoglycoside-modifying enzymes (AGMEs) is reported to be the major mechanism involved in aminoglycoside resistance. This isolate was found to harbour *aac(6')-Ie-aph(2")-Ia* and *aph(3')-IIIa.* The latter encodes the aminoglycoside phosphotransferase, APH(3')III which confers high-level resistance to kanamycin while, the former encodes AAC(6')-APH(2")-Ia, a bifunctional enzyme that confers resistance to all clinically available aminoglycosides except streptomycin.

Multidrug resistance is a matter of great concern among nosocomial uropathogens with the associated increase in the health care cost compared to the community strains. Currently, the use of synergistic combinations of a cell wall-active agent, a penicillin or a glycopeptide, with an aminoglycoside is claimed to be optimal in the treatment of enterococcal infections [11]. However, certain *Enterococci* have acquired resistance genes that encode AGMEs, which eliminate this synergistic bactericidal effect. This isolate was found to exhibit high level aminoglycoside resistance and was resistant to synergism with cell wall active agents.

Biofilm formation along all surfaces of the catheter is reported to be a major factor that promotes catheter associated UTI (CAUTI). Enterococcal surface protein (Esp) is reported to enhance biofilm formation [3]. Also, biofilm specific counterparts are reported to exhibit enhanced antibiotic tolerance [12]. This isolate was capable of forming *in-vitro* biofilm but did not harbour *Esp*. This is in line with the recent finding which has documented that Esp independent biofilm formation can occur in *Enterococci* [13].

Taking into account the possibility of VRE associated UTI among hospitalized, non-ambulatory patients, empirical treatment for UTI with vancomycin needs to be administered with caution. Previous Indian reports have documented increased case fatality among catheterized patients who developed bacteremia and sepsis caused by VRE of urinary origin [14].

CONCLUSION

Prompt diagnosis, identification of the aetiology of UTI and the antibiotic resistance pattern of the isolate is critical in the management of UTI. Prudent use of third generation cephalosporins could prevent the emergence of VRE and linezolid is currently effective in the treatment of UTI caused by VRE with a *vanA* genotype.

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