Article published online: 2021-08-09

CASE REPORT

The first linezolid-resistant *Enterococcus faecium* in India: High level resistance in a patient with no previous antibiotic exposure

Simit Kumar, Maitreyi Bandyoapdhyay, Mitali Chatterjee, Prabir Mukhopadhyay, Sumon Poddar, Parthajit Banerjee

Department of Microbiology, R.G. Kar Medical College and Hospital, Kolkata, West Bengal, India

Access this article online

Website: www.avicennajmed.com

DOI: 0.4103/2231-0770.127416

Quick Response Code:

ABSTRACT

Linezolid provides high rates of the clinical cure and microbiological success in complicated infections due to Enterococcus spp., including vancomycin-resistant *Enterococcus faecium*. However, the emergence of resistance during linezolid treatment has been reported for clinical strains of Enterococcus, which is alarming given the fact that, this leaves the clinician with very few treatment options. We report the first case of linezolid resistant *Enterococcus faecium* from India, which was isolated from the blood culture of a hypoglycemic encephalopathy patient. There have been previous reports of linezolid resistant enterococci from different parts of the world, with minimum inhibitory concentration (MIC) ranging from 16 to 64 μg/mL and most of them were associated with vancomycin resistance but the isolate reported over here had an MIC of 1024 μg/mL and interestingly was sensitive to vancomycin.

Key words: *Enterococcus faecium*, linezolid resistant, testing methods for detecting linezolid minimum inhibitory concentration

INTRODUCTION

Linezolid, a member of the oxazolidinone class of antibiotics, exerts antibacterial activity by inhibiting the formation of the 70S initiation complex. This ultimately prevents the translation and replication of bacterial proteins. Linezolid provides high rates of clinical cure and microbiological success in complicated infections due to Enterococcus spp., including vancomycin-resistant *Enterococcus faecium*.[1] However, the emergence of resistance during linezolid treatment has been reported for clinical strains of Enterococcus. Clinical resistance to linezolid is associated with a G2576T mutation in domain V of 23S ribosomal ribonucleic acid (rRNA) genes of Enterococcus and the level of linezolid resistance is directly related to the number of 23S rRNA genes containing this mutation. Both laboratory and clinical strains of *E. faecium* with linezolid minimum inhibitory concentrations (MICs) of 4 μg/mL have been shown to carry the G2576T mutation.[1-3] Although other mutations have been reported under experimental conditions, only this mutation has been seen in clinical isolates found to date. Previously reported patient factors that might pre-dispose to the development of linezolid resistance include indwelling intravascular devices, under dosage, immunosuppression after transplantation and long courses of linezolid therapy (20-40 days).[3] Accurate detection by susceptibility testing methods of decreased susceptibility due to G2576T mutation in one or two genes is necessary since this can be a prelude to higher levels of linezolid resistance associated with extensive use of the antibiotic.[2]

The different suggested modalities of acquisition of linezolid-resistant VREF (LR VREF): (i) An independent event of *de novo* selection of resistant mutants in colonizing/infecting VREF (patients who carried genetically unrelated strains), (ii) possible patient to patient spread (patients who carried genetically related strains) and (iii) emergence of LR mutants from linezolid intermediate vancomycin resistant enterococci (LI VRE) during the linezolid therapy.[4] Nosocomial transmission of LR VREF from a linezolid-treated patient to several untreated patients,

Address for correspondence: Dr. Simit Kumar, Department of Microbiology, R.G. Kar Medical College and Hospital, Kolkata - 700 037, West Bengal, India. E-mail: simitkumar@yahoo.co.in

resulting in asymptomatic colonization, has also been documented.[5]

We describe a fatal case of linezolid resistant enterococci associated sepsis. The MIC of the isolate for linezolid was 1024 μg/mL and the isolate was susceptible to vancomycin unlike the previous reported cases, in which linezolid resistance was usually detected in VRE. The report is also unique, given the fact that the patient did not have any previous exposure to linezolid.

CASE REPORT

We report a case of a 72-year-old female, known diabetic for last 16 years, was admitted to the intensive care unit (ICU) of a tertiary care hospital with sudden onset unconsciousness for approximately 12 h first noticed when patient was not responding when her family tried to wake her up from sleep, patient was on oral hypoglycemic drugs with poor glycemic control. The patient was also hypertensive, which was controlled on medication. Patient did not have any history of convulsions, fever, vomiting, headache, trauma or any neurodeficit in the past.

At the time of admission, the patient neurological score was 5/15 as per Glasgow Coma scale. The pulse was 88/min, blood pressure-140/80 mm Hg, respiratory rate of 16/min and was irregular with bilateral vesicular breath sounds. The capillary blood glucose was 28 mg/dL. The other blood investigations were as follows: Total leukocyte count-16,200/mm^3 with neutrophil predominance, serum alanine aminotransferase (ALT) -31 IU/mL, serum aspartate aminotransferase (AST) -71 IU/mL, alkaline phosphatase-22 IU/L, total protein-4.8 g/dL, albumin-2.4 g/dL, urea-54 mg/dL, creatinine-1.2 mg/dL, total bilirubin-0.6 mg/dL, conjugated bilirubin 0.3 mg/dL. Computerized tomographic scan on admission showed signs of focal ischemia. She was diagnosed as a case of hypoglycemic encephalopathy with hypoxic brain damage. She was intubated and put on ventilator support. The patient's glycemic status was restored and the patient was started on piperacillin + tazobactam empirically, to cover against any aspiration pneumonia, given the long period of unconsciousness of the patient.

On day 3 of treatment, she was showing some response to a painful stimulus, but the blood sugar was remaining uncontrolled fluctuating in between 221 mg/dL and 350 mg/dL. On day 4 of treatment, she developed low grade fever for which the endotracheal secretions were sent for culture. *Acinetobacter baumanii* with significant colony count (>10^6 colony forming units) was isolated, which was sensitive to netilmicin and polymixin B. The blood cultures sent on day 3 showed no growth and the chest X-ray showed mild right sided basal opacity. Patient was started on netilmicin and cefepime.

On day 7 of treatment, the patient developed high grade fever. The blood investigations were showed total leukocyte count of 8400/mm^3 with neutrophilic predominance and serum creatinine of 2 mg/dL. The catheterized urine sample and blood were sent for culture. The urine culture showed a significant growth of *Escherichia coli* which was sensitive to meropenem, poymyxin B, cotrimoxazole and nitrofurantoin. On the basis of the culture reports, meropenem was added to the treatment regimen, but the general condition of the patient deteriorated, with total leukocyte counts dropping to 4000/mm^3.

The blood cultures sent on day 7 and day 8 showed growth of *E. faecium,* which was identified by standard laboratory procedures.[6] The antibiotic susceptibility of the isolate was performed by Kirby Bauer disc diffusion technique as per Clinical and Laboratory Standards Institute (CLSI guidelines), which showed the organism to be sensitive to vancomycin, but more remarkably the isolate was resistant to linezolid showing no zone around the disc by the disc diffusion technique [Figure 1].[7] Disk diffusion testing was performed with 30-μg linezolid disks (BBL, Becton Dickinson). *E*-test linezolid strips with a concentration gradient corresponding to 0.016-256 μg/mL were utilized with Mueller-Hinton agar as described by the manufacturer (Hi Media laboratories Mumbai) to determine the MIC of theisolate, but the isolate did not show any zone of inhibition, indicating that the MIC of the isolate was more than 256 μg/mL [Figure 1]. The MIC of the isolate was further determined by agar dilution method (using 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1024 μg/mL) was

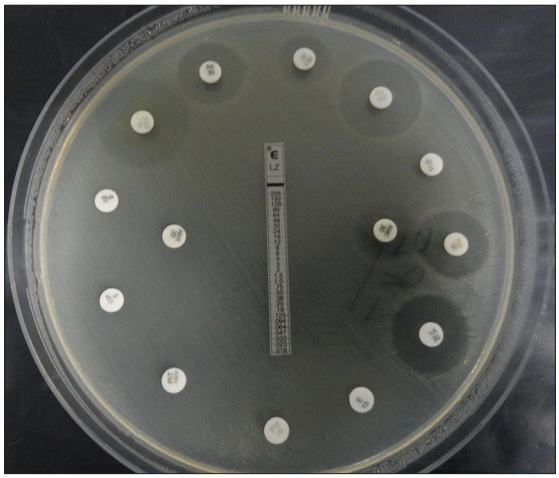

Figure 1: The antibiotic susceptibility plate showing no zone of inhibition around the linezolid *E*-test strip along with the susceptibility pattern for other antibiotics by Kirby Bauer disc diffusion method for the linezolid resistant enterococcus isolate

conducted in accordance with CLSI standards[7] using a linezolid preparation obtained from the manufacturer (Pfizer, India) which showed the MIC of the isolate was 1024 µg/mL. Automated susceptibility testing by the Vitek 2 system using the antimicrobial susceptibility testing (AST) GP-61 card (bioMérieux) was performed according to the manufacturers' instructions. The categorical interpretation of results was based on CLSI guidelines.

Patient was started on vancomycin but her condition further deteriorated and she was declared dead on day 15 of admission.

The cultures from the other patients in the ICU, did not reveal any similar organisms, unlike other case reports where isolation of linezolid resistant enterococci from one patient was usually associated with clonally related isolates, being isolated from other patients in the same wards.[5]

DISCUSSION

We report the first case of linezolid resistant enterococci from India in a patient who had no previous reported history of linezolid medication. The previous studies have identified exposure to linezolid as a risk factor for linezolid resistance among enterococci, but some studies have identified linezolid resistant enterococci from patients without prior exposure to linezolid.[1,5] These patients in previously reported cases appear to have been infected with closely related strains of LR VREF, which was possibly transmitted nosocomially via the hands of health-care workers or through contaminated fomites. In our case, also the patient did not have any previous history of linezolid medication.

The previous studies have established an ecological link between increasing incidence of LI or resistant strains in the rectal surveillance cultures paralleling increasing linezolid consumption.[1] Our patient was the only isolated case of linezolid resistant enterococci, with no similar isolation from the clinical samples from the same or different ICUs throughout the hospital. The rectal cultures collected from the patients also did not reveal any linezolid resistant *Enterococcus faecium* (LREF) during that period.

There have been different methods documented for detecting linezolid resistance in *Enterococcus* strains. The Vitek 2 system demonstrated poor correlation of MICs in the susceptible and intermediate range with the presence or absence of the G2576T mutation, likely reflecting a lack of validation of the Vitek AST GP-61 card with LR strains of *Enterococcus*. Disk diffusion testing appears to be somewhat less sensitive than dilution methods for detection of decreased linezolid susceptibility due to G2576T mutation, but specific for detection of fully susceptible strains without the G2576T mutation.[2] Variability in *E*-test results likely reflects the inherent difficulty in interpretation by visual examination of 80% growth inhibition end points with the *E*-test method. Agar and broth dilution methods were in concordance with polymerase chain reaction detection of the mutation and disk diffusion was somewhat less sensitive, but equally specific.[2] In our case, we employed the disc diffusion method for the initial detection of linezolid resistance followed by detection of MIC by Vitek 2 and confirmed by the *E*-test method and the agar dilution method.

The alarming finding of rapid emergence of resistance to linezolid in *E. faecium* isolates during the linezolid therapy contradicts previous reports indicating that such resistance arises only after prolonged therapy with this antibiotic.[8]

Most of the previous reported strains of LREF were also resistant to vancomycin and teicoplanin, to ampicillin and to high concentrations of gentamicin and streptomycin; all were susceptible to quinupristin-dalfopristin; and all carried the *vanA* gene.[4] LR enterococci are usually resistant to vancomycin and to other antimicrobial agents, though rare cases of clinical enterococcal isolates that are linezolid resistant, but vancomycin susceptible have been identified. Despite resistance to linezolid, the *E. faecium* isolate from our patient was susceptible to vancomycin, ampicillin, tetracycline, aminoglycosides and teicoplanin.

Linezolid is active against *Enterococcus faecalis* and *E. faecium*, whereas quinupristin dalfopristin is active against *E. faecium* isolates, but not against *E. faecalis* isolates. This highlights the importance of the role of clinical microbiology laboratories in speciation of *Enterococcus* isolates in order to provide the clinician with the correct choice of antibiotics. The practice of indiscriminate administration of linezolid to treat methicillin-resistant *Staphylococcus aureus* and eneterococcal infections, given the fact that the drug can be administered orally, could be the accelerated the process of development of resistance to linezolid.[9]

An additional concern is the risk of nosocomial spread of LR organisms. There is a little experience with these infections and specific infection control measures have yet to be formulated. We suggest that the issues that the emergence of resistance to linezolid should be considered as a warning signal, especially considering the fewer armamentarium of antibiotics, effective against enterococci, which is one of the leading causes of nosocomial infections. The clinicians should also be aware that the indiscriminate prescribing of

this oral drug in the outdoor as well as indoor patients may lead to the emergence of LI and linezolid resistant cases of *Enterococcus* in the hospital or even the community.

REFERENCES

1. Scheetz MH, Knechtel SA, Malczynski M, Postelnick MJ, Qi C. Increasing incidence of linezolid-intermediate or -resistant, vancomycin-resistant *Enterococcus faecium* strains parallels increasing linezolid consumption. Antimicrob Agents Chemother 2008;52:2256-9.
2. Qi C, Zheng X, Obias A, Scheetz MH, Malczynski M, Warren JR. Comparison of testing methods for detection of decreased linezolid susceptibility due to G2576T mutation of the 23S rRNA gene in *Enterococcus faecium* and *Enterococcus faecalis*. J Clin Microbiol 2006;44:1098-100.
3. Auckland C, Teare L, Cooke F, Kaufmann ME, Warner M, Jones G, *et al*. Linezolid-resistant enterococci: Report of the first isolates in the United Kingdom. J Antimicrob Chemother 2002;50:743-6.
4. Bonora MG, Solbiati M, Stepan E, Zorzi A, Luzzani A, Catania MR, *et al*. Emergence of linezolid resistance in the vancomycin-resistant *Enterococcus faecium* multilocus sequence typing C1 epidemic lineage. J Clin Microbiol 2006;44:1153-5.
5. Rahim S, Pillai SK, Gold HS, Venkataraman L, Inglima K, Press RA. Linezolid-resistant, vancomycin-resistant *Enterococcus faecium* infection in patients without prior exposure to linezolid. Clin Infect Dis 2003;36:E146-8.
6. Winn WC Jr, Allen SD, Janda WM, Koneman EW, Procop GW, Schreckenberger PC, *et al*., editors. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Ch. 13. Baltimore: Lippincott Williams and Wilkins; 2006. p. 348-9.
7. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: 20th Informational Supplement. CLSI Document M100-S20. Wayne, PA: Clinical and Laboratory Standards Institute; 2010.
8. Seedat J, Zick G, Klare I, Konstabel C, Weiler N, Sahly H. Rapid emergence of resistance to linezolid during linezolid therapy of an *Enterococcus faecium* infection. Antimicrob Agents Chemother 2006;50:4217-9.
9. Dibo I, Pillai SK, Gold HS, Baer MR, Wetzler M, Slack JL, *et al*. Linezolid-resistant *Enterococcus faecalis* isolated from a cord blood transplant recipient. J Clin Microbiol 2004;42:1843-5.

Cite this article as: Kumar S, Bandyoapdhyay M, Chatterjee M, Mukhopadhyay P, Poddar S, Banerjee P. The first linezolid-resistant Enterococcus faecium in India: High level resistance in a patient with no previous antibiotic exposure. Avicenna J Med 2014;4:13-6.

Source of Support: Nil, **Conflict of Interest:** None declared.