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Letter to Editor

Microbiology Section

Linezolid Resistant *Enterococcus*: An Important Emerging Pathogen Isolated from a Non Healing Ulcer of a Diabetic Foot Patient

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Dear Editor,

Linezolid resistance is on increase from last few years. In April 2000, linezolid (an oxazolidinone class of antimicrobial) was approved by US Food and Drug Administration (FDA) for clinical use. It acts by inhibiting bacterial protein synthesis by binding to V domain of 23S rRNA of 50S subunit of bacterial ribosome [1]. The main use of this antimicrobial agent was in the treatment of infections due to Methicillin Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Resistant *Enterococcus* (VRE) because of drug's convenience, good bioavailability and no requirement for blood level or renal dose adjustment [2].

Till date only three cases of Linezolid Resistance *Enterococcus* (LRE) have been reported in India [3-5]. Here, we describe a 50-year-old diabetic male with non healing foot ulcer, from which LRE was isolated. The emergence of LRE is an unfortunate problem, which could lead to disastrous results such as *Enterococcus* species, which is an important cause of both healthcare associated as well as community acquired infections where linezolid has been used as a reserve drug.

A 50-year-old diabetic male, presented to the diabetic clinic of our tertiary care hospital, with a history of non healing ulcer at the base of left foot, with pus formation since two months. The patient was admitted at a private hospital where the culture of pus discharge showed growth of MRSA. He was given linezolid therapy 100 mg twice daily and discharged with healed wound. After one month, the patient came with complain of the ulcer at the same site, which had increased in size and pus formation started again. This was associated with fever, pain and discomfort in the local area.

Patient was a known case of diabetes mellitus from last 22 years and had been on oral hypoglycaemic agents. On examination, the ulcer on the dorsal aspect of left foot was 1×1 cm in size, with punched out margin, tender and had pus discharge. All investigations were within normal range except HbA1c 7.9%, and fasting blood sugar 132 mg/dL. Pus and blood sample were sent for culture and sensitivity testing to Department of Microbiology.

The culture of pus sample showed pure growth of *Enterococcus faecium* which was identified according to standard microbiological procedures [6]. The antimicrobial sensitivity testing of the isolate was performed by Kirby-Bauer disc diffusion testing as per Clinical and Laboratory Standards Institute (CLSI) guidelines [7]. The isolate showed susceptibility to vancomycin and teicoplanin and was resistant to penicillin, linezolid, erythromycin, tetracycline, ciprofloxacin, gentamicin and chloramphenicol. The drug resistance to linezolid was confirmed by putting linezolid E-test strip (AB BioMerieux, India) and the isolate showed the Minimum Inhibitory Concentration (MIC) > 256 µg/mL. The patient was treated with intravenous vancomycin for seven days and oral hypoglycaemic drugs were continued. He responded well to the treatment, as the fever subsided and the wound healed with no pus discharge thereby he was discharged with contact cast.

Kumar S et al., reported the first case of linezolid resistant *Enterococcus faecium* from India. This bacterial strain was isolated from blood culture of a hypoglycaemic encephalopathy patient. The MIC for linezolid was 1024 µg/mL as detected by agar dilution method. Here the patient had no history of previous exposure to linezolid and moreover this isolate was sensitive to vancomycin [3].

Bhatt P et al., in 2014 conducted a cross-sectional study to look for drug resistance in 200 non repeat clinical strains of *Enterococcus* species. Out of 200 isolates, four strains (one *Enterococcus faecalis*, three *Enterococcus faecium*) were reported to be linezolid resistant by both disc diffusion method and E-test. The author didn't comment on the history of previous exposure to linezolid [4].

In another study by Rai S et al., linezolid resistant *Enterococcus faecium* was isolated from a patient on short-term linezolid therapy. This strain was from blood and showed MIC for linezolid by VITEK 2 as ≥4 mg/L. Moreover, the patient also harboured linezolid resistant *S. aureus* in the anterior nares and linezolid resistant *Enterococcus faecium* was also isolated from the patient's stool. The patient had history of linezolid intake a week before the isolation of LRE species [5].

The most common mechanism of drug resistance in linezolid is mutation, involving genes encoding 23S rRNA. *Enterococcus faecalis* has four copies of this gene and *E. faecium* has six copies [1]. It has been hypothesised that there occurs recombination between susceptible and resistant strains with multiple mutated copies. This is said to occur in persistent linezolid selective pressure. The most common mutation in 23S rRNA is G2576U, though a variety of mutations have been described [8]. Marshall SH et al., showed that with the increase in number of mutated genes there is corresponding increase in linezolid MIC [8]. They showed that if 17% of total number of 23S genes were mutated than MIC of *E. faecium* strains was between 8-16 µg/mL and if this increased to 83-100% then, the corresponding linezolid MIC increases to 64-128 µg/mL [8].

The second mechanism of drug resistance to linezolid is because of the presence of plasmid *cfr*, which also confers resistance to lincosamide and Streptogramin A [9]. The gene *cfr* encodes an rRNA methyltransferase that modifies an adenosine in the linezolid binding region on 23S rRNA, preventing antibiotics binding. This gene was first identified in the year 2000 in *S. aureus* and in 2010 in clinical strain of *Enterococcus faecalis* and *Enterococcus faecium*. The gene *cfr* has been associated with mobile transposable element IS256, thus mediating transfer of the antibiotics resistance. There could be a possibility of spread of *cfr* across various *S. aureus* and *Enterococcal* strains in the hospital environment [9].

Pogue JM et al., determined various risk factors associated with linezolid resistance [10]. These are peripheral vascular diseases, solid organ transplant and total parental nutrition, use of piperacillin/tazobactam and cefepime. Previous exposure to linezolid was not a risk factor associated with linezolid resistant infection [10]. Kainer MA et al., in a hospital outbreak of LRE performed a case control study to identify independent predictors for LRE infections. Multivariate analysis identified the following predictors of LRE infections like isolation of MRSA, duration of hospitalisation and duration of preceding linezolid therapy [11]. Our patient had a history of short duration of linezolid intake. Also the patient was diabetic from last 22 years, peripheral vascular disease and history of previous hospitalisation was also there.

Clinicians should be aware of the fact that resistance may arise following prolonged treatment with linezolid but there is the possibility of LRE arising as primary resistance due to presence of *cfr* plasmid in patients who have no history of linezolid exposure. Mutschler M et al., reported LRE from a 10-year-old child with severe sepsis and multiple trauma, who had no history of linezolid intake. Therefore, three different routes of acquisition of resistance to linezolid in Enterococci have been postulated. First being a de novo selection of resistant mutants, secondly possible patient to patient spread and lastly during linezolid therapy emergence of LRE from linezolid intermediate *Enterococcus* [12].

Siegel JD et al., suggested additional interventions such as active surveillance culture and contact precautions if either of the following two conditions are met: 1) the incidence or prevalence of Multidrug Resistant Organisms (MDROs) not decreasing despite implementation of rigorous infection control practices; or 2) the first case or outbreak of an epidemiologically important MDRO (e.g., VRE, LRE, MRSA) is identified within a healthcare setting [13].

In conclusion, microbiologist and clinicians should remain aware that LRE may arise due to prolonged linezolid treatment. Also, dissemination of *cfr* gene may occur if good infection control practices are not adhered to. Further, there should be judicious use of linezolid and accurate identification of LRE.

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