

Toxicity of Oxazolidinone Linezolid on Pathogenic Microorganism *Listeria ivanovii*

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Abstract

Oxazolidinone, a new class of antimicrobial agents is active against various Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis* (MRSE), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin-resistant *Enterococci* (VRE). Sensitivity of Oxazolidinone Linezolid was studied against pathogenic microorganism *Listeria ivanovii* and Minimum Inhibitory Concentration (MIC) determined by broth dilution method and Hi-Comb method was 10 µg/ml. Morphological alterations on *Listeria ivanovii* cell surface were seen by Scanning Electron Microscopy (SEM) after treatment with Linezolid. Inhibition on the activity of dehydrogenases of organism by Linezolid was also studied in the present study.

Keywords: Linezolid, *Listeria ivanovii*, Scanning Electron Microscopy (SEM), Dehydrogenases, Hi-Comb Method, Minimum Inhibitory Concentration (MIC)

1. Introduction

Listeria monocytogenes has been found in number of food-borne disease outbreaks from past decade and several sporadic episodes of *Listeria* illness (Farber and Peterkin 1991). Apart from *Listeria monocytogenes*, *Listeria ivanovii* is only other *Listeria* sp which is considered to be pathogenic. They both invade host cells, replicate in the cytosol after phagosomal escape and spread from cell to cell by polymerizing actin (Vazquez-Boland *et al.*, 2001). *L. ivanovii* has been reported to infect animals, causing abortions, neonatal sepsis and enteritis (Cooper *et al.*, 1973; Seeliger *et al.*, 1986; Seeliger *et al.*, 1984), human infections are very rare. This organism has also been isolated from healthy animals, human carriers and the environment (Seeliger *et al.*, 1986; Seeliger *et al.*, 1984).

Guillet *et al.* (2010) studied two species of *Listeria* which were pathogenic and infected both humans and animals. They found that *L. monocytogenes* infects humans and animals and *L. ivanovii* infects ruminants only and it was associated with gastroenteritis and bacteremia in man. According to this recent study, it was shown that *L. ivanovii* was an enteric opportunistic human pathogen. The pathogenic changes associated with *L. ivanovii* in humans appear similar to those in ruminants, i.e., fetoplacental infections and septicemia (often accompanied by enteritis). Lack of central nervous system involvement could be a general characteristic of *L. ivanovii* infection regardless of host species (Seeliger *et al.*, 1986).

In present investigation, sensitivity of *L. ivanovii* against Oxazolidinone Linezolid was studied by broth dilution method and Hi-Comb method (Hi-media) and MIC was determined. Morphological alterations on the cell

surface of *L. ivanovii* were studied after treatment with Linezolid by Scanning Electron Microscope (SEM). Emphasis was also given on inhibition of dehydrogenases enzymes of organism by the antibiotic.

2. Materials and methods

2.1 Organisms, Culture Media and Antibiotic

L. ivanovii ATCC 19119 was obtained from Hi-media, India, for the present study. Organism was grown at 37 °C for 18 hours in Nutrient broth medium (Hi-media, India) and maintained at 5 °C. Linezolid powder was gifted by Glenmark Pharmaceuticals Ltd., India for experimental purpose.

2.2 Determination of MIC

Minimum Inhibitory Concentration (MIC) of Linezolid against pathogenic microorganism *L. ivanovii* was determined by Broth dilution method (NCCLS 2001) and by Hi-Comb (Hi-media, India) method (CLSI 2008). In Broth dilution method, different concentrations of antibacterial agents were prepared. Inoculums were adjusted to 0.5 Macfarland turbidity standards and an aliquot of 0.1 ml of inoculums was added to each tube of dilution. The tubes were incubated at 37° C overnight. MIC was read visually following 24 hours of incubation and was defined as the lowest concentration that produced no visible turbidity (NCCLS 2001).

In Hi-Comb method, at least 4 to 5 well isolated colonies of same morphological type from agar plate were touched with a wire loop and growth was transferred to tube containing 5 ml of broth. Turbidity was compared with 0.5 Macfarland standards and adjusted with sterile saline or broth if required. Organism was spread on agar plates by spread plate method and Hi-Comb strip was placed on medium in sterile condition. Plate was incubated for 24 hours at 37 °C and zone of inhibition was observed. According to Hi-Comb MIC test, MIC value is the value at which the zone converges on the comb-like projections of the strips and not at the handle and zone of inhibition below the lowest concentration is to be considered (CLSI 2008).

2.3 Effect of Linezolid on morphology of *L. ivanovii*

After determination of MIC, surface morphology was studied by SEM on Quanta 200 ESEM system (Icon Analytical Equipment Pvt. Ltd., India). Specified concentration of Linezolid (10 µg/ml) was added to culture in the logarithmic phase of growth (12 hour culture) at 37 °C. SEM was done after different incubation time period, 3 hrs, 6 hrs and 24 hrs with Linezolid. Treated and untreated cells after incubation were washed by centrifugation in 0.9 % NaCl and fixed in 2 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and images were taken by SEM (Klainer and Perkins 1974).

2.4 Effect of Linezolid on Dehydrogenases activity

Inhibition of Linezolid on dehydrogenase enzymes activity of *L. ivanovii* was studied by the procedure followed by Guha and Mookerjee (1979). After the cells grown for 48hrs at 37 °C, they were used as samples. According to the procedure, chloramphenicol was added to disrupt cell wall and MIC concentration of Linezolid was added. 0.005 M Substrates of TCA cycle, α -Ketoglutaric acid, Succinic acid, Isocitric acid and Glutamic acid was added to each tube. 0.5 M Potassium phosphate buffer at pH 7.0, 0.3 M MgCl₂ and Triphenyl Tetrazolium Chloride solutions (9 mg/ml) were finally added and OD values of the control tubes and percentage inhibition of the activity of enzymes was calculated.

3. Results

In present investigation, MIC of *Listeria ivanovii* against oxazolidinone linezolid was determined by broth dilution method and Hi-Comb method and was found to be 10 µg/ml. The inhibition in the growth of *L. ivanovii* was read visually by broth dilution method and in Hi-Comb method, zone of inhibition was seen after 24 hour incubation (figure 1).

Morphological alterations on cell surface of *L. ivanovii* after treating with Linezolid for 3 hours, 6 hours and 24 hours are shown in Figures 2 and 3. Images of control organism which was not treated by Linezolid (figure 2) and test organism treated by Linezolid (10 µg/ml) (figures 3a-c) were taken by SEM. Normal control cells were seen as rod shaped while cells treated with Linezolid showed changes at different time intervals. The effect of linezolid (10 µg/ml) on *L. ivanovii* strain resulted in elongation of cells after 3 hours (figure 3a); formation of spheroplasts after 6 hours (figure 3b) and ultimately spheroplasts and small broken pieces of cells were seen after 24 hours incubation time period (figure 3c).

Inhibition of dehydrogenase enzymes was seen after exposing them to Linezolid. Percentage inhibition of dehydrogenases activity was Glutamic 65%, Succinic 50%, α -ketoglutaric 69%, and Isocitric dehydrogenases 62% (table 1). The percentage inhibition of the activity of enzymes was calculated by comparing the O. D. values of the control tubes and the tubes containing Linezolid.

4. Discussion

During the past decade, emergence of bacterial resistance to antibiotics has posed serious concern to medical professionals. Oxazolidinones represent a new synthetic class of antibacterial agents with activity against gram-positive organisms (Brickner *et al.*, 1996). The antimicrobial activities of the oxazolidinones were first described by scientists at E. I. Dupont de Nemours and Co., Inc (Daly *et al.*, 1988; Slee *et al.*, 1987). The Oxazolidinones Linezolid and Eperezolid have shown activity against methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae* and vancomycin-resistant *Enterococcus faecium* (Ford *et al.*, 1996; Jones *et al.*, 1996; Kaatz and Seo 1996; Mason *et al.*, 1996; Zurenko *et al.*, 1996).

Swaney *et al.* (1998) demonstrated that Oxazolidinone Linezolid inhibit the formation of the initiation complex in bacterial translation systems by preventing formation of the N-formyl-methionyl-tRNA-ribosome-mRNA ternary complex. Lin *et al.* (1997) concluded that the Oxazolidinone Eperezolid inhibited protein synthesis by binding to the 50S ribosomal subunit at a site close to the site(s) to which chloramphenicol and lincomycin bind but that the oxazolidinones were mechanistically distinct from these two antibiotics. Cui *et al.* (2005) synthesized a series of oxazolidinones and compared their activities against a panel of gram-positive bacteria with linezolid.

Troxler *et al.* (2000) described a database on susceptibility of *Listeria* species strains to a wide range of antibiotics. Scortti *et al.* (2006) studied fosfomycin susceptibility against *L. monocytogenes* and found resistant in vitro, although they are in fact susceptible to the antibiotics during infection. In present study, Linezolid susceptibility was studied against *L. ivanovii* and MIC determined by both broth dilution method and Hi-Comb method was found to be 10 µg/ml. Hi-Comb MIC test (Himedia) was used for cross checking MIC of the antimicrobial and was found to be 10 µg/ml. Menezes *et al.* (2008) also used Hi-Comb test for determination of MIC on *S. hemolyticus* and *S. aureus* against vancomycin. Hi-comb test is useful for determining the antimicrobial susceptibility of aerobes and anaerobes, non-fastidious and fastidious organisms. Surve and Bagde (2010b) studied susceptibility of *Streptococcus agalactiae* against methicillin with the help of Hi-Comb MIC test and determined its MIC.

Morphological changes observed by SEM in the present study are in substantial agreement with the previous reports of surface disruption of antibiotics treated organisms (Klainer and Perkins 1974; Klainer and Perkins 1972). Formation of spheroplast represents a final common pathway of drug induced injury at many sites within or on bacterial cells. The present study demonstrates that antimicrobial agent whose site of action is thought to be intracellular may cause morphological alterations which are similar to those induced by cell-wall active drugs.

Surve and Bagde (2009; 2010a; 2010b) reported inhibition effects of silver, arsenic and methicillin on dehydrogenase activity of pathogenic microorganisms. Effect of trivalent and hexavalent chromium on a freshwater fish *Anabes scandens* was studied by Venugopal and Reddy (1992) and found that activities of lactate dehydrogenase, malate dehydrogenase and isocitrate dehydrogenase were inhibited. In present investigation, inhibition of four dehydrogenases involved in the TCA cycle, glutamic, succinic, α -ketoglutaric, and isocitric dehydrogenases was found to a greater extent, when bacterial cells were exposed to linezolid.

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Table 1. Effect of Linezolid on the activity of dehydrogenases of *Listeria ivanovii*

Dehydrogenases enzymes	Control			Activity with Linezolid		
	O.D.	Activity %	Inhibition %	O.D.	Activity (%)	Inhibition (%)
Glutamic	0.17	100	0	0.06	35	65
Succinic	0.16	100	0	0.08	50	50
α Ketoglutaric	0.26	100	0	0.08	31	69
Isocitric	0.16	100	0	0.06	38	62



Figure 1. Determination of the MIC of Linezolid against *L. ivanovii* using Hi-Comb method. The clear area indicated the growth inhibition zone of the bacterium (10 µg/ml)

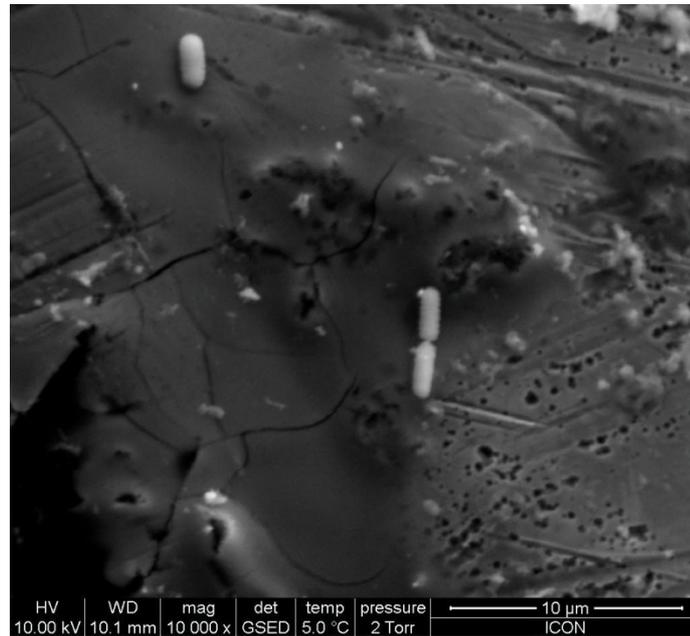


Figure 2. *L. ivanovii* under the untreated condition showing rod-shaped bacilli

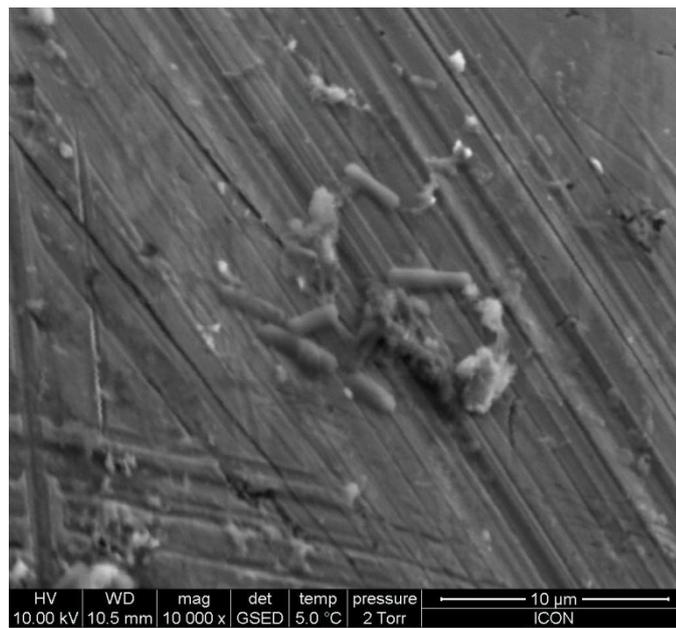


Figure 3a. *L. ivanovii* following treatment with Linezolid (10 μg/ml), exposed for 3 hrs

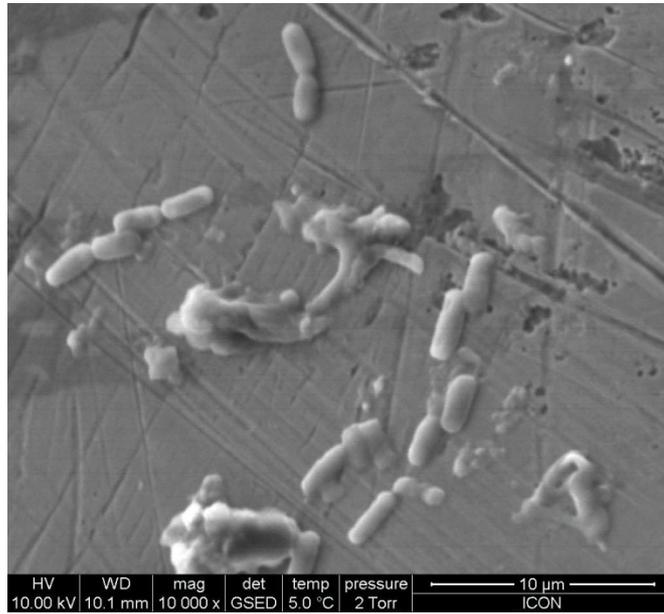


Figure 3b. *L. ivanovii* following treatment with Linezolid (10 µg/ml), exposed for 6 hrs

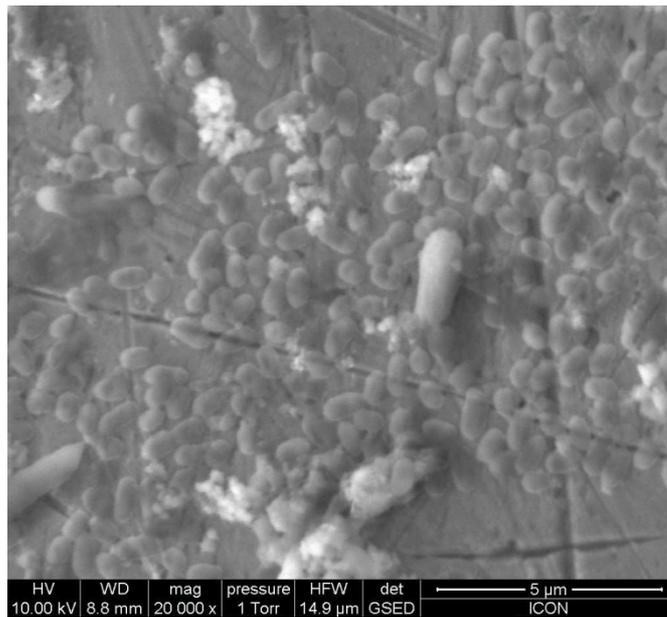


Figure 3c. *L. ivanovii* following treatment with Linezolid (10 µg/ml), exposed for 24 hrs