

# Linezolid Study on Isolates of *Staphylococcus aureus* and *Escherichia coli* Through Disc Diffusion Method

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## Authors' Contributions

1 Conception & Study Design, Data Collection, Data Analysis.

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## ABSTRACT

**Objective:** The objective of this study was to evaluate the effectiveness of Linezolid on *Staphylococcus aureus* and *Escherichia coli* through disc diffusion method.

**Methods:** Discs of Linezolid were used for the evaluation of antibacterial activity of the Linezolid. Antibacterial activity was investigated against gram positive *Staphylococcus aureus* and gram negative *Escherichia coli*. The disc content of Linezolid was composed of 30 µg.

**Results:** The antimicrobial susceptibility test demonstrated that Linezolid was effective against *Staphylococcus aureus* and *Escherichia coli*. Two isolates of *Staphylococcus aureus* showed susceptibility (zones were found 26mm and 22mm), while one of the isolates of *Escherichia coli* showed susceptibility and the other showed resistance (zones were found 21mm and 18mm, respectively).

**Conclusion:** It is concluded that Linezolid have better antibacterial activity on *Staphylococcus aureus* as well as on *Escherichia coli* so can be used in treatment of different infections.

**Keywords:** *Staphylococcus aureus*, *Escherichia coli*, linezolid, disc diffusion, oxazolidinone.

## INTRODUCTION

Antibiotic was seeing wonder drugs when they appeared because antibiotic can treat every infection. As soon, it was observed that the treated bacteria can build resistance in contrast to them and the resistance can be intrinsic or acquired [1]. The antibiotics are chemical compounds, which minimize the production of microorganism and ultimately kill microorganism. Natural fermentation or chemical synthesis may help in the production of these drugs. The antibiotics are the drugs, which are obtained from the compounds by different microbial flora. It is also noted that not every antibacterial compounds are antibiotics and obtained completely by chemical synthesise [2]. Though moldy materials helped in healing the wounds and infections, but it was observed in late 19th century that this was due to the microbes. Fleming's, Chain's

and Florey's clinical observations, development studies made a new innovation in antibiotics in the twenty century [2].

The new addition in the antimicrobial world is the oxazolidinone group of antibiotics which can play various significant roles in order to combat the infections occurred by Gram positive bacteria. The oxazolidinone provides greater result in contrast to Gram positive microorganisms and produces high resistance against microbial in clinical situations. Oxazolidinone is very useful and also shows a moderate microbial effectiveness in contrast to Gram negative bacteria.

Oxazolidinone substances are structurally available with 2-oxazolidinone. Normally oxazolidinone group of antibiotics comprises the 2-oxazolidinone along four alternative ring of phenyl at three locations [3].

DuPont in the year 1980 introduced the oxazolidinone



antibacterial substances very first time. Oxazolidinone antibacterial compounds were seen problematic because they produced toxic effects in clinical studies. After few years oxazolidinone class was studied more for enhancing efficacy and safety, with the introduction of two vital and useful compounds like Eperezolid and Linezolid. Bioactive and toxic examination of both of a Linezolid and Eperezolid were almost same, so clinical testing was performed. But Linezolid was found to be more advantageous as it can be taken twice a day while Eperezolid should be taken thrice/ day. So, Linezolid was the prime oxazolidinone compound which was permitted by Food Drug Administration [3].

### Linezolid

Linezolid chemically known as S-N-[3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl]-acetamide [4].

Linezolid was recently registered for use clinically in more than fifty countries. Food drug administration (FDA) allowed indications include *Vancomycin-resistant enterococci* infections, consisting *bacteremia*, *nosocomial pneumonia* occurred by *Staphylococcus aureus* and *Streptococcus pneumoniae*, complicated skin and skin structure infections occurred by *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus agalactiae*, uncomplicated skin and skin structure infections occurred by *Staphylococcus aureus*, and community acquired pneumonia from *Streptococcus pneumoniae* and or *Staphylococcus aureus*. There are various phases like phase II and III trials assessing Linezolid activity in these infections [5]. Previous investigations evaluated that Linezolid was very effective in terms of a number of significant gram positive cocci, like *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus* species, and streptococci [6]. Time kill evaluation showed that Linezolid was effective in respect of staphylococci and enterococci and determined bactericidal effectiveness versus few streptococci [6]. The *in vitro* investigation of Linezolid and eperezolid demonstrated that resistance take place hardly by simple mutation in *Staphylococcus aureus* and include few problems or create no issue with adopted antimicrobial force, with a spiral gradient protocol [6]. Linezolid is synthetic in nature that is why susceptibility of this substance was great uniform in large observations of ingenuous populations of target

organisms [7]. The recent demonstration of a process to Linezolid acquired resistance, [8] evaluated that Linezolid or Eperezolid-resistant *E. faecalis* and *S. aureus* isolates of from laboratory origin resulted single G → U mutation at area 2447 / 2576 of the central loop of domain V of twenty three S rRNA [9]. The antibacterial treatment helps the body to terminate infectious microorganisms without producing any toxic effect to the host. The patient's natural defense process should be known to avoid the infections [2]. Generally, antibacterial are classified on the basis of their mode of action, their bacteriostatic and bactericidal activity. Actually, the inhibition method of bacteriostatic substances involves inhibition of protein synthesis or few bacterial metabolic passages. As bacteriostatic substance just prevents the growth of the pathogenic bacteria, sometimes it is problematic to mark a clear boundary between bacteriostatic and bactericidal, especially when high concentrations of few bacteriostatic substance are used then they may work as bactericidal [10]. Researchers assessed the *in vitro* studies in contrast fifty four methicillin-resistant *Staphylococcus aureus* (MRSA) strains using agar dilution method in conjunction with scanning electron microscopy of only Linezolid and in combination of vancomycin or teicoplanin. Their study revealed that Linezolid as a single agent over vancomycin and teicoplanin in contrast to MRSA isolates. Linezolid and vancomycin shows better activity than Linezolid and teicoplanin at all concentrations [11]. The comparison of Minimum inhibitory concentrations and Disc inhibition zones was done as per suggested by the National Committee for Clinical Laboratory Standards and the British Society for Antimicrobial Chemotherapy for one ninety eight strains of gram-positive cocci. Zones were found to be 4-5 mm larger by the British Society for Antimicrobial Chemotherapy process, but MICs showed no variations, except for pneumococci, which was found to be very sensitive when the British Society for Antimicrobial Chemotherapy method was utilized. The activity of Linezolid depresses due to the incubation in CO<sub>2</sub> against this species only [12].

### Antibiotic Susceptibility Testing

The most common laboratory test to determine the effectiveness of antimicrobials is the susceptibility that evaluates the ability of an antibiotic to inhibit the growth of microorganisms. Susceptibility test is performed in order to recommend the physicians /



pharmacist to select ideal and best antibiotic for a particular patient and to collect the epidemiological data within the society [13]. Antimicrobial susceptibility testing methods are classified depend on the principle applied in each system. They include:

### Disc Diffusion Method

The agar diffusion test (Kirby-Bauer antibiotic testing, KB testing, or disc diffusion antibiotic sensitivity testing) is a test used to determine the antibiotic. With the help of antibiotic discs, the bactericidal or bacteriostatic nature of the antibiotics can be measured. The method is divided into two types:

I- Kirby-Bauer method

II- Stokes method

KB testing is basically base on antibiotic permeated disc, which was implanted on agar already inoculated with bacteria. The moisture was picked up and diffused the antibiotic rapidly through the agar medium, resulting in antibiotic concentration gradient. The concentration of antibiotic at the corner of the disc is greater and constantly lower where the distance from the disc enhances to mark. At this area it is no longer shows any inhibitory response for the organism, so it then grows easily. A visible zone appears around an antibiotic disc after incubation, if the compound suppresses bacterial growth. [14]. KB testing is done with Mueller Hinton Agar for susceptibility test on a regularly basis due to the better reproducibility, low in sulphonamide, trimethoprim, and tetracycline inhibitors, and shows adequate growth of most bacterium. KB testing Inoculum was made with appropriate broth like Trypticase soya broth. The medium was manufactured followed the guidelines of manufacturer's, dispenses in tubes at 4-5 ml and sterilized [14].

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## METHODOLOGY

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The current study was studied in Department of Pharmaceutics, Federal Urdu University. Linezolid discs were purchased by Musajee Adam and Sons Company while 70% Isopropyl Alcohol, Sterile Distilled Water (WFI), Nutrient broth, Mueller- Hinton Broth (MHB) were also used. The interest of this study was to perform antibiotic susceptibility test in order to establish the *in vitro* activity of Linezolid against infection caused by *Staphylococcus aureus* and *Escherichia coli*.

### Preparation of Agar Media

- I. With the help of manufacturer's guidelines, Mueller Hinton Agar was made by dehydrated medium using distilled water or de-ionised water.
- II. The medium was heated till boiled with constant stirring in order to liquefy complete.
- III. The medium was sterilized in autoclave at 121 °C for 15 minutes.
- IV. After sterilization, pH of every preparation was checked at room temperature (should be in between 7.2 and 7.4).
- V. After that agar medium was allowed to cool to 40-50 °C and was transferred into glass or petri dish, with a uniform depth of 4mm.
- VI. The medium was allowed to become solid.
- VII. Dry plates with lids were kept in an incubator at 30 °C to 37 °C for 30 minutes or when the unnecessary moisture is evaporated. Media should be moist and free from water droplets. Droplets of water may favor the growth of bacteria which results in contamination.

### Inoculum Preparation

1. Four or five colonies were streaked out with the help of wire loop from the culture of bacteria. (The culture should be not more than 48 hours as the old cultures result in slow growth).
2. Colonies were transferred to 5 ml of Trypticase soya broth or 0.9% saline.
3. The broth was incubated at 30 °C until it was matched the turbidity of 0.5 McFarland standards.
4. The test bacterial suspension's turbidity was compared with 0.5 McFarland (it was vigorously shaken before use) in contrast to white background with black line using proper light. Arrow points to tube with correct turbidity.
5. If the test sample's turbidity exceeds the turbidity of McFarland's turbidity, then dilute it with the help of sterile saline or broth.

### Standardizing Inoculum

At adequate turbidity, bacterial culture suspension was made and incubated as well as to standardize the inoculum density 0.5 McFarland was used.



**Steps Involved in Kirby-Bauer Method**

1. The colonies of both the cultures of *Staphylococcus aureus* and *Escherichia coli* were streaked from the petri plates with the help of inoculating loop.
2. After that these colonies were transferred in a Mueller-Hinton Agar broth tube, and were incubated at 37°C.
3. The inoculums turbidity was maintained, similar to that of the McFarland standard's turbidity and was incubated with their lids at 10-15 minutes. The depth of the Mueller-Hinton Agar plate should be 4 mm. The culture suspension was vortexed properly in order to attain maximum mixing.
4. Sterile cotton swab was dipped into bacterial suspension and eliminated the extra liquid by pressing the cotton swab against the sides of the tube.
5. Through streaking, the agar was inoculated using cotton swab consisting of the inoculum.
6. The Discs consisting of 30µg of Linezolid on the inoculated MHA plate. Every disc pushed down in a manner that these were fully interacted with agar.
7. Agar plates were incubated at inverted position.
8. The zone of inhibition was observed from

the back of the plate with reflected light and the zone was measured with the help of ruler.

9. The antimicrobial susceptibilities standard tables clinical and laboratory institute standard [15] were used in order to find out the susceptibility, intermediate or resistance of the strain to the Linezolid.

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**RESULT**


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The antibacterial activity of Linezolid disc against different isolates of *Staphylococcus aureus* and *Escherichia coli* was shown in Table 1.

*Staphylococcus aureus* showed susceptibility and zones were found to be 26mm and 22mm, while *Escherichia coli* showed susceptibility as well as resistance and zones were found to be 21mm and 18mm, respectively.

Linezolid disc showed diameters of zone inhibition (susceptible / resistance) in a range of 18-26 against different bacterial strains which is as per CLSI standard. The results achieved from Linezolid disc showed susceptibility to two isolates of *Staphylococcus aureus* and one isolate of *Escherichia coli*. Moreover, Linezolid disc showed resistance against one isolate of *Escherichia coli* as well.

**Table1. Result of antibacterial activity of Linezolid Disc against two isolates of different bacteria.**

Isolates	Disc Content	Susceptible (S)	Resistance (R)	Intermediate (I)
<i>Staphylococcus aureus</i>	30 µg	26 mm	-	-
<i>Staphylococcus aureus</i>	30 µg	22 mm	-	-
<i>Escherichia coli</i>	30 µg	21 mm	-	-
<i>Escherichia coli</i>	30 µg	-	18 mm	-



## DISCUSSION

Assessment of antimicrobial susceptibility testing study indicated that Linezolid is very effective and helpful to treat infections caused by gram positive and gram negative bacteria.

The important function of the clinical microbiology laboratory is the role of antimicrobial testing of bacterial isolates. The main purpose of this testing is to evaluate the resistance of certain bacteria against the drug and to observe whether the particular bacterial isolate is susceptible, resistant or bactericidal against the infections. The most important benefit of Disc diffusion method is that it is very simple and it does not require any distinctive equipment, the clinicians can easily elucidate the results and also the selection of this method is flexible [16]. Linezolid inhibited the protein synthesis in a unique fashion and used to treat the community-acquired pneumonia, skin and soft-tissue infections and other infections caused by Gram-positive bacteria including vancomycin resistant enterococci (VRE) and methicillin-resistant staphylococci. Linezolid shows less resistant against these pathogens i.e., <1.0%, although it is widely spread in many countries. Due to the resistance produced by clinical isolates, regular susceptibility test of Linezolid should be performed in clinical laboratories as well as, considered critically the therapeutic uses of Linezolid. As for the treatment of infections produced by Gram-positive bacteria, Linezolid is a very important bactericidal agent, this review should be considered to provide maximum clinical use of Linezolid [17]. Stevens in 2000 demonstrated that the Linezolid is too effective like oxacillin - dicloxacillin to eradicate *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus agalactiae* [18].

## CONCLUSION

It is concluded from current study that the Linezolid is an important choice to tackle infections caused by *Staphylococcus aureus* and *Escherichia coli*. Linezolid showed the best bactericidal activity against *Staphylococcus aureus* and a much better response was also observed with *Escherichia coli*.

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