"Invitro Investigation of Linezolid Susceptibility against Clinical Samples of Staphylococcus Species"

Nashra A[1], Deepak S[2], R. Sujatha[*], Arunagiri D.[3]

Abstract:

Introduction: The expanding incidence of antimicrobial resistance among Gram-positive pathogens is becoming a major concern. Linezolid is a synthetic antimicrobial, belonging to the class of oxazolidinones that acts in the protein synthesis, specifically in the binding to the 50S subunit of the ribosome, with bacteriostatic action and great importance on treatment of MRSA.
Aim and Objective: Invitro Investigation of Linezolid susceptibility against Clinical Samples of Staphylococcus aureus.
Material and Methods: Total 100 Staphylococcus species was isolated from clinical samples. Identification of Staphylococcus species was done by standard conventional microbiological methods. Antibiotic susceptibility testing was done by using disk diffusion method and MIC as per CLSI guidelines.
Results: Out of total 100 samples, the isolates were MSSA (18), CoNS (28), MRSA (38), and MR-CoNS (16).The Males was more in number as compared to Females. The isolates was more in the age group of 21-40 years and minimum in age group above 61 years.. All strains were 100% sensitive to linezolid.
Conclusion: The expanding incidence of antimicrobial resistance among Gram-positive pathogens is becoming a major concern. All the isolates were sensitive to linezolid hence remains the drug of choice for gram positive organisms. Hence, regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of resistance pattern.

Keywords: Staphylococci, MRSA, MIC, CLSI

Introduction

Staphylococcus genus is a heterogeneous group of bacteria consisting of 30 species. Staphylococcus aurous has been found to be the most clinically important species, with broad presence in nature. It is part of the normal flora of human body and commonly carried on the skin or in the nose of healthy individuals, which makes it easy to be transmitted by air or fomites from patients or carriers [1,2]. It been recognized as one of the most common cause of human infections, such as skin infects, wound infections and bacteremia. Nevertheless, the introduction of antibiotics has lowered the mortality rate of S. aureus infections. However, the bacteria have rapidly developed resistance mechanisms against many antimicrobial agents [1, 3]. Methicillin-Resistant Staphylococcus aureus (MRSA) has been isolated and recognized more than 50 year ago. MRSA is a specific strain of the S. aureus, which is resistant to methicillin and all β-lactams. Later use of Oxacillin as an alternative to methicillin in susceptibility tests resulted in the term 'Oxacillin-resistant S. aureus' (ORSA) [2], which is resistant to numerous antibiotics. Before the Development of antibiotics, invasive infections caused by Staphylococcus aureus have often been fatal [4].

The global spread of MRSA constitutes one of the most serious contemporary challenges to the treatment of hospital-acquired infections [5]. MRSA carries a uniquely effective antibiotic resistance mechanism that can protect the microorganisms against all members of β-lactam antibiotics. This makes infections caused by these pathogens very difficult to manage and costly to treat [6, 7]. Linezolid can be considered as the first member of the class of oxazolidinone antibiotics that was approved for clinical use in 2000 for the treatment of nosocomial and community-acquired pneumonia, uncomplicated and complicated skin and skin structure infections, and infections caused by vancomycin-resistant Enter ococcus faecium [8, 9]. The unique mode of action of linezolid involves binding of the agent to the ribosomal 50S subunit in domain V of the 23S rRNA. As a result, the 50S subunit is prevented from interacting with the 30S subunit for the formation of the 70S initiation complex. The unique inhibition of protein synthesis initiation by linezolid confers potent antibacterial properties. This mechanism of action is refractory to cross-resistance from the presence of resistance mechanisms that impact other agents that target ribosome-mediated protein synthesis (e.g., macrolides, lincosamides, streptogramins, and chloramphenicol) [10]. Plasma concentrations of intravenous and oral linezolid are equivalent [11], with average concentrations exceeding the MICs for

[1]*Research Assistant, Central research lab, Rama Medical College Hospital and Research Center Kanpur (India)*
[2]*Tutor, Department of Microbiology, Rama Medical College Hospital and Research Center Kanpur (India)*
[*]*Prof and Head*, Department of Microbiology, Rama Medical College Hospital and Research Center Kanpur (India)*
[3]*Professor & HOD, Dept of Endodontics, MDC, Kanpur (India)*

susceptible pathogens throughout the 12-h dosing interval [12]. Clinical trials demonstrate that linezolid is well tolerated and that it is as effective as standard therapies [13]. We report the results of the largest randomized, comparator-controlled, open-label clinical trial to date comparing the safety and efficacy of linezolid with that of vancomycin in treating patients with presumed MRSA infections. In comparative clinical trials, linezolid was as effective as vancomycin for treating nosocomial pneumonia [14] and MRSA and as effective as oxacillin-dicloxacillin for the treatment of complicated skin and soft tissue infections [15].

Material and Methods

This study was conducted in the Department of Microbiology; Rama Medical College Hospital & Research centre Kanpur. It was a Prospective study design with Observational study conducted for a period of 1 year from January 2018 to December 2018. The sample size calculated was 100. A suitable statistical test was carried out according to the study.

Inclusion criteria: The entire clinical sample from IPD and OPD patients like pus, urine, sputum, blood and body fluids was included in the study.

Exclusion criteria: Patients on antibiotics more than one week was excluded in this study.

Procedures Data Collection: Data included age, Sex, Medical history (including transfer and length of stay), Medication history, as well as questions focusing on degree of illness.

Sampling Method

Specimen Collection: Specimens like Pus collected using sterile cotton swabs, moistened with sterile normal saline immediately before collection , Blood where Blood samples was collected and transported to the laboratory in the brain heart infusion broth, sputum which was collect in a disposable, wide-mouthed, screw-capped plastic container of about 100 ml capacity, urine in which about 20 ml of clean catch mid stream urine sample was collected from all patients with urinary tract infection in sterile, dry, wide-necked and leak proof containers and Body fluids about only 3-5 ml of body fluids like CSF was collected by the lumber puncture only by the well trained physicians. Care was taken to avoid contaminating the specimen with commensally organisms from the skin and transported immediately to the Laboratory.

Mic Test: MIC test was performed for all the isolates as per the CLSI guideline (CLSI) 2018 **[17]**.

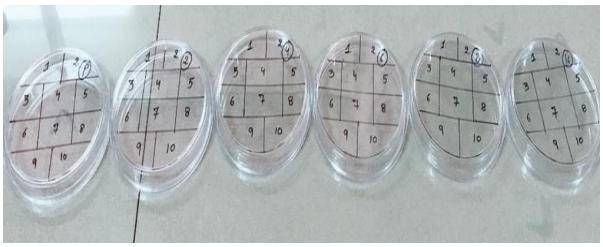


Figure 1: Labelling and pouring in different concentrations (µg/ml)

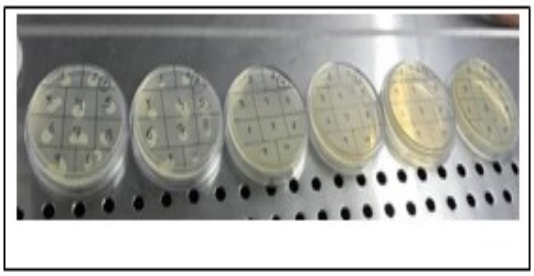


Figure 2:- Micro dilution detection of linezolid

Results

A Total of 100 *Staphylococcal* species was studied in our study.

Table 1: Age Wise Distribution

Age	No. of Patients	Percentage
0-20	14	14%
21-40	52	52%
41-60	28	28%
61-80	6	6%
Total	100	

Out of 100 cases, the maximum age group was in 21-40 years and minimum age group was in the age group of 61-80 and above

.

Table 2: Gender Wise Distribution

S. No.	Sex	No. of Patients
1.	Male	70
2.	Female	30
3.	Total	100

Table 3: Types of Samples Collected

Type of Sample	No. of Samples
Pus	**72**
Blood	**12**
Urine	**8**
Body Fluids	**4**
Sputum	**4**
Total	**100**

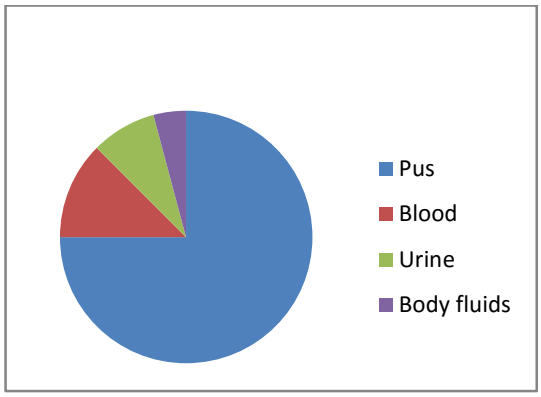
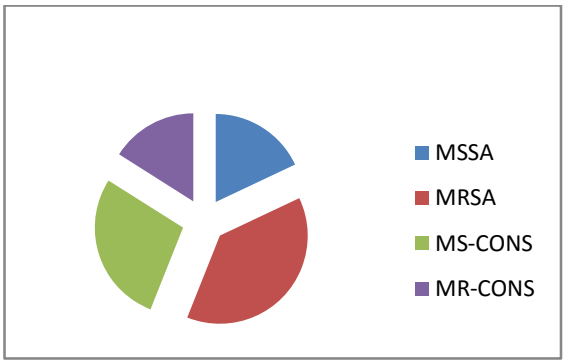


Figure 3: Types of sample collected

The highest sample was from the Pus.

Staphylococcus aurous		*Coagulate Negative Staphylococcus*	
MSSA	MRSA	MS-CoNS	MR-CoNS
18	38	28	16



Graph 4: Total no. of organisms isolated

Among all the isolated, the most common isolates was MRSA i.e, 38.

Table 5: Antibiotic Sensitivity Pattern of MSSA Isolates

Antibiotics	S. Aureus
Penicillin	4 (22.2%)
Erythromycin	6 (33%)
Amikacin	10 (55%)
Clindamycin	8 (44%)
Cefoxitin	4 (22.2%)
Oxacillin	6 (33%)
Tetracycline	18 (100%)
Teicoplanin	18 (100%)
Nitrofurantoin (urine)	8 (44%)
Norfloxacin(urine)	8 (44%)
Linezolid(MIC)	**18 (100%)**

Graph 6: Antibiotic sensitivity pattern of MSCoNS

In case of MSCoNS, Linezolid, Vancomycin and Teicoplanin were 100% sensitive, followed by Oxacillin (85%), Cefoxitin (78%) and Tetracycline (64%). So above three mentioned can be the drug of choice for the treatment of MSCoNS.

Antibiotics	MRSA(38)	MRCoNS (16)
Penicillin	14(36%)	4(25%)
Erythromycin	28(73%)	6(37%)
Amikacin	26(68%)	16(100%)
Clindamycin	12(31%)	10(62%)
Cefoxitin	0(0%)	0(0%)
Oxacillin	0(0%)	0(0%)
Tetracyline	38(100%)	0(0%)
Teicoplanin	38(100%)	16(100%)
Nitrofurantoin (urine)	0(0%)	0(0%)
Norfloxacin (urine)	0(0%)	0(0%)
Linezolide (MIC)	**38(100%)**	**16(100%)**

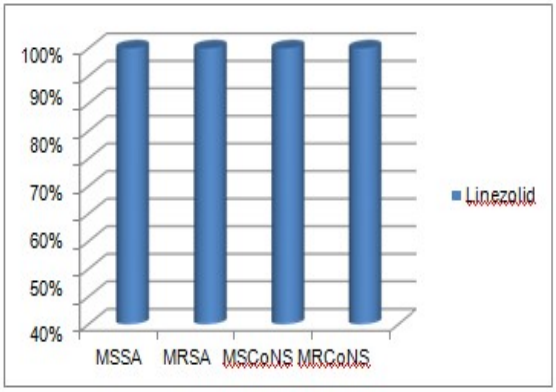
Graph 7: Antibiotic sensitivity pattern for MRSA and MR-CoNS

In case of MRSA and MR-CoNS Vancomycin, Teicoplanin, and Linezolid were 100% sensitive.

Table 11: Sensitivity Pattern of Linezolid for All the Isolates

No.of isolates	Action of linezolid
MSSA	100%
MRSA	100%
MSCoNS	100%
MRCoNS	100%

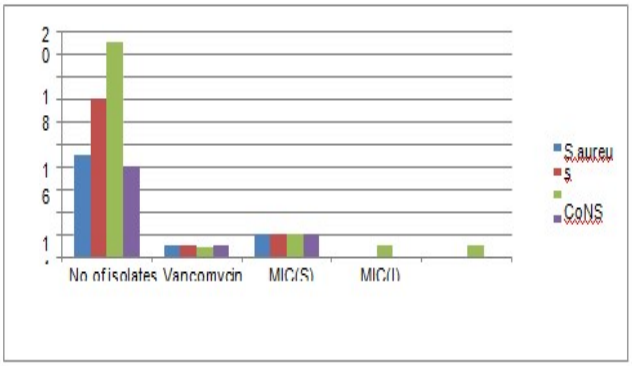
Graph 8: Sensitivity Pattern of linezolid in all the isolates.



Linezolid was 100% sensitive to all the isolates

Table 9: Mic of Linezolid for the Isolates

Antimicrobial Agents			MIC interpretive criteria (µg/ml)		
Types of isolates	No. of isolates	Linezolid	Sensitive (≤4)	Intermediate	Resistant (≥8)
S. aureus	18	100%	≤4	-	-
CoNS	28	100%	≤4	-	-
MRSA	38	100%	≤4	-	-
MR-CoNS	16	100%	≤4	-	-



Graph 10: MIC of Vancomycin for all the Isolates

Among the MRSA isolated from blood, 1 was found to be VISA with the MIC of 8µg/ and the other 1 was VRSA with the MIC of 32µg/ml.

Discussion

Increasing prevalance of Gram positive cocci that are resistant to antimicrobials has complicated the treatment of infections due to these microorganism. The spread of methicillin- resistant *Staphylococci* underline the need for therapeutic alternatives. Linezolid is the first of a new group of agents, the oxazolidinones, which are synthetic antibacterial agents. The good clinical outcome and low side effects associated with linezolid indicates that it might be an appealing option for the treatment of infections caused by MDR Gram-positive pathogens.

Table 11: Age Wise Distribution

S. No	Study	Year	Result
1	Archana Bhimrao et.al **[18]**	2017	In this study 61% cases were in the age group of 15-65 years
2	Ruby Thomas et.al **[19]**	2017	In this study 28% cases were in the age group of 16-30 years.
3	**Present study**	**2018**	**In in study 52% cases were in the age group of 21-40.**

In our present study 52% cases was in the age group of 21-40 years which is similar to Archana Bhimrao et.al.

Table 12: The Gender Wise Distribution

S.No.	Study	Year	Male	Female	Total
1	Ruby Thomas et.al [19]	2016	60 (43%)	79 (56.83%)	69
2	Archana Bhimrao et.al [18]	2017	42 (61%)	27 (39%)	69
3	Wilfed Gitau et.al **[20]**	2018	511 (54%)	433 (45%)	944
4	Present study	2018	70 (70%)	30 (30%)	100

In present study male ratio was more 70(70%) then female 30(30%) in correlation with the study of Wilfed Gitau et.al and Archana Bhimrao et.al, but was in contrast with Ruby Thomas el al.,

Table 13: Distribution According to the Types of Samples

S. No.	Study	Year	Result
1	Muhammad et.al [21]	2013	Isolates were from blood, and followed by husband other clinical samples
2	Cennet et.al [22]	2016	Isolates were from pus, ear, blood followed by urine & abscess.
3	Ruby Thomas et.al [19]	2016	Isolates were from pus sample, sputum, urine, & blood.
4	Archana Bhimrao et.al [18]	2017	Isolates were from pus, followed by urine, blood and body fluid.
5	**Present study**	**2018**	**Isolates was from pus, blood, then followed by urine, sputum and body fluid.**

In the present study the most of the strains was isolated from the pus sample. Which is accordance with the study of Ruby Thomas et.al and others but was in contrast with Muhammad Murad et.a

Table 14: Distribution According to Antibiotic Sensitivity Pattern

S.N0	Study	Year	Result
1	Archana Bhimrao et.al**[18]**	2016	All the isolates were 100% sensitive to Vancomycin. Followed by Teicoplanin(87%) and Linezolid(80%).
2	Ruby Thomas et.al **[19]**	2018	In case of MRSA, Linezolid and Vancomycin worked the best with 100% sensitivity.
3	**Present study**	**2018**	**In all the isolates, Linezolid, Teicoplanin and Vancomycin showed 100% sensitivity.**

In present study all the isolates was 100% sensitive to Vancomycin, Teicoplanin and Vancomycin. Which is accordance with the study of Ruby Thomas Et.al?

Table 15: Distribution According to the Linezolid Mic Test

S. No.	Study	Year	Result
1	Jones RN et.al **[23]**	2009	All isolates had an MIC of <4µg/Ml
2	**Present Study**	**2018**	**All isolates were sensitive to linezolid and had an MIC of ≤4µg/Ml**

In present study the MIC of the linezolid was ≤4µg/mL, which is in correlation with the study of Jones RN et.al

Table 16: Distribution According to the Vancomyc in Mic Test

Sl. No	Study	Year	Result
1	Ramana et al.**[24]**	2012	75 were Vancomycin sensitive (MIC ≤2µg/ml) and 4 were VISA (MIC 4-8µg) from blood
2	Rashedul et al.**[25]**	2016	11 were Vancomycin sensitive (≤2µg/ml), 8 were resistant to Vancomycin (3µg/ml) from burn wound.
3	**Present study**	**2018**	**36 were Vancomycin sensitive, 1 was VISA (8µg/ml) and 1 was VRSA (32µg/ml) from blood.**

In present study, out of 38 MRSA isolated. 36 was Vancomycin sensitive, 1 VISA with an MIC of 8µg/ml and 1 VRSA with an MIC of 32µg/ml was also isolated from blood, which is in correlation with the study of Ramana et al.

Conclusion

Resistance mechanisms have been present in bacteria for millennia, while antibiotics have been in clinical use for approximately 80 years. Faced with selective pressure from increasing antibiotic use, bacteria have adapted and developed complex mechanisms in order to survive. This, along with decreasing interest in antibiotic development by the pharmaceutical industry, makes it clear that preserving our current antibiotic armamentarium through wise antibiotic stewardship is paramount.

Although linezolid is efficient against multidrug-resistant gram-positive, researchers must strive and optimize infection-control measures to inhibit their spread.This will be beneficial to preserve the effectiveness of antibiotics and for better patient management. Further development of novel compounds, identification of additional drug targets, better stewardship, and more informed choices about combination therapy will hopefully allow us to continue to treat MRSA infections for the foreseeable future.

References

1. Hardy KJ, Hawkey PM, Gao F, Oppenheim BA. Methicillin resistant Staphylococcus aureus in the critically ill. Br J Anaesth. 2004; 92:121-130.

2. Brown DF, Edwards DI et al. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant Staphylococcus aureus (MRSA). Jounal Antimicrobial Chemotherapy. 2005; 56:1000-1018.
3. Anupurba S, Sen MR ET. Al. Prevalence of methicillin resistant Staphylococcus aureus in a tertiary referral hospital in eastern Uttar Pradesh. Indian Journal Medical Microbiology. 2003; 21:49-51.
4. Palavecino E. Clinical, epidemiological, and laboratory aspects of methicillin-resistant Staphylococcus aureus (MRSA) infections. Methods Molecular Biology. 2007; 391:1-19.
5. Szczepanik A, Koziol-Montewka M, Al-Doori Z, Morrison D, Kaczor D. Spread of a single multiresis tant methicillin-resistant Staphylococcus aureus clone carrying a variant of staphylococcal cassette chromosome mec type III isolated in a university hospital. European Journal Clinical Microbiology Infection Disease. 2007; 26:29-35.
6. Hiramatsu K, Katayama Y, Yuzawa H, Ito T. Molecular genetics of methicillin-resistant Staphylococcus aureus. International Journal Medical Microbiology. 2002; 292:67-74.
7. Aires de Sousa M, de Lencastre H. Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant Staphylococcus aureus clones. FEMS Immunology Medical Microbiology. 2004(8)40: 101-11.
8. Livermore, D.M. Linezolid in vitro mechanism and antibacterial spectrum. Journal of Antimicrobial Chemotherapy. 2003; 51(2):9-16.
9. Moellering. R. C., Jr. Linezolid: the first oxazolidinone antimicrobial. Annal International Medicine. 2003; 138:35-42.
10. Hershberger, E., S. Donabedian, K. Konstantinou, and M. J. Zervos. Quinupristin-dalfopristin resistance in gram-positive bacteria: mechanism of resistance and epidemiology. Clinical Infect. Dis.2004; 38:92–98.
11. Welshman IR, Sisson TA, Jungbluth GL, et al. Linezolid absolute bioavailability and the effect of food on oral bioavailability. Biopharmacautic Drug Dispisition. 2001; 22:91-7.
12. Stalker DJ, Wajszczuk CP et.al. Linezolid safety, tolerance, and pharmacokinetics following oral dosage twice daily for 14.5 days. American Society for Microbiology. 1997; 115:462-56.
13. Bone, R.C., Balk, R.A., et.al. Defination for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine.1992; 101:1644-1655.
14. Stevens, D.L., Lampiri, H et al. Linezolid versus Vancomycin for the treatment of Methiciliin-resistant Staphylococcus aureus infections. Clinical Infectious Diseases. 2001; 34:1481-90.
15. Smith, L.G., et al. Randomized comparison of linezolid versus oxacillin- dicloxacillin for the treatment of complicated skin and soft tissies infections. Antimicrobial Agents and Chemotherapy. 2002; 44:3408-13.
16. Mackie & McCartney, Practical Medical Microbiology, Churchill Livingstone. 2006; 14 th edition: 796-798, 135-141,152.
17. CLSI. Performance standard for Antimicrobial susceptibility testing 28th ed. CLSI supplement M100.Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
18. Archana Bhimrao et al. Study of antimicrobial resistance profiling of Staphylococcus aureus isolated from clinical specimen. International Journal of Research in Medical Sciences .2017; 5(11): 4808-4811.
19. Ruby Thomas et, al. Prevalence and Recent Trend in the Antibiogram of Staphylococcus aureus isolated from clinical specimen. International Journal of Current Microbiology and Applied Sciences. 2016; 5(12):644-649.
20. James, D., Warner, M. & Gardiner, Antimicrobial susceptibility pattern of Staphylococcus aureus isolated from clinical specimens. Bio Medical Central. Research. 2018; .11:226.
21. Muhammad Murad et, al. Identification and antibiotic susceptibility pattern of coagulase-negative Staphylococci in various clinical specimens. International Medicine Sciences. 2013; .29:6.
22. Cennet Ragbetli et, al. Evalution of Antimicrobial Resistance in Staphylococcus aureus Isolates. Interdisciplinary Perspectives on Infections Diseases. 2016; 22: 4.
23. Jones R N, Kohno S, Ono Y, Ross JE et al. ZAAPA International Surveillance Program linezolid resistance. Diagnosis Microbiology Infection Diseases. 2009; 64:191-201.
24. Ramana BV et al. Antibiotic therapeutic options for infections caused by drug- resistant Gram-positive cocci. Journal of Infetion Public Health. 2009; 2:62-73
25. Rashedul Hasan et al. Prevalence of vancomycin resistant Staphylococcus aureus (VRSA) in methicillin resistant S.aureus (MRSA) starins isolated from burn wound infections.Clinical medical Journal. 2016; 28:49-53