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Research Article

PREVALENCE OF LINEZOLID RESISTANCE IN GRAM POSITIVE PATHOGENIC COCCI ISOLATED FROM CLINICAL SAMPLES IN A TERTIARY CARE CIVIL HOSPITAL, AHMEDABAD

SONI S.T.*, PATEL K.J., RADADIYA R.P., PRAJAPATI V.K., MISTRY D.J. AND SHARMA H.D.
Department of Microbiology, B. J. Medical College, Gujarat University, Ahmedabad, Gujarat, 380016, India
*Corresponding Author: Email - riddhi.pr.rp@gmail.com , drsumeetasoni@gmail.com

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Abstract- Introduction: The first approved oxazolidinone group of drugs, Linezolid is nowadays have been used for multidrug resistant bacterial infection, including *Staphylococci, Streptococci, Enterococci* infections. Resistance to Linezolid is not common but now a days some cases of Linezolid resistance have been reported. So, this study is done to see the prevalence of Linezolid resistance in gram positive pathogenic cocci isolated from various clinical samples in tertiary care Civil Hospital, Ahmedabad. Material and Methods: The present study was conducted over a period of 20 months (January 2020 to August 2021). Various samples like blood, pus, swabs, CSF, body fluids, *etc.* are included in this study. From positive growth of these samples gram positive pathogenic cocci were isolated and confirmed. Antimicrobial susceptibility tests were done according to CLSI guidelines 2021. Results: Total 54266 samples were tested out of which gram-positive cocci were 5548 (10.22%). Out of these only 53 (0.95%) isolates were Linezolid resistant. The most commonly found Linezolid resistance is in *Enterococcus fecium*-29(54.72%). Conclusion: Linezolid which was the promising agent against multi drug resistant gram-positive cocci such as VRE, MRSA is now showing resistance. Its detection in clinical samples is necessary for deciding treatment protocols by clinicians.

Keywords- *Linezolid resistance, Staphylococcus aureus, Enterococcus faecium, Streptococcus spp*

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Introduction

Linezolid is the first approved drug of newer antimicrobial group, the oxazolidinones, to be approved for clinical use in the United States and other places [1]. It was first introduced in 19802 and US Food and Drug Administration (FDA) approved the use of Linezolid, in April 20003. Linezolid shows activity against most of the Gram-positive organisms and *mycobacteria* but not Gram-negative organisms [3]. It also shows activity against aerobic and anaerobic gram-positive bacilli, anaerobic gram-positive cocci, some gram-negative anaerobes and Nocardia species also [1,2]. Important function of Linezolid is its activity against multidrug resistant bacteria *i.e.*, *methicillin-resistant staphylococci, penicillin-resistant pneumococci, macrolide-resistant streptococci*, and *vancomycin-resistant enterococci* [1]. It is approved drug for the treatment of MRSA for >40 years and the first oral agent approved for *vancomycin-resistant enterococcal* infections [3].

Linezolid is made up of totally synthetic materials with 100% bioavailability and were thought to be not likely to develop natural drug resistance [2]. The exact mechanism of action has not been concluded, but many studies showed that oxazolidinones group of drugs inhibit bacterial ribosomal protein synthesis [5-8]. Established resistance to other ribosomal agents do not cause cross-resistance to mechanism of action of Linezolid [9]. These show that Linezolid resistance is not something which is commonly found.

In 2000, Linezolid was approved for clinical use, then only one year after that the first case of Linezolid resistance was identified and reported in the US in a patient receiving Linezolid for the treatment of peritonitis [10]. After that many cases have been reported in North and South America, Europe and Asia. From past few years prevalence of Linezolid resistance is increasing. So, this study is done to see the prevalence of Linezolid resistance in *Staphylococcus, Enterococcus* and *Streptococcus* spp.

Material and methods

This study was carried out in Microbiology Department, B. J. Medical college, Ahmedabad. The study period is from January 2020 to August 2021. Total of 54266 different samples like blood, urine, pus, swabs, CSF, sputum *etc.* were received from Civil hospital, Ahmedabad from OPD, various wards and ICU. They are cultured on MacConkey agar and Blood/Chocolate agar as per standard guidelines. From various isolated growths, gram-positive cocci were identified by colony morphology, gram staining and biochemical reactions such as catalase test, coagulase test, Bile esculin hydrolysis test, PYR test and sugar fermentation test. Automated identification was and AST was done by VITEK 2.0 compact automated system. Antimicrobial susceptibility tests were done using standard Kirby-Bauer disc diffusion method using Linezolid (30 µg) disc on Mueller-Hinton agar according to MacFarland standards. The zone of inhibition was determined after 18-24 hours of incubation at 35-37°C. Antimicrobial susceptibility tests were also done using MIC method with VITEK 2.0 Automated system.

Interpretation of antibiotic sensitivity was done according to latest CLSI guidelines 2021.

The Linezolid resistance screened by the disc diffusion and the automated AST is confirmed by the broth microdilution testing.

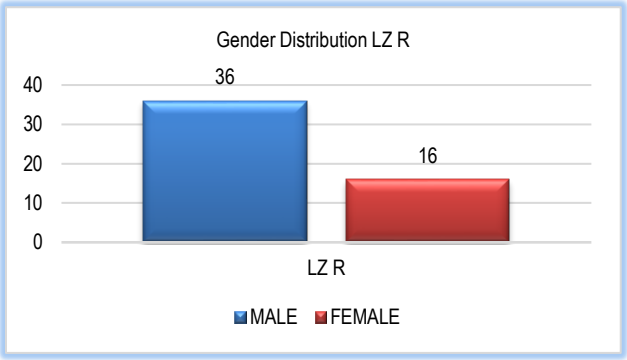
The quality control of antibiotic discs and MHA used was performed with S. aureus ATCC 25923 and E. faecalis ATCC 29212 and ATCC 29212 for VITEK AST cards. The Linezolid resistance is confirmed by the broth microdilution testing

Results

Total 54266 samples from patients of Civil hospital, Ahmedabad are screened for Linezolid (LZ) resistance, between January-2020 to August-2021. Among these total samples, 5548 (10.22%) Gram Positive cocci are isolated. Blood, Pus, Ascitic fluid, CSF, other body fluids, swab, sputum, urine *etc.* are included in this study.

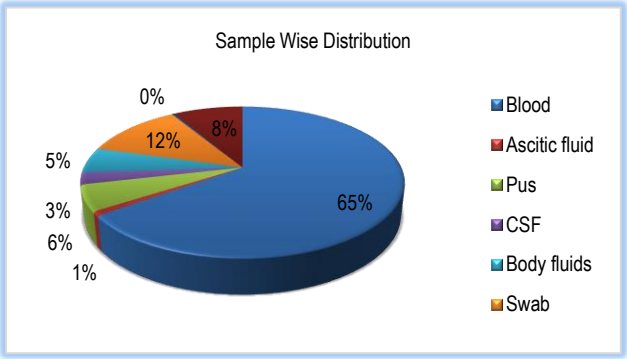
Out of these, there are 3605 (65%) blood samples.2920 (52.63%) organisms are pathogenic Gram-positive cocci. Among 2920 isolate 52 (1.78%) organisms are Linezolid resistant. It is more common in males than females with sex ratio is 2.25:1.

Gender	LZ R
Male	36
Female	16
Total	52

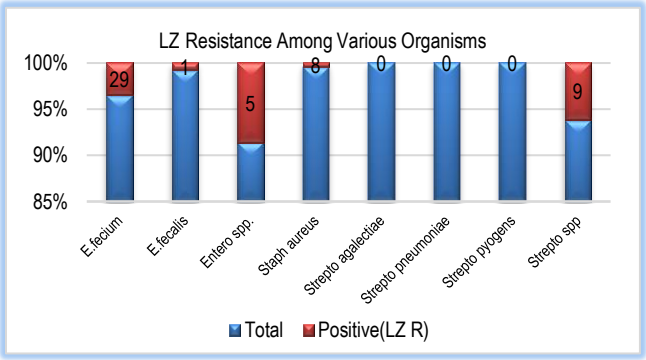


Positivity is highest in *Enterococcus faecium* (29;54.71%) followed by *Streptococcus* species (9;16.98%), *Staphylococcus aureus* (8;15.09%), other *Enterococcus* species (6;11.32%).

Sample	Number	Percentage (%)
Blood	3605	64.97
Ascitic fluid	50	0.90
Pus	306	5.50
CSF	141	2.54
Body fluids	302	5.4
Swab	662	11.93
Sputum	20	0.36
Urine	462	8.32



Organism	Total	Positive(LZ R)	Percentage
E.fecium	807	29	3.59
E.fecalis	126	1	0.79
Entero spp.	53	5	9.43
Staph aureus	1785	8	0.45
Strepto agalectiae	2	0	0.00
Strepto pneumoniae	4	0	0.00
Strepto pyogens	6	0	0.00
Strepto spp	137	9	6.57
Total	2920	52	1.78



Discussion

Linezolid is one of the antimicrobial agents which is highly active against Gram positive organisms. Resistance in Linezolid is increasing worldwide [11-16,18-23]. Studies in Europe laboratories are showing rise in Linezolid resistance [7]. Although, the LEADER surveillance programme, 2014 of the USA stated the Linezolid susceptibility rate of >99.78%, the emergence of the resistance is of great concern [24]. ZAAPS study of Europe shows 0.02% *S. aureus*, 0.03% E. faecium, 0.92% E. faecalis, isolates were Linezolid resistant. In LEADER study of the US shows 0.05% S. aureus are Linezolid resistant. As compared to this study, Linezolid resistance is higher in *S. aureus* and *E. faecium* in our study, suggesting rise in the resistance of Linezolid nowadays.

Cefoxitin was used as a surrogate marker for Methicillin resistance and it was found that 12.5% of all *S. aureus* isolates were Methicillin-resistant. It was found that 28.30% of all *Enterococcus* isolates were VRE. In Ireland, more no of Linezolid resistance have been reported from ICU patients [25]. As compared to this study, our study shows the significant resistance from both ICU (38%) and wards (58%) and very low in OPD based patients (3.77%). In our study, there is no resistance activity of Linezolid is seen in *Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogens.*

Conclusion

In summary, various levels of Linezolid resistant strains have emerged in Civil Hospital, Ahmedabad in various gram-positive isolates. Multiple resistance patterns (MRSA, VRE) were involved in this Linezolid resistant strains. Although the prevalence remains low overall, but the emergence of rise in Linezolid resistance in *Staphylococcus* and *Enterococcus* in clinical samples is of great concern. Robust antimicrobial stewardship and strengthened infection control measures are required to prevent spread and reduce further emerging resistance.

Application of research: Linezolid which was the promising agent against multi drug resistant gram-positive cocci such as VRE, MRSA is now showing resistance. Its detection in clinical samples is necessary for deciding treatment protocols by clinicians

Research Category: Antimicrobial agents

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****Principal Investigator or Chairperson of research: Dr Sumeeta Soni**
University: Gujarat University, Ahmedabad, Gujarat, 380016, India
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Author Contributions: All authors equally contributed
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Study area / Sample Collection: Civil Hospital, Ahmedabad

Strain name: *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and ATCC 29212

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
Ethical Committee Approval Number: Nil

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