ORIGINAL RESEARCH PAPER
Volume-8 | Issue-12 | December - 2019 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

LINEZOLID RESISTANCE IN STAPHYLOCOCCUS AUREUS: AN EMERGING PROBLEM

Microbiology

Dr Kiranjeet Kaur MD, Assistant Professor, Deptt. Of Microbiology, Adesh Institute Of Medical Sciences And Research, Bathinda

Dr Mandeep Kaur* MD, Assistant Professor, Deptt. Of Microbiology, Adesh Institute Of Medical Sciences And Research, Bathinda *Corresponding Author

Dr Amarjit Kaur Gill MD, Professor And Head, Deptt. Of Microbiology, Adesh Institute Of Medical Sciences And Research, Bathinda

ABSTRACT

BACKGROUND: Linezolid is a precious drug used to treat infections by resistant Gram positive cocci. Resistance to linezolid is either by G2576T mutation or plasmid mediated cfr gene.

AIM: This study aimed to find out the prevalence of linezolid resistance among S. aureus. Methods & Materials: The study was conducted in a tertiary care hospital from July 2016 to June 2017. All clinical isolates of S. aureus were subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method. The confirmed LRSA were included in the study. Clinical details of the patients were analyzed.

RESULTS: Out of 162 S. aureus isolates, eight showed resistance for linezolid. Most LRSA strains were isolated from soft tissue infections. Vancomycin showed susceptibility in 87.5% LRSA strains while teicoplanin and tigecycline were 100% susceptible.

CONCLUSION: Linezolid resistance in S. aureus poses a significant challenge in the treatment of infectious diseases. The continued antimicrobial surveillance and stopping injudicious antibiotics use is necessity.

KEYWORDS

Bacterial Infection, Drug Resistance, Linezolid Resistance, Antimicrobial Agents

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is the pathogen of greatest concern because it causes both hospital-acquired and community associated infection.[1] This organism has high incidence of resistance to ß lactam and glycopeptides.[2] Linezolid, a novel antibiotic, is the representative drug of oxazolidinone antibiotic group which is effective against MRSA infections like skin and soft tissue infections, osteomyelitis, pneumonia and bacteremia.[3] It is available in both oral and perenteral formulation and can be used in patients of all ages, liver disease and poor kidney function. This drug has high bioavailability and post antibiotic effect. Linezolid acts by inhibiting bacterial protein synthesis by binding to peptidyl transferase center of 50S ribosomal subunit.[4] Its mechanism of action is different from other protein synthesis inhibitors which usually act at inhibiting chain elongation in protein biosynthesis. With this novel mechanism of action, frequency of spontaneous resistance to linezolid in *S.aureus* is very low.[5] Also because of its completely synthetic nature, no natural reservoir of resistance genes would be expected to favour the presence of clinical resistance. Data from the USA and global surveillance studies reported <1% of *S.aureus* and 2% of Coagulase Negative Staphylococci (CONS) are linezolid resistant.[6] However, nowadays linezolid resistance is being increasingly reported which is a cause of concern.[7] As per CLSI (clinical laboratory standard institute) and EUCAST (European Committee on Antibiotic susceptibility testing) criteria, linezolid resistance in *Staphylococcus* is defined as a linezolid MIC of ≥8mg /l and with inhibition zone size ≤20mm when tested with a 30µg linezolid disc whereas the isolates with MIC ≤4mg/l and zone diameter ≥21mm are considered as linezolid sensitive.[8,9] Three mechanisms of oxazolidinone resistance have been described- a) mutations in the domain V region of 23S rRNA genes; b) acquisition of the ribosomal methyltransferase gene cfr (chloroamphenicol, florfemucol resistance); and c) mutations in rplD, and rplC genes encoding 50S ribosomal proteins L4, and L3 respectively. [10] Among these mechanisms, most concerned is the cfr gene mediated resistance which is usually plasmid mediated or transposon born and can easily disseminate among to susceptible population.[10,11] Furthermore, this *cfr* encodes resistance to a group of chemically distinct antibiotics like phenicol, lincosamides, pleuromutilin and streptogramin A as well.[10,11]

There are many *in vitro* antibiotic susceptibility testing methods available for determining linezolid susceptibility like disk diffusion, broth microdilution, agar dilution, E (Epsilometer) test and other automated antimicrobial testing systems. Single method for testing linezolid resistance is not considered reliable hence two or more methods should be used for confirming the resistance.[10] Linezolid is a precious drug used in the treatment of gram positive bacteria and should be used judiciously. Furthermore, the better understanding of epidemiology, mechanism of drug resistance and effective surveillance would prevent the emergence of linezolid resistant Staphylococcal strains. The present study was planned to observe the prevalence of linezolid resistance among *S. aureus*, associated factors and treatment modalities available.

MATERIAL AND METHODS

The study was conducted in a tertiary care hospital for a period of one year (July 2016 to June 2017) after taking approval from institutional ethical and research committee. All clinical samples like blood, urine, pus, ETT, CSF, swabs (throat, ear, vaginal) and sterile body fluids received in microbiology lab during our study period were included in study. Samples were processed using standard microbiological techniques. All *S. aureus* strains isolated from these clinical samples were identified by colony morphology, Gram staining and biochemical tests (catalase test and coagulase test). Antibiotic susceptibility testing for *S. aureus* strains was performed by the Kirby-Bauer disc diffusion method and the interpretation was done following CSLI criteria.[11] The minimum inhibitory concentration (MIC) of linezolid against various Staphylococcal strains was determined using VITEK 2 compact system. The *S. aureus* strains showing ≤20mm zone size on disk diffusion test and MIC value of >8mg/l were considered linezolid resistant.[8] The clinical data for all the patients infected with LRSA pathogen was collected and analysed.

RESULTS

Out of 162 *S. aureus* strains, 8 (4.94%) were linezolid resistant. Maximum *S. aureus* strains were isolated from pus samples 62(38%) followed by swabs (ear,throat and vaginal) 34(21%), urine 30 (18.5%) and blood 21(%). LRSA were isolated maximum from pus 5 (62.5%) followed by swabs (ear and vaginal) 2 (25%) and blood 1(12.5%) [Table 1].

Table 1.Distribution of S. aureus and LRSA in various clinical samples

Sample	No.(%) of Staphylococcus aureus	No.(%) of LRSA
Pus	62 (38.2)	5 (62.5)
Swabs(Ear, throat,Vaginal)	34 (20.9%)	2 (25)
Urine	30 (18.5%)	0 (0)
Blood	21 (12.9%)	1 (12.5)
Sputum	07 (4.3%)	0 (0)
Body fluids	05 (3.0%)	0 (0)
Other	03 (1.8%)	0 (0)

The clinical characteristic of the affected patients showed more isolation of LRSA from male 5(62.5%), adult age group 6 (75%) and hospitalized patients 7(87.5%). Previous exposure of linezolid was recorded in 2(25%) patients infected with LRSA, whereas 6(75%) were having exposure to broad spectrum antibiotics. In 5(62.5%) patients LRSA caused skin and soft tissue infection, whereas in others it was associated with respiratory tract 1(12.5%), bacteremia 1(12.5%), and genitourinary 1(12.5%) tract. [Table 2].

Table 2. Characteristics of patients with LRSA

Characteristics	Variables	Number (%)
Gender	Male	5 (62.5)
	Female	3 (37.5)
Age group	Pediatric	1 (12.5)
	Adult	6 (75)
	Old	1 (12.5)
Hospital group	ICU	2 (25)
	WARD	5 (62.5)
	OPD	1 (12.5)
Prior antibiotic exposure	Broad spectrum	6 (75)
	Linezolid	2 (25)
Site of infection	Skin and soft tissue infection	5 (62.5)
	Respiratory tract infection	1 (12.5)
	Blood stream infection	1 (12.5)
	Genitourinary tract	1 (12.5)

These linezolid resistant strains showed considerable susceptibility against other second line agents like vancomycin (87.5%), tigecycline (100%) and tiecoplanin (100%)[Table 3]. The antimicrobial susceptibility pattern in LRSA strains towards other antimicrobials is shown in table 3.

Table 3.Antimicrobial susceptibility pattern of LRSA strains

Antibiotic	No. of susceptible strains/	Percentage
Penicillin	0	0
Oxacillin	3	37.5
Gentamicin	6	75.0
Cephalexin	4	50.0
Erythromycin	3	37.5
Clindamycin	5	62.5
Ciprofloxacin	3	37.5
Levofloxacin	3	37.5
Cotrimoxazole	4	50.5
Vancomycin	7	87.5
Tigecyclin	8	100
Tecoplanin	8	100

4. DISCUSSION

The expanding incidence of antimicrobial resistance among Gram-positive pathogens particularly resistance to glycopeptides, which are considered the last line of defence against infections with these organisms has posed a major therapeutic challenge for the treating clinicians and has also resulted in prolonged hospital stays and increased hospital expenses. Other group of antibiotics such as aminoglycosides, third-generation cephalosporins, tetracyclines, fluoroquinolones and lincosamides do have considerable activity against community-acquired MRSA strains, but are not sufficiently effective for clinical use in all infections.[12] Linezolid usually becomes the drug of choice against the vast majority of clinically important Gram-positive cocci, including VRE, as well as MRSA and high-level penicillin-resistant *Streptococcus pneumoniae*, which may potentially acquire resistance genes from enterococci.[13]Its favorable antimicrobial spectrum, safety profile, pharmacodynamics and pharmacokinetics makes it a useful drug to be used in critical care setting[14] and for the treatment of severe infections in adults like bacteremia, endocarditis, osteomyelitis, nosocomial pneumonia and severe soft tissue infections.[15]

In 2001, the first linezolid resistant clinical strain was reported in US patient who had received one month linezolid treatment for dialysis associated peritonitis.[16] In 2004, Endimiani and co-worker from Cleveland, Othio, reported their first LRSA, with a total 11 LRSA infected Cystic fibrosis patients being identified by 2009.[17] First ever outbreak of LRSA was documented from a teaching hospital in Madrid, Spain with 12 LRSA patients identified within a 3 month period.[14] In Japan, linezolid was introduced for treatment in 2006 and within 2 years of its introduction, eleven LRSA cases were detected.[18] The overall global prevalence of linezolid resistance among Staphylococci was documented in 0.05% of *S. aureus* and 1.4% of CONS.[6] The reported incidence of linezolid resistance in India range from 2.2% as reported by Norma et al[19] to a much higher prevalence (20.3%) reported by Singh et al in Rajasthan.[20]The prevalence of LRSA in our study was 4.94% which is above the global prevalence and as compared to other Indian studies.[21] Similar results were obtained in a study by Lyra et al with linezolid resistance observed in 5.7% of MRSA isolates.[22] The increasing prevalence of LRSA strains and its rapid spread is becoming a matter of great concern. In our study, maximum *S. aureus* strains were isolated from pus samples 62(38%) and same trend was obsereved for LRSA also[Table1]. Another Indian study also reported higher isolation of *S.aureus* and LRSA from pus sample.[20] The factors associated with the emergence and spread of linezolid resistance in *S. aureus* are treatment with linezolid for extended period, non adherent to antimicrobial therapy or presence of foreign devices.[23] in our study LRSA was most commonly isolated from male and adult age group patient this most active population group is most prone to injuries and infections. In our study, among LRSA cases, most common factors were the prolonged hospital stay (7 out of 8 patients) and previous exposure to antibiotics other than oxazolidinone i.e. amikacin, gentamicin and fluoroquinolone (in 6 patients out of 8 patients) [Table 2]. In four of these cases, no previous linezolid intake could be documented whereas 2 cases had a history of linezolid exposure in the past one year. The record of past linezolid exposure in other two patients couldn't been traced. In our study 62.8% (n=5) LRSA were detected from complicated skin and soft tissue infections followed by respiratory (n=1) and blood stream (n=1) infections[table 2] while Bing Gu et al reported LRSA strains from respiratory tract infections in 60% cases, in 10% from BSI, 10% from surgical site infection and rest 20% from other sites.[8] Colonization with LRSA found in one patient who was operated for fibroid uterus and vaginal swab was sent for routine post operative investigation. These types of infections are caused by highly resistant type of pathogens and spread of drug resistance has been facilitated by selective pressure induced by intensive use of antibiotics both in hospital and outpatient. In such type of infections the chances of cross resistance by *cfr* harbouring organisms like CONS may also have occurred.[24]

The testing of resistance to linezolid against staphylococci is a growing challenge. Linezolid resistance may be under reported based on technical difficulties in the interpretation of both MIC and disc diffusion results. To address this concern it is advisable that clinical laboratory should confirm their findings of LRSA, preferably by a second method, prior to reporting. Bing GU et al noted that the majority of studies (67.4%) used E test, broth microdilution and disc diffusion, however 19.6% of studies used VITEK or VITEK -2, 10.2% agar dilution, 8.7% microscan and 2.2% broth microdilution.[8] Also the second method confirmation of LRSA was done in only 74% of studies conducted globally.[8] In our study, we used disc diffusion as the first method to detect resistance followed by MIC determination by VITEK 2 compact systems.

Treatment options for LRSA are limited because as such organisms carrying *cfr* gene are resistant to an array of antibiotics. As per available *in vitro* susceptibility data for LRSA the universal susceptibility to vancomycin, daptomycin and tigecycline remains. In our study also, LRSA showed 83.33% (n=1) susceptibility to vancomycin and 100%(n=8) towards teicoplanin and tigecycline. All LRSA strains (n=8) were resistant to penicillin whereas only 37.5%(n=3) were sensitive to erythromycin, ciprofloxacin and levofloxacin and 50.0 % (n=4) to cephalexin and cotrimoxazole [Table 3]

The results from our study indicate the rising trend of linezolid resistance among *S. aureus* isolated from hospitalized patients. Various preventive measures like timely surgical debridement or drainage of collection, use of combination therapy, judicious use of linezolid and most importantly an effective time to time surveillance may be helpful.

LIMITATIONS

In our study, molecular analysis of LRSA strains was not done to find out the genetic mechanism of drug resistance. Future

recommendations of our study is to do time to time analysis of antibiotic resistance in *S. aureus* and to incude molecular analysis of resistant strains.

CONCLUSION

Linezolid, a precious drug used to treat severe infections like bacteremia, endocarditis, osteomyelitis and severe soft tissue infections. There is an increasing trend of linezolid resistance in *S. aureus* and occurrence of LRSA infections among patients with no previous linezolid indicate the emergence of *cfr* gene carrying LRSA strains that confers resistance to array of antibiotics. The management in such cases poses a significant challenge to the clinicians; hence minimizing the risk factors along with an effective antimicrobial surveillance mechanism is the need of the hour.

REFERENCES

1. MacKenzie FM, Bruce J, Struelens MJ, Goossens H, Mollison J, Gould IM, et al. Antimicrobial drug use and infection control practices associated with the prevalence of methicillin-resistant Staphylococcus aureus in European hospitals, Clin Microbiol Infect. 2007;13(3):269-76.
2. National Nosocomial Infection Surveillance (NNIS) system report, data summary from January 1992-june 2001, issued August 2001. Am J Infect Control 2001;29:404-21.
3. Diekema DJ, Jones RN. Oxazolidinone antibiotics. Lancet 2001;358(9297):1975-82.
4. Aoki H, Ke L, Poppe SM, Poel TJ, Weaver EA, Gadwood RC, et al. Oxazolidinone antibiotics target the P site on Escherichia coli ribosomes. Antimicrob Agents Chemother. 2002;46(4):1080–5.
5. Kaatz GW, Seo SM. In vitro activities of oxazolidinone compounds U100592 and U100766 against Staphylococcus aureus and Staphylococcus epidermidis. Antimicrob Agents Chemother. 1996;40(3):799–801.
6. Ross JE, Farrell DJ, Mendes RE , Sader HS, Jones RN. Eight-year (2002–2009) summary of the linezolid (Zyvox(R) Annual Appraisal of Potency and Spectrum; ZAAPS) program in European countries. J Chemother. 2011;23(2):71-6.
7. Campanile F, Mongelli G, Bongiorno D, Adembri C, Ballardini M, Falcone M, et al. Worrisome trends of new multiple mechanisms of linezolid resistant in staphylococcal clones diffused in Italy. J Clin Microbiol. 2013;51):1256-9.
8. Clinical and Laboratory standard institute (CSLI). Performance standards for antimicrobial susceptibility testing. 26th ed. CLSI supplement. M100S.
9. Bing G, Thedoros K, Sotios T. The emerging problem of linezolid –resistant Staphylococcus. J Antimicrob Chemother. 2012;69(1):1-37.
10. Long KS, Vester B. Resistance to linezolid caused by modification at its binding site on the ribosome. Antimicrob Agents Chemother. 2012;56(2):603-12.
11. Kehrenberg C, Aarestrup FM, Schwarz S. IS 21-558 insertion sequences are involved in mobility of multiresistance gene cfr. Antimicrob Agents Chemother. 2007;51(2):483-7.
12. Diekema D, Pfaller M, Schmitz F,Smayevsky J, Bell J, Jones RN et al. Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY antimicrobial surveillance program, 1997–1999. Clin Infect Dis. 2001;32(Suppl2):S114–32.
13. Mark H. Wilcox. Efficacy of linezolid versus comparator therapies in Gram-positive infections. Journal of Antimicrobial Chemotherapy. 2003;51(S2):27–35.
14. Sánchez García M, De la Torre MA, Morales G, Peláez B, Tolón MJ, Domingo S, et al. Clinical outbreak of Linezolid-resistant Staphylococcus aureus in an Intensive Care Unit. JAMA. 2010;303(22):2260-4.
15. Stefani S, Bongiorno D, Mongelli G, Campanile F. Linezolid resistance in staphylococci. Pharmaceuticals.2010;3(7):1988-2006.
16. Tsiodras S, Gold HS, SakoulasG, et al. Linezolid resistance in a clinical isolate of Staphylococcus aureus. Lancet. 2001;358(9277):207-8.
17. Endimiani A, Blackford M, Dasenbrook EC, Reed MD, Bajaksoudszian A, Hujer AM, et al. Emergence of Linezolid –Resistance Staphylococcus aureus after Prolong Treatment of Cystic Fibrosis Patients in Cleveland, Ohio.Antimicrob Agent Chemother. 2011;55(4):1684-92.
18. Ikeda –Dantsuji Y, Hanakin H, Sakai F, Tomono K, Takesue Y, Honda j, et al. Linezolid-resistant Staphylococcus aureus isolated from 2006 through 2008 at six hospital in japan. J infect chemother. 2011;17(1):45-51.
19. Norma V, Juan Carlos V, Gerado EV. Resistance to linezolid of methicillin-resistant Staphylococcus aureus and Enterococcus with high level resistance to aminoglycosides at a third level pediatric hospital. Bol Med Hosp Infant Mex. 2010;67:17-24.19.
20. Harcharan Singh, Meena Atray, Pankaj Kumar Modi. Antibiotic susceptibility pattern of methicillin resistant Staphylococcus aureus in tertiary care center at Southern Rajasthan. IJPSR. 2014;5(2):607- 11
21. Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandam P. Prevalence and antimicrobial susceptibility pattern of Methicillin resistant Staphylococcus aureus: A multicenter study. Indian J Med Microbiol. 2006;24(1):34-8.
22. Lyra PR, Anuradha K, Shilpa A and Venkateshha D. Linezolid resistance in isolates of methicillin resistant Staphylococci from blood cultures. Int J Pharma Bio Sci. 2013;4(4):1085-90.
23. Wilson P, Andrews JA, charlesworth R. Linezolid resistance in clinical isolates of Staphylococcus aureus. J Antimicrob chemother. 2003;51(1):186-8.
24. Mendes RE, Deshpande L, Rodriguez- Noriego E et al. First report of staphylococcal clinical isolates in Mexico with linezilide resistance caused by cfr: evidence of in vivo cfr mobilization. J Clin Microbial. 2010:48(8):3041-3.