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LINEZOLID RESISTANCE IN ISOLATES OF METHICILLIN RESISTANT *STAPHYLOCOCCI* FROM BLOOD CULTURES

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ABSTRACT

Linezolid is one of the few effective ways to treat Methicillin Resistant *Staphylococcus aureus* infections. However resistance has been reported recently. Hence we conducted this study to determine the methicillin susceptibility of *Staphylococci* isolated from blood culture samples and to know their susceptibility to Linezolid. A total of 252 *Staphylococci* were subjected to antibiotic susceptibility testing by Kirby- Bauer disk diffusion method; MRSA was detected by using cefoxitin disk (30µg) and linezolid (30µg) was included to know susceptibility. Methicillin resistance was observed in 53 (69.74%) of the *Staphylococcus aureus* and 131(74.43%) of the Coagulase negative *Staphylococci*. Linezolid resistance was observed in 3 (5.66%) of the MRSA and 2 (1.53 %) of the Methicillin Resistant *Coagulase Negative Staphylococci*. Linezolid is the therapeutic choice in methicillin resistant *Staphylococci* as it could be administered orally and comparatively cheaper than glycopeptides. So this emergence of resistance may pose problem in the treatment.

KEYWORDS: **Linezolid resistance, MRSA, LRSA**

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INTRODUCTION

S. aureus is perhaps the pathogen of greatest concern because of its intrinsic virulence, its ability to cause a diverse array of life-threatening infections, and its capacity to adapt to different environmental conditions [1].The mortality of *S. aureus* bacteremia remains approximately 20–40% despite the availability of effective antimicrobials . *S. aureus* is the leading cause of nosocomial infections and as more patients are treated outside the hospital setting, is an increasing concern in the community also [2,3,4] *S.aureus* has its ability to develop resistance to antimicrobial agents, thus evoking significant concern in both the public and the health care communities. One year following the introduction of methicillin for the treatment of penicillin-resistant *S aureus*, the first strain of Methicillin Resistant *Staphylococcus aureus* (MRSA) was identified. Over the past two decades, MRSA rates have increased dramatically in both the community and hospital settings [5]. Available treatment options for serious invasive disease due to *S. aureus* are limited because of increasing antimicrobial resistance [6]. Linezolid is an oxazolidinone antibiotic with a novel mechanism of action. It inhibits bacterial protein synthesis by blocking formation of the initiation complex [7.8]. It is active against Gram-positive organisms resistant to other antibiotics, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), Penicillin-Resistant *Streptococcus pneumoniae* and Vancomycin Resistant Enterococci (VRE) [9,10]. It was thought that the pre-existing mechanisms of resistance to Linezolid would not be common in nature, so resistance would be slow to emerge as Linezolid is a purely synthetic antimicrobial [11].The first case of Linezolid resistant *Staphylococci* appeared within 1 year after Linezolid was approved for treatment [12]. There are various reports of emergence of resistance from different places. Linezolid is the common choice of drug in our set up to treat infections with methicillin resistant *staphylococci*. So we decided to take up this study to know the linezolid susceptibility of methicillin resistant and methicillin susceptible *staphylococci* isolated from blood cultures.

MATERIALS & METHODS

Blood samples received from different departments in the hospital were processed by conventional blood culture method. Staphylococci were identified as per the standard protocol [13].Tube Coagulase test was performed to identify coagulase positive and negative (CoNS) species. A suspension of each isolate was prepared so that the turbidity was equal to 0.5 McFarland standards and then plated onto Mueller–Hinton agar plate. Kirby-Bauer disc diffusion method was adopted to know the antibiotic susceptibility pattern. Antibiotics included were Penicillin(10units),Gentamicin (10µg),Erythromycin (15µg),Clindamycin(2µg),Ciprofloxacin(5µg),Trimethoprim-Sulfamethoxazole (1.25/23.75µg),Linezolid(30µg).S.aureusATCC 25923 was used as control.

Methicillin resistance was detected by disc diffusion method using cefoxitin disc (30µg).After incubation at 37°C for 24 hrs zone sizes were measured. Results were interpreted according to the criteria of Clinical and Laboratory Standard Institute (CLSI). *S.aureus* showing inhibition zone sizes <21 mm and CoNS isolates <24 mm were considered methicillin resistant [14]. *Staphylococcus* spp showing inhibition zone size ≥21 mm was considered as Linezolid sensitive and ≤20 mm was considered as resistant.[14]

RESULTS

Out of the 252 *staphylococci* isolated, 76(30.16%) were *Staphylococcus aureus* and 176 (69.84%) were coagulase negative *staphylococci* (CoNS)

Table 1
Comparisonof antibiotic susceptibility pattern of S.aureus and CoNS

ANTIBIOTICS	STAPHYLOCOCCUS AUREUS (n=76)	CoNS (n=176)
Penicillin(10 units)	13 (17.10%)	21 (11.93%)
Gentamicin(10µg)	31(40.79%)	65 (36.93%)
Erythromycin(15µg)	12 (15.79%)	36 (20.45%)
Clindamycin(2µg)	54 (71.05%)	112 (63.64%)
Ciprofloxacin(5µg)	27 (35.53%)	39 (22.16%)
Co-trimoxazole(1.25/23.75µg)	58 (76.31%)	147 (83.52%)

Table II
Distribution of Methicillin resistant isolates

Isolate	Methicillin resistance
Staphylococcus aureus (n=76)	53 (69.74%)
CoNS (n=176)	131 (74.43%)

Table III
Linezolid susceptibility pattern of the Methicillin resistant isolates

ISOLATE	SENSITIVE	RESISTANT
MRSA (n=53)	50 (94.34%)	3 (5.66%)
MRCoNS (n=131)	129 (98.47%)	2 (1.53%)

DISCUSSION

Drug-resistant Gram-positive bacteria, especially *S.aureus*, are emerging as the predominant organisms involved in both nosocomial and community-acquired infections. Clinicians are restricted to select a few antimicrobial agents with limited efficacy to treat these antibiotic-resistant pathogens [5]. MRSA were reported by (Mayhall,2004) [15], and methicillin resistant coagulase negative staphylococci (MRCoNS) have become the predominant pathogen in hospitalized patients with the number of infections caused by these pathogens increased dramatically [16,17]. The global emergence of MRSA has been the biggest setback in the history of antibiotic therapy [18]. Despite the introduction of antimicrobial therapy and the recent improvements of medical services, MRSA and MRCoNS are recognized as a major cause of nosocomial infections which result in significant morbidity and mortality. These organisms have the extraordinary ability to adapt to antibiotics stress [19]. In the present study Methicillin resistance was observed in 53 isolates (69.74%) of *Staphylococcus aureus* which was comparable to the previous studies [20].However our data showed a high prevalence rate of MRSA with regard to other previous studies (Anupama et al., 2003; Stefani and Varaldo, 2004; Adegoke et al., 2009) [21, 22, 23] and low prevalence rate compared to the study done by Verma et al., 2000 [24]. Methicillin resistance was observed in 131 isolates (74.43%) of CoNS which is higher than MRSA. A study conducted by Khadri et al has reported a slightly higher MRSA compared to MRCoNS strains [19].

The trend of antibiotic resistance to a large number of commonly prescribed antibiotics observed in this study is similar to earlier observation [23, 25]. MRSA treatment options are limited. Tigecycline, Linezolid, Quinupristin-Dalfopristin are few available alternatives, resistance to these agents looms on the horizon [26]. Linezolid is the widely used therapeutic choice because it can be administered orally and comparatively cheaper than glycopeptides, has a favourable antimicrobial spectrum, short term safety profile, pharmacodynamics and pharmacokinetics [27]. Researchers assumed that resistance to

linezolid would never develop. Endimiani and co-workers from Cleveland, Ohio, reported their first Linezolid Resistant *Staphylococcus aureus*(LRSA) in 2004, with a total of 11 LRSA-infected cystic fibrosis patients being identified by 2009 [28]. Linezolid treatment started in Japan in 2006 and by 2008, 11 patients were detected positive for LR-MRSA [29]. A worldwide program, the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) (2007) for linezolid resistance, revealed the overall resistance to linezolid in 23 countries to be 0.03% [30]. The first ever outbreak from a teaching hospital in Madrid, Spain documented 12 LRSA patients during a short span of approximately a three-month period from April 13 to June 26, 2008 [31]. Similarly, 0.34% LRSA was also reported in the LEADER 2009 programme that monitors and tracks linezolid resistance in the United States [31, 32].

In the present study all the Methicillin sensitive *Staphylococci* were found to be Linezolid sensitive, whereas 3 (5.66%) MRSA were found to be resistant to Linezolid. Rajaduraipandi reported 2.4% of LRSA in South India [33]. In a study conducted by Deep A et al the resistance to linezolid was noted in 9 percent of all isolates of *S. aureus,* with linezolid resistance being seen only in MRSA isolates [34]. We have observed slightly higher rate probably over a period of time there is an increase in the number of strains acquiring resistance as it has become routine to use Linezolid. Linezolid resistance was observed in 2 (1.53 %) MRCoNS in this study. Peer, et al in 2009 isolated 2 Linezolid resistant CoNS from cases of sepsis from Kashmir [35]. Kalawat, et al in 2010 isolated 2 Linezolid resistant CoNS from Andhra Pradesh [11].The resistance to Linezolid is not only limited to MRSA, it is also observed in MRCoNS. A study suggested that resistance is probably acquired following the prior Linezolid exposure [27].We also observed that resistance was seen in three isolates from patients hospitalized for a prolonged period and treated with Linezolid. Other two isolates were from neonatal intensive care unit where they use this antibiotic for staphylococcal infections. The study we performed is by the disc-diffusion method. We were not able to confirm the resistance by determining the MIC.

CONCLUSION

Emergence of Linezolid resistance is a matter of great concern.It is better we recognise the far reaching consequences posed by linezolid resistance in *Staphylococci* which is a great threat. Resistant strains can be detected by simple disk diffusion method even though MIC is ideal.Rapidity and simplicity of the test helps in implementing this in any centers which will help in early detection of these strains and to take proper measures to prevent the potential outbreak with these strains. The prospective resistance surveillance studies are needed to closely monitor and track resistance, particularly where frequent and extended linezolid therapy is used. Paucity of newer antimicrobials demands the judicious use of linezolid to preserve its clinical utility, more than ever before. Prescribing antibiotics with a different mode of action, effective surveillance, rational use of antibiotics, and appropriateness in antimicrobial therapy may reduce the increasing selection pressure for resistance to linezolid.

REFERENCES

1. Waldvogel, F.A. 2000. Staphylococcus aureus (including staphylococcal toxic shock). In Principles and practice of infectious diseases. G.L. Mandell, J.E. Bennett, and R. Dolin, editors. Churchill Livingstone. Philadelphia, Pennsylvania, USA. 2069–2092.
2. CDC NNIS System: National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992–

April 2001, Am. J. Infect. Control, 29:400-421, (2001).
3. Diekema, DJ, 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, 32(Suppl. 2):S114-S132, (2001).
4. Franklin D. Lowy. Antimicrobial resistance: the example of *Staphylococcus aureus.* J Clin Invest.; 111 (9):1265–1273, (2003).
5. Schwalm JD, El-Helou P, Lee CH. Clinical outcome with oral linezolid and rifampin following recurrent methicillin-resistant *Staphylococcus aureus* bacteremia despite prolonged vancomycin treatment. Can J Infect Dis, Mar-Apr; 15(2): 97–100, (2004).
6. Richard H. Drew, Pharm.D.Emerging Options for Treatment of Invasive, Multidrug-Resistant Staphylococcus aureus Infections. Pharmacotherapy; 27(2):227-249, (2007).
7. Swaney SM, Aoki H, Ganoza MC, ShinabargerDL."Theoxazolidinone linezolid inhibits initiation of protein synthesis in bacteria''.Antimicrob AgentsChemother; 12: 3251-5, (2008).
8. Garazzino S ,Tovo PA. Clinical experience with linezolid in infants and children. Journal of Antimicrobial Chemotherapy,66(4):iv23-iv41.
9. Mark H. Wilcox.Efficacy of linezolid versus comparator therapies in Gram-positive infections. Journal of Antimicrobial Chemotherapy ,51, Suppl. S2, ii27–ii35, (2003).
10. Jones RN, Fritsche TR, Sader HS, Rosse JE. LEADER Surveillance program results for 2006: An activity and spectrum analysis of linezolid using clinical isolates from the United States (50 medical centers).DiagnMicrobiol Infect Dis, 59: 309-17, (2007).
11. Kalawat U, Sharma KK, Reddy S.Linezolid – resistant Staphylococcus spp. at a tertiary care hospital of Andhra Pradesh. Ind J Of Med Microbiol;29(3): 314-315, (2011).
12. TsiodrasS, GoldHS, SakoulasG, Eliopoulos GM, etal. Linezoild resistance in a clinical isolate of Staphylococcal aureus.Lancet; 358:207-8, (2001).
13. J.G. Collee, Barrie P. Marmion, AG Fraser, A. Simmons. Mackie and McCartney Practical Medical Microbiology, 14th ed. Edinburgh: Churchill Livingstone; (2007).
14. Clinical Laboratory Standards Institutes. Performance Standards for antimicrobial susceptibility testing, XXI International Supplement (M100-S21). Wayne, Pennsylvania, USA : National Committee for Clinical Laboratory Standards 2011.
15. Mayhall C. Hospital epidemiology and infection control. 3rd ed.Philadelphia: Lippincott William and Wilkins, p. 2069, (2004).
16. Sohn A, Garrett D, Sinkowitz-Cochran R. Prevalence of nosocomial infections in neonatal intensive care unit patients: results from the first national point-prevalence survey. J. Pediatr. 136: 821-827, (2001).
17. Koksal F, Yasar H, Samasti M. Antibiotics resistant patterns of coagulase-negative staphylococcus strains from blood cultures of septicemic in Turkey. Microbiol. Res. 16:31-34, (2007).
18. I.M Gould.Antibiotic resistance: the perfect storm.International J Of Antimicrob Agents 34; 53. S2-S5. (2009).
19. Khadri H, Alzohairy M.Prevalence and antibiotic susceptibility pattern of methicillin-resistant and coagulase-negative staphylococci in a tertiary care hospital in India. International Journal of Medicine and Medical Sciences; 2(4): 116-120, April 2010
20. Yadav S, Yadav A, Sharma M, Chaudhary U. Prevalence and sensitivity pattern of *Staphylococcus aureus* in surgical wound infections. International Journal of Pharma and Bio Sciences.Vol.1/Issue-3/Jul-Sep.2010.
21. Anupama S, Sen M, Nath G, Sharma B, Gulati A, Mohapatra T. Prevalence of Methicillin resistant *Staphylococcus aureus*

in a tertiary referral hospital in Eastern Utter Pradesh. Indian J. Med. Microbiol.21: 49-51, (2003).

22. Stefani S, Varaldo P. Epidemiology of methicillin-resistant *staphylococci* in Europe. Clin. Microbiol. Infect. 9(12): 1179-1186, (2004).
23. Adegoke, Ayodeji A, Komolafe, Omoniyi A, Multi-drug resistant *Staphylococcus aureus*in clinical cases in Ile-Ife, Southwest Nigeria. International Journal of Medicine and Medical Sciences Vol 1. (3) pp. 068-072, March, 2009.
24. Verma S, Joshi S, Chitnis V, Hemwani M, Chitnis D.Growing problem of methicillin resistant *Staphylococc*i. Indian Scenario. Indian J. Med. Sci. 54: 535-540, (2000).
25. Grisold AJ, Leitner E, Muhlbauer, G., Marth, E. and Kessler, H. H. Detection of Methicillin-resistant *Staphylococcus aureus*andsimultaneous confirmation by automated Nucleic acid extraction andreal time PCR. J. Clin. Microbiol. 79: 143-6, (2002).
26. MahmoodK,Tahir T,Jameel T,Ziauddin A, Aslam H.F.Incidence of Methicillin-resistant Staphylococcus Aureus (MRSA) Causing Nosocomial Infection in a Tertiary Care Hospital. Annals vol 16. no. 2 apr. – jun. 2010.
27. Sánchez García M, De la Torre MA, Morales G, Peláez B, TolónMJ,Domingo S, *et al*. Clinical outbreak of Linezolid-resistant *Staphylococcusaureus*in an Intensive Care Unit. JAMA ; 303:2260-4, (2010).
28. Endimiani A, Blackford M, Dasenbrook EC, Reed MD, BajaksouszianA,Hujer AM, *et al*. Emergence of Linezolid-Resistant *Staphylococcus aureus* after Prolonged Treatment of Cystic Fibrosis Patientsin Cleveland, Ohio. Antimicrob Agents Chemother; 55:1684-92, (2011).
29. Ikeda-Dantsuji Y, Hanaki H, Sakai F, Tomono K, Takesue Y, Honda J, *et al*. Linezolid-resistant *Staphylococcus aureus*isolated from 2006 through 2008 at six hospitals in Japan. J Infect Chemother; 17:45-51,(2011).
30. Jones RN, Kohno S, Ono Y, Ross JE, Yanagihara K. ZAAPS International Surveillance Program (2007) for Linezolid resistance: Results from5591 Gram-positive clinical isolates in 23 countries. DiagnMicrobiol Infect Dis;64:191-201,(2009).
31. Farell DJ, Mendes RE, Ross JE, Sader HS, Jones RN. LEADER Program Results for 2009: An Activity and Spectrum Analysis of Linezolid Using 6,414 Clinical Isolates from 56 Medical Centers in the United States.Antimicrob Agents Chemother ;55:3684-90,(2011).
32. Thool VU, Bhoosreddy GL, Wadher BJ. Detection of resistance to linezolid in *Staphylococcus aureus* infecting orthopedic patients.Indian J Of Pathol and Microbiol; 55(3): July- Sep 2012.
33. 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; 24:34-8, (2006).
34. Deep A, Goel N, Sikka R, Chaudhary U et al. quinupristin-dalfopristin resistance in gram-positive bacteria: experience from a tertiary care referral center in north india. J Infect Dis Antimicrob Agents Sep.-Dec; vol. 25 no. 3, (2008).
35. MA Peer, RA Nasir, DK Kakru,et al. Sepsis due to linezolid resistant *Staphylococcus cohnii* and *Staphylococcus kloosii*: First reports of linezolid resistance in coagulase negative staphylococci from India.Indian Journal of Medical Microbiology,29(1),60-62,(2011).