IN VITRO ACTIVITY OF LINEZOLID AGAINST CLINICAL ISOLATES OF METHICILLIN RESISTANT STAPHYLOCOCCUS

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Background: Staphylococcus is responsible for a variety of medical problems, including skin and soft-tissue infections (SSTIs), surgical site infections (SSIs), endocarditis and hospital acquired bacteraemia. Methicillin resistance in staphylococcus has become a global problem limiting the treatment modalities to a large extent. **Methods:** The aim of this study was to evaluate the *in vitro* activity of linezolid and other antibiotics against clinical isolates of methicillin resistant staphylococcus (n=163); including 105 methicillin resistant *Staphylococcus aureus* and 58 methicillin resistant coagulase negative staphylococci. Antibiogram of these isolates was determined by the Kirby-Bauer disc diffusion method and minimum inhibitory concentration of linezolid was determined by standard agar dilution method. **Results:** Overall methicillin resistant *S. aureus* showed high multi-drug resistance. ATCC 25923 *Staphylococcus aureus* and ATCC 29213 *Staphylococcus aureus* were used as the standard control strains. MIC_{90} of linezolid was comparable for methicillin resistant coagulase negative staphylococci and methicillin resistant *S. Aureus* (4.0 mg/L); however at MIC_{50} linezolid was two fold more active against methicillin resistant coagulase negative staphylococci (1mg/L) than methicillin resistant *S. aureus* (2mg/L). **Conclusion:** It is concluded that linezolid has excellent activity against methicillin resistant staphylococci including multidrug resistant strains.

Keywords: Linezolid, MIC, MRSA, MRCoNS

INTRODUCTION

Staphylococcus is responsible for a variety of medical problems, including skin and soft-tissue infections (SSTIs), surgical site infections (SSIs), endocarditis and hospital acquired bacteraemia.[1] An increasing number of infections are related to developments in medicine, including the use of joint prosthesis, immunosuppressants and catheters etc. Staphylococci are inherently susceptible to most of the antibiotics in use except those with purely anti-gram-negative spectrum. The organism, adept at developing resistance both by mutation and by DNA transfer[2] is difficult to treat and remains a frequent cause of morbidity and mortality. A concurrent growth in resistance among coagulase negative staphylococci (CoNS) is partly due to the increasing use of broad-spectrum antibiotics that promote selection of multiresistant strains.[3]

Methicillin resistance in staphylococcus has become a global problem limiting the treatment modalities to a large extent. As with methicillin resistant *Staphylococcus aureus* (MRSA), meticillin-resistant coagulase negative staphylococcal isolates show cross-resistant to all β-lactams *in vivo*, despite some isolates indicating apparent susceptibility during *in vitro* testing.[4]

Once the β-lactam fails, the mainstay against methicillin resistant staphylococcus (MRS) infections is the use of glycopeptides, vancomycin and teicoplanin. However, the emergence of clinical infection due to MRSA with decreased susceptibility to vancomycin is a recent and certainly a worrying fact. Since 1996 vancomycin-intermediate *S. aureus* (VISA) strains have been increasingly reported in Europe, Asia and the USA. At least seven of these vancomycin-resistant *S. aureus* (VRSA) strains have also been reported in the USA since 2002. VISA strains, however, represent an important public health threat, having been implicated in nosocomial infections. These strains tend to be multidrug-resistant against a large number of currently available antibiotics, compromising treatment options and increasing the likelihood of inadequate antimicrobial therapy.[5]

Oxazolidinone class of antibiotics includes synthetic compounds unrelated to other antimicrobials; inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit. They block the initiation complex formation, resulting in a bacteriostatic action and linezolid is the first licensed antibiotic of this class.[6] It possess limited activity against selected gram-negatives and anaerobes but is highly active against gram-positive bacteria, including various resistant strains. It is bactericidal against streptococci but bacteriostatic against MRSA and vancomycin resistant enterococci (VRE) *in vitro*. The excellent oral bioavailability of linezolid makes it an extremely attractive antibiotic for the treatment of suspected or confirmed staphylococcal infections.[7]

This study was designed to evaluate *in vitro* activity of linezolid against clinical isolates of MRSA and MRCoNS as no data is so far available from Pakistan regarding linezolid resistance in methicillin resistant staphylococci.

MATERIALS AND METHODS

The study was carried out in Department of Microbiology, University of Health Sciences, Lahore Pakistan from November 2007 to October 2008.

Antimicrobial Agent:
Linezolid (Lot# K20007005) was provided by Continental Pharmaceuticals Pakistan.

Bacterial Strains:
A total of 163 clinical isolates, including MRSA (n=105) and MRCoNS (n=58) were collected during November 2007 to November 2008. The isolates were identified by their morphology, cultural and biochemical characteristics. The isolates were preserved in 20% glycerol in brain heart infusion (v/v) at -70 °C.

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was determined by modified Kirby-Bauer disk diffusion method as per recommendation of Clinical Laboratory Standards Institute (CLSI) 2007 guidelines.[8]

The following antibiotic disks; cefoxitin (30 µg), linezolid (30 µg), vancomycin (30 µg), teicoplanin (30 µg), ciprofloxacin (30 µg), amikacin (30 µg), azithromycin (30 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg), were used. Methicillin resistance was determined using cefoxitin disc (30 µg) (Oxoid Basingstoke, UK).

The test was performed on Mueller-Hinton agar. Bacterial suspension was made in isotonic saline (0.85%) and turbidity was visually adjusted to 0.5 McFarland standards. Prior to inoculation the swab stick was dipped into the saline suspension. The swab stick was then squeezed on the inner wall of the tube to get rid of extra suspension. The agar surface was then inoculated by using dipped swab stick. Inoculated plates were then incubated at 35 ºC for 24 hours. On the subsequent day, plates were read by measuring zones of inhibition and were interpreted according to interpretive standards, CLSI, 2007 guidelines. ATCC 25923 *Staphylococcus aureus* was used as standard strain to monitor the procedure.

MIC determination by agar plate dilution method
MIC is the lowest concentration of the antimicrobial agent that completely inhibits a bacterial isolate. The protocols were followed as previously reported.[9,10] Staphylococcal isolates were sub cultured on blood agar and after overnight incubation at 35 °C; three to five morphologically similar colonies were then emulsified in sterile isotonic saline. The suspension was adjusted to 0.5 McFarland standard (10^8 CFU/ml). The suspension was then diluted 1:10 in sterile saline. This gave an inoculum concentration of 10^7CFU/ml. The inocula were spot inoculated onto Mueller Hinton agar plates containing serial 2-fold dilutions in duplicates from 0.015 to 8.0 mg/L of linezolid, using a multipoint inoculator. This instrument holds 35 pins each with a diameter of approximately 3 mm; delivering approximately 3 µl/spot.

After 18–24 hours of incubation, the MIC was read against dark non reflecting surface as the first antibiotic concentration that inhibits the growth of the organism completely. The presence of faint haze caused by the inoculum or a single colony was disregarded as growth. Results of MIC were interpreted according to the breakpoints given by CLSI 2007.[8] ATCC 29213 *S. aureus* was used as reference strain.

RESULTS

Antibiogram:
MRSA showed highest resistance to ciprofloxacin (96.2%) followed by azithromycin (80%), amikacin (76.2%) and 65.7% for cotrimoxazole while MRCoNS showed highest resistance to azithromycin (98.3%) followed by cotrimoxazole (88.8%), ciprofloxacin (87.9%). and 20.7% for amikacin (Figure-1).

Multidrug resistance among MRSA and MRCoNS
Out of 105 MRSA isolates 83.80% (n=88) were multidrug resistant, with 40.95% (n=43) being resistant to more than three drugs. Multidrug resistance among MRCoNS (n=58) isolates was 81.03% (n=47) with 10.34% (n=6) being resistant to more than three drugs (Figure-2).

MIC determination
Linezolid possessed an effective spectrum of activity which covered all the most important gram positive organisms including those resistant to methicillin and glycopeptides.[7,11]

Table-1 shows the MIC range, cumulative % inhibition, MIC_{50} and MIC_{90} of linezolid against MRSA (n=105 and MRCONS (n=58). Linezolid inhibited all strains of MRSA and MRCoNS in the range of 1.0–4.0 mg/L and 0.5–4.0 mg/L, respectively.

Although MIC_{90} of linezolid was comparable for MRCoNS and MRSA (4.0 mg/L) but MIC_{50} of linezolid was two fold more active against MRCoNS isolates than MRSA.

Table-1: Minimum inhibitory concentrations of linezolid against MRSA (105) and MRCoNS (58)

Isolates (n)	Antimicrobial Agent	% of Isolates Susceptible at MIC (mg/L)										Concentration (mg/L)		
		0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	Range	MIC_{50}	MIC_{90}
MRSA	Linezolid	0	0	0	0	0	0	24	52	100	-	1.0-4.0	2	4
MRCoNS		0	0	0	0	0	2	62	84	100	-	0.5-4.0	1	4

n= Number of isolates; MIC_{50} MIC_{90} , MIC at which 50% and 90% of the isolates are inhibited, respectively

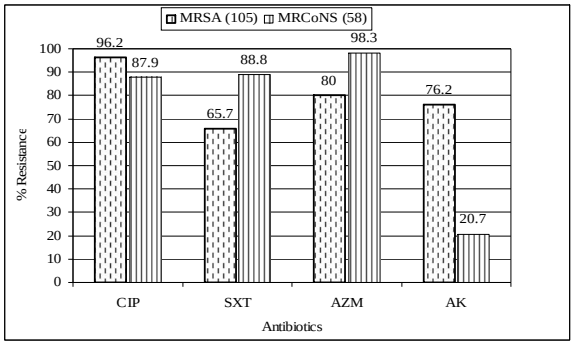

Figure-1: Percent resistance among methicillin resistant staphylococcal isolates

MRSA: Methicillin resistant *S. aureus*
MRCoNS: Methicillin resistant coagulase negative staphylococci
CIP: Ciprofloxacin; SXT: Cotrimoxazole
AZM: Azithromycin AK: Amikacin

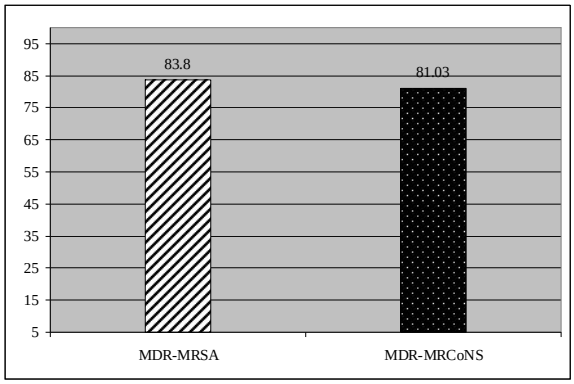

Figure-2: Percent multidrug resistance (MDR) among methicillin resistant staphylococci

MDR: Multidrug resistant; isolates which were resistant to three or more than three non β-lactam antibiotics tested were considered to be multidrug-resistant
MDR-MRCoNS: Multidrug resistant methicillin resistant coagulase negative staphylococci
MDR-MRSA: Multidrug resistant methicillin resistant *S. aureus*

DISCUSSION

MRSA represents a major challenge to hospitals in all countries due to the emergence and spread of isolates with decreased susceptibilities to several antibiotic classes. Treatment of Staphylococcus infections has become more difficult because of multidrug-resistant strains (resistance of an isolate ≥3 antibiotics tested at the same time (ciprofloxacin, azithromycin, cotrimoxazole, amikacin, vancomycin, teicoplanin, linezolid).[11]

In this study we found a high percentage of MDR-MRSA and MDR-MRCoNS, i.e., 83.80% and 81.03%, respectively. A comparable result (86%) of multidrug resistance in staphylococci spp. has been reported from Bolan Medical Complex Hospital Quetta in 2006–2007.[13] Another study conducted at Rawalpindi reported almost 75% MDR-MRSA.[14] The high percentage of multidrug resistance in MRSA and MRCoNS in this study should be a matter of serious concern in the present therapeutic scenario in the developing countries including Pakistan.

Linezolid proved to be very active against all staphylococcal strains, irrespective of susceptibilities to other antibiotics. In our study, all clinical isolates were fully susceptible to linezolid. The MIC_{90} values for MRSA and MRCoNS were comparable (4.0 mg/L). Our findings are comparable with those previously reported by Tunger A *et al* from Turkey[15] and AK James *et al* from Virginia[16].

These results suggest that linezolid may have an important role in the treatment of severely ill patients especially in areas where drug resistance is the main problem.

Finally, it is concluded that resistance to linezolid was not observed in any MRSA and MRCoNS isolates. Therefore, it could be a suitable therapeutic option for the treatment of highly resistant nosocomial infections but it should not be used empirically without proper laboratory evaluation. For future, we recommend that to deal with the ever-increasing antimicrobial resistance, it is necessary to monitor resistance patterns carefully and continuously.

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