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Susceptibility profile of methicillin-resistant *Staphylococcus aureus* to linezolid in clinical isolates

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ABSTRACT

Objective: To determine the resistance and sensitivity pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates to linezolid (LZD) along with its prevalence in a tertiary care hospital of Karachi, Pakistan.

Materials and Methods: A cross-sectional study was carried out. This study lasted for about 1 year. Prevalence and sensitivity of LZD, vancomycin, and oxacillin was tested against isolates of MRSA.

Results: Out of total 369 specimens 165 were found to be MRSA making the prevalence in our study 44.7%. All of the isolates which were tested positive for MRSA were susceptible to LZD and no resistance was noted when compared with previous studies performed in Europe and USA.

Conclusion: Stringent implementation of infection control measures along with screening for resistance in patients on prolonged LZD therapy or who previously went under LZD therapy should be performed, coupled with judicious usage of the aforementioned antibiotic should be undertaken, as sufficient data is not available at this point for the clinical spectrum of LZD resistant *S. aureus,* antimicrobial resistance.

Keywords: Linezolid resistant *Staphylococcus aureus,* methicillin-resistant *Staphylococcus aureus, Staphylococcus aureus*

Introduction

Staphylococcus aureus is a Gram-positive bacterium and the major pathogenic organism of genus staphylococcus.[1] It is responsible for causing hospital acquired and community acquired infections.[2] The very first isolate of methicillin-resistant *S. aureus* (MRSA) was described in 1961 in the UK, however, during that era, the prevalence of MRSA in Europe and elsewhere was negligible and was not really considered of as a significant threat. Although during the last two decades it has proven itself to be a significant threat to the patients well-being, especially those patients who are admitted to the hospital for an extended period of time. It has come to the observation that the overall prevalence of MRSA changed from 29% to 59% between 1991 and 2003.[3]

Regarding resistance, in 1950 *S. aureus* acquired resistance against β-lactams by producing an enzyme called β-lactamase. This was shortly followed by the introduction of methicillin, to which these bacteria developed resistance as well a decade later, as mentioned earlier.[4] It was not before 1980s that MRSA strains became multidrug resistant with a very high endemicity in certain parts of the word, proving itself to be a very difficult pathogen to eradicate, especially in hospital setups all over the world. Ever since then, a restricted amount of therapeutic options have been available for treating MRSA patients.[5] Until now glycopeptides such as intravenous vancomycin are one of the only few cost-effective options available for the treatment of MRSA infections in developing countries.[6]

The first available oxazolidinone antibiotic, linezolid (LZD), has also been clinically regarded as broadly active against multi drug-resistant strains of Gram-positive bacteria. By preventing formation of the 70S initiation complex, LZD prevents the bacterial protein synthesis.[7] Moreover, clinical trials have also proved us that LZD is generally well tolerated for up to 28 days in patients with minimal adverse effects on hematological parameters.[8] LZD has also showed superior

efficacy in the eradication of skin and soft tissue infections.[9] Intravenous and oral LZD show equal plasma concentrations thereby allowing switch over between both administration routes, proving to be highly cost effective.[7] On the other hand, newer drugs such as tedizolid, telavancin, and dalbavancin, been used for the treatment of MRSA infections are also possess higher efficacy, but due to being very costly, their use and availability in developing countries is not that common, hence making LZD a better choice than the aforementioned antibiotics. The objective of our study is to determine the resistance and sensitivity pattern of methicillin-resistant *S. aureus* (MRSA) isolates to LZD and also report the prevalence of the aforementioned pathogen in our setup and compare it with national and international data that has been published in South Asia and Europe.

Materials and Methods

A prospective cross-sectional study was performed and a total of 369 clinical specimens, which comprised eye, ear, wound and pus swabs, blood, urine, sputum samples, and tracheal aspirates which were cultured for a time frame of March 2012 to November 2013. Positive cultures for *S. aureus* were identified. Specimens (wound swabs, blood, ear swabs, eye swabs, sputum, and aspirates) were inoculated onto sheep blood agar and chocolate agar plates and incubated at 37°C for 18-24 h. Standard procedures were used to identify the isolates. Antibiotic discs, oxacillin (1 μg), vancomycin (30 μg), LZD (30 μg), teicoplanin (30 μg), and fusidic acid 10 μg (oxoid) were positioned at appropriate distances on the bacterial lawns and incubated at 37°C for 24 h. The growth inhibition zones were carefully measured with calipers and recorded according to the standard Kirby-Bauer disc diffusion method and NCCLS guidelines.[10] *S. aureus* ATCC 25923 strain was used for control.

MRSA strains were screened by following the CLSI guideline.[10] Suspension with similar concentration to 0.5% McFarland was prepared and streaked on the Mueller-Hinton agar (MHA) plates containing 6 μg oxacillin. Spot inoculation of 0.5 McFarland suspension onto MHA agar plates containing 6 μg of vancomycin was also performed to check for resistance to vancomycin, and the isolate was incubated for 24 h at 35°C by following the CLSI guidelines. Two or more colonies showing growth indicated resistance.

For statistical analysis SPSS version 22 was utilized and Chi-square test was applied for *P* value. Out of total 369 specimens 165 specimens were found to be MRSA. Sensitivity of LZD along with vancomycin, fusidic acid, teicoplanin, and oxacillin to MRSA isolates was deduced as well. The data used in this research were also a part of a previous research, which was performed to determine the resistance pattern of MRSA to trimethoprim/sulfamethoxazole and clindamycin and compared it with similar studies which were performed in the past.[11]

Results

Out of a total of 369 isolates of *S. aureus* 165 isolates were found to be resistant to methicillin/oxacillin, hence making the prevalence of MRSA in our study 44.71% and the prevalence of methicillin susceptible *S. aureus* 55.28%. While on the other hand only 10 (6.06%) of the isolates were resistant to fusidic acid and none of the isolates were resistant to teicoplanin, vancomycin or LZD, essentially signifying 0% resistance to all three antibiotics.

Discussion

In 1959, to treat infections caused by penicillin-resistant *S. aureus*, methicillin was introduced to in the drug markets. However, in 1961, the U.K. reported that *S. aureus* isolates had acquired resistance to methicillin (MRSA).[12] Since 1987, the prevalence of MRSA has increased up to 25-fold (16%) in the intensive care units (ICUs) of USA.[13] *S. aureus* spreads mainly through skin contact with an infected individual or contaminated surface.

An infection occurring within 48 h of hospital administration or 3 days of discharge is defined as nosocomial infection.[14] The European Prevalence of Infection in Intensive Care Study (EPIC), involving over 4500 patients, has also demonstrated that the nosocomial infection prevalence rate in ICU was 20.6%, which is also alarmingly high.[14] Annually, these results in over 5000 deaths. On average, nosocomial infections force the patients to spend 2.5-times longer in hospital, incurring additional costs of £3000 compared to an uninfected patient.[14]

MRSA can be carried out in the nose and skin of about 1-2% of people and some strains of MRSA can be very aggressive that cause staphylococcal infection. A research in Saudi Arabia found out that the most common site of infection was surgical wounds followed by chest and central venous catheter.[15] According to an American article, conditions most commonly caused by MRSA are sepsis and pneumonia.[16]

Prevalence of MRSA varies significantly throughout the world.[17] An investigation carried out in Nigeria, Kenya, and Cameroon deduced the prevalence rate to vary between 21% and 30%,[18] while studies performed in different countries of Europe reported the overall prevalence of 20%[19] and in India, Mehta presented a rate of 33% from wound swabs and pus.[20] In our study, the prevalence rate was 41.71% whereas in Rawalpindi it was 60.40% which can be considered a regional high[21] and 41.9% in Lahore which is almost similar to our study.[22]

MRSA isolates were tested for their sensitivity to LZD in Iran and it was proven that all of the isolates (100%) to be susceptible to it.[23] Similar results were found in Kenya.[24] In major cities of Pakistan like Peshawar and Rawalpindi, LZD, when tested, appeared to be an admirable therapeutic choice as

all isolates were susceptible to it as well.[25,26] However a study conducted in 2011 in Cleveland, Ohio by Endimiani *et al*. showed 10.4% resistance of MRSA to LZD in patients who were suffering from cystic fibrosis and had a prolonged therapy with the aforementioned antibiotic,[27] On the other hand, a study conducted in 2008 in Madrid, Spain by Sánchez García *et al*. reported one of the first known clinical outbreaks of LZD resistant *S. aureus* (LRSA) in which 12 patients admitted to the ICU were reported and all of whom went under a short treatment of LZD.[28] When we tested LZD on MRSA isolates in our setup, we found out that 100% of the samples were susceptible to it as shown in Table 1.

S. aureus is infamous for acquiring resistance to almost any antibiotic. Resistance to penicillin was reported in hospitals shortly after its introduction into clinical practice.[29] These penicillin resistant *S. aureus* strains produce a plasmid-encoded enzyme, called penicillinase, which cleaves the beta-lactam ring of penicillin vital for its antibacterial activity. Infections caused by these strains rose by the mid-1940s but largely disappeared with the introduction of Methicillin.[29]

The earliest reports of a Methicillin resistant *S. aureus* strain were published in 1961.[29] Methicillin resistance in *S. aureus* involves integration of a staphylococcal cassette chromosome mec element composed of the mecA gene and the ccr gene complex.[30] The mecA gene encodes an altered penicillin binding protein 2a having reduced affinity for β-lactams thereby providing resistance to practically all β-lactam antibiotics.[31] Staphylococcus species which develop resistance to LZD is fairly uncommon, and very limited number of cases have been documented so far.[32] To date, the following mechanisms responsible for LZD resistance have been reported in clinical isolates of *S. aureus*: (i) Mutations in the domain V region of one or more of the five or six copies of the 23S rRNA gene (e.g., T2500A *Escherichia coli* numbering system)[33] (ii) acquisition of the plasmid-mediated ribosomal methyltransferase cfr gene,[34] and (iii) deletions or mutations in the ribosomal protein L3 of the peptidyl transferase center.[35]

LZD is the first broad-spectrum oxazolidinone available and has been effectively used to treat central nervous system infections, MRSA acute bacterial endocarditis and MRSA hospital-acquired pneumonia.[36] LZD can be administered either orally or intravenously and shows limited side effects. The oral bioavailability approaches 100%, thereby offering economic benefits.[37] LZD treatment has been associated with shorter length of stay (all $P < 0.01$), reduced intravenous duration (all $P < 0.0001$) and greater discharge rates (all $P < 0.05$).[38]

Table 1: Antibiotic resistance and sensitivity pattern of MRSA and MSSA to antibiotics

Antimicrobial drug	Number of resistant (%)		*P*-value
	MRSA isolates *n*=165	MSSA isolates *n*=204	
Oxacillin	165 (100)	0 (0)	0.0000*
Vancomycin	0 (0)	0 (0)	0.000*
LZD	0 (0)	0 (0)	0.000*
Fusidic acid	10 (6.06)	0 (0)	0.0000*
Teicoplanin	0 (0)	0 (0)	0.0000*

MRSA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin susceptible *S. aureus*, LZD: Linezolid

A wide range of measures have been suggested by infectious disease specialists for the control and prevention of MRSA including decolonization and isolation of MRSA-positive patients, hand washing, increased glove use, and shorter stay in the hospital.[39] Having guidelines for hospitals and nursing homes regarding environmental cleaning, use of personal protective equipment and MRSA screening are also identified as essential preventative ways of MRSA spread.[40] Additional efforts are being made toward more direct intervention, such as the use of anti-MRSA antibacterial and vaccines, followed by surveillance and annual reporting of MRSA cases to further decrease the global burden of MRSA.[39]

Conclusion

In our study, it was determined that the prevalence of MRSA was fairly high when compared with the previous studies conducted in Europe and South Asia. There are a multitude of reasons responsible for such a high prevalence of MRSA especially in the developing countries, the most common being substandard infection control policies in hospital combined with lack of screening and improper antibiotic usage and prescription. Hence, effective infection control policies and regular screening for MRSA should be performed on infected inpatients who previously went under LZD therapy or are currently under one, especially long-term therapy, to determine whether the strains are developing a resistance to LZD or not, as it is the only available antibiotic to show a good activity against MRSA with minimal side effects when compared with other drugs. Since the data available on the patients who are infected with LRSA is scarce, prolonged usage of LZD in patients with MRSA should be avoided and strict and monitored usage of antibiotics should be performed globally.

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