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Research article

Antimicrobial sensitivity testing of levonadifloxacin – A novel benzoquinolizine drug against MRSA isolates in a tertiary care hospital

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) adds a significant burden for health-care workers. In India there is a significant rise in prevalence of Methicillin resistant *Staphylococcus aureus* recently. Treatment has become very difficult among the resistant Staphylococcal isolates. Currently vancomycin and linezolid are the commonly used antibiotics. But these drugs have their adverse effects. Hence improved bactericidal antibiotics with increased tissue penetration and low possibility of developing resistance and safe to be used in chronic cases should be used for management of Methicillin resistant *Staphylococcus aureus* infections. The drug Levonadifloxacin acts actively against Methicillin resistant *Staphylococcus aureus* and Quinolone - resistant *Staphylococcus aureus* phenotypes. Recently in India, levonadifloxacin has been approved for the treatment of various infections like acute bacterial skin and soft tissue infections with complicating blood stream infections and also for diabetes complicating infections. Hence we evaluated the activity of levonadifloxacin (10µg) by Kirby–Bauer disk diffusion assay against Methicillin resistant *Staphylococcus aureus* isolates in the central laboratory of a tertiary care centre for a period of 1year between May 2021 to April 2022. 296 isolates of Staphylococcus aureus were identified from various clinical samples. Based on the results of disc diffusion test using Cefoxitin disc, Of the 296 *Staphylococcus aureus* isolates 104 were methicillin resistant and the rest of the 192 were methicillin sensitive. Methicillin resistant Staphylococcus aureus isolates were tested against levonadifloxacin by disc diffusion which yielded 100% susceptibility rate. Hence this study displays potent activity of the drug levonadifloxacin against Methicillin resistant Staphylococcus aureus isolates and is recommended for therapeutic use.

Keywords: Levonadifloxacin, Methicillin Resistant Staphylococcus aureus, *Staphylococcus aureus*, Gram positive infection

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INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) adds a significant burden for health-care workers [1]. According to WHO (World Health Organisation) mortality caused by MRSA is high compared to the Staphylococcal infections that are sensitive to Methicillin [2]. In India there is a significant rise in prevalence of MRSA to 37.3%(2017) from 29% (2009) [3]. Treatment has become very difficult among the resistant staphylococcal isolates. Currently vancomycin & linezolid are the generally used antibiotics whereas teicoplanin and daptomycin are rarely used to treat methicillin resistant staphylococcal infections. But all the drugs have their adverse effects.

In critically sick patients vancomycin is not used as the first choice of drug. This is attributed to the decreased bactericidal activity, less tissue penetration (lung), renal toxicity and clinical failure due to MIC creep (Minimum Inhibitory Concentration) [4-6]. Linezolid is not used in Blood Stream Infections (BSI) as it is a bacteriostatic agent and has side effects like bone marrow Suppression & thrombocytopenia. Thereby usage for a shorter period and by monitoring the vitals, it is recommended [7]. Though daptomycin is highly bactericidal, it cannot be used in pneumonia which is a major part of gram-positive BSI. This can be attributed to the pulmonary surfactant which inactivates the drug and the cross-resistance with vancomycin in hVISA (thermo resistant vancomycin -intermediate *Staphylococcus aureus)* strains [8]. Hence improved bactericidal antibiotics with increased tissue penetration and low possibility of developing resistance and safe to be used in chronic cases should be used for management of MRSA infections.

The drug levonadifloxacin (WCK 771) is a novel broad-spectrum benzoquinolizine fluoroquinolone. Levonadifloxacin acts actively against MRSA & QRSA(quinolone- resistant *Staphylococcus aureus*) phenotypes [9]. Recently in India alalevona difloxacin (WCK 2349) and levonadifloxacin are authorized for the treatment of various infections like acute bacterial skin and

soft tissue infections with complicating blood stream infections and also for diabetes complicating infections [10]. The action mechanism responsible for bactericidal activity is by inhibition of both DNA gyrase and topoisomerase IV [11]. Nor A efflux pumps aids the bacteria to expel the fluroquinolones out of cell and are associated with quinolone resistance in *S.aureus*, but levonadifloxacin is minimally affected invitro and does not have any impact on bacterial potency in *S.aureus*. This was validated by MIC determination of levonadifloxacin which was unaffected by Nor A efflux [12]. MRSA isolates, including the Bengal Bay clones, has been claimed to respond in vitro for levonadifloxacin [9]. It was also shown to have improved bactericidal effect on biofilm embedded QRSA [13].

Hence, we evaluated the activity of levonadifloxacin (10µg) phenotypically by Kirby–Bauer disk diffusion assay against Methicillin resistant *Staphylococcus aureus* isolates which were collected and tested for in central laboratory of a tertiary care centre in South India.

MATERIAL AND METHOD

The study was conducted in a tertiary care center, Sree Balaji Medical college and Hospital after obtaining ethical clearance for a period of 1 year between May 2021 to April 2022. 296 isolates of Staphylococcus aureus were identified from various clinical samples.

The *Staphylococcus aureus* strains were tested by the modified Kirby Bauer disc diffusion method against different antibiotic discs according to the CLSI (Clinical and Laboratory Standards Institute guidelines) for gram positive cocci [14]. Antibiotic discs were procured from HiMedia Laboratories Ltd commercially and used. The antibiotic panel of drugs used include the following discs, ampicillin (10µg), amikacin (30µg), amoxycillin-clavulanic acid (30/10µg), cefotaxime (30µg), ciprofloxacin (10µg), cotrimoxazole (25µg), erythromycin (5µg), linezolid (30µg), penicillin (10units), and vancomycin (30µg).

For detecting methicillin resistance, disk diffusion by Cefoxitin was done. Cefoxitin (30µg) disc was used for the test and the interpretation of results were done based on latest CLSI guidelines [14]. If the zone of inhibition of *S. aureus* is ≤21 mm then it is resistant. If the zone of inhibition of *S. aureus* is ≥21 mm then it is Sensitive. The results were also confirmed by Vitek-2 compac. of the 296 *Staphylococcus aureus* isolates 104 were methicillin resistant (MRSA) and the rest of the 192 were methicillin sensitive (MSSA).The methicillin resistant *Staphylococcus aureus* strains were subjected to susceptibility testing by disc diffusion with Levonadifloxacin (10µg).Following overnight incubation at 37°C,

Table 1: Levonadifloxacin Disk Diffusion

Pathogen	Levonadifloxacin Disk Diffusion (zone diameter in mm)		
	Sensitive	**Intermediate**	**Resistant**
Methicillin resistant Staphylococcus aureus	≥17	14-16	≤13

The results were interpreted using the following interpretive criteria (Table 1).

This was formulated based on a pharmacodynamic target attainment analysis which was enabled by Monte Carlo simulation and also the population pharmacokinetic model [15,16]. Quality control for antibiogram was performed using *Staphylococcus aureus* ATCC 25923 with zone size between 32-39 mm as interpretive criteria.

RESULTS

A sum of 296 isolates of *Staphylococcus aureus* were isolated from diverse clinical samples such as pus, Skin & soft tissue, Blood & Tissue fluids. Of which 104 were found to be Methicillin resistant Staphylococcus aureus and the rest 192 were methicillin sensitive isolates. The samples distribution among MRSA isolates is described in Table 2.

Table 2: Sample distribution of MRSA isolates

Specimen	Number of samples	Percentage of samples
Pus	45	42.72%
Skin & soft tissue	25	24.25%
Blood	18	17.64%
Tissue fluids	16	15.39%

Figure 1: sex ratio

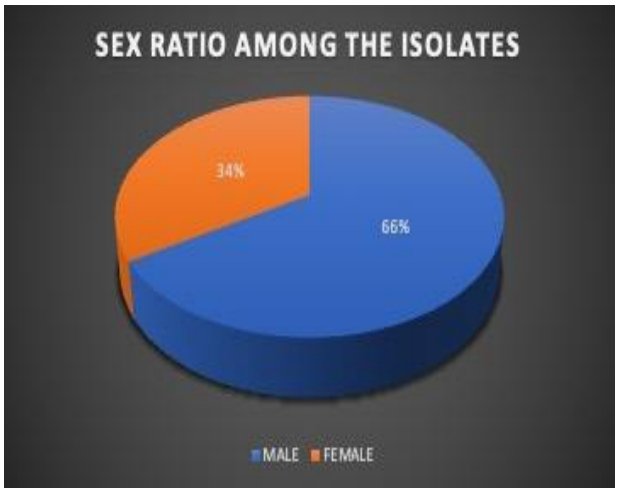


Table 3: Antibiotic profile of MRSA isolates

ANTIBIOTIC DISC	SENSITIVE	RESISTANT
Penicillin	-	104 (100%)
Cefoxitin	-	104 (100%)
Linezolid	104 (100%)	-
Ciprofloxacin	66 (63.4%)	38 (36.5%)
Cotrimoxazole	86 (82.69%)	18 (17.30%)
Tetracycline	88 (84.61%)	16 (15.38%)
Gentamycin	82 (78.84%)	22 (21.15%)
Erythromycin	18(17.30%)	86 (82.69%)
Clindamycin	52 (50%)	52 (50%)
Rifampicin	98 (94.23%)	6(5.76%)
Chloramphenicol	98 (94.23%)	6 (5.76%)
Levonadifloxacin	104 (100%)	-

The MRSA isolates were further analysed. The age of the patient population ranged from 17 to 73 years. The number of males and females enrolled in the study were 69 and 35, respectively with a

M:F ratio of 1.5:1. Detailed antibiotic profile of the patients is is shown in Figure 1.

Figure 2: Antibiotic resistance pattern among MRSA isolates.

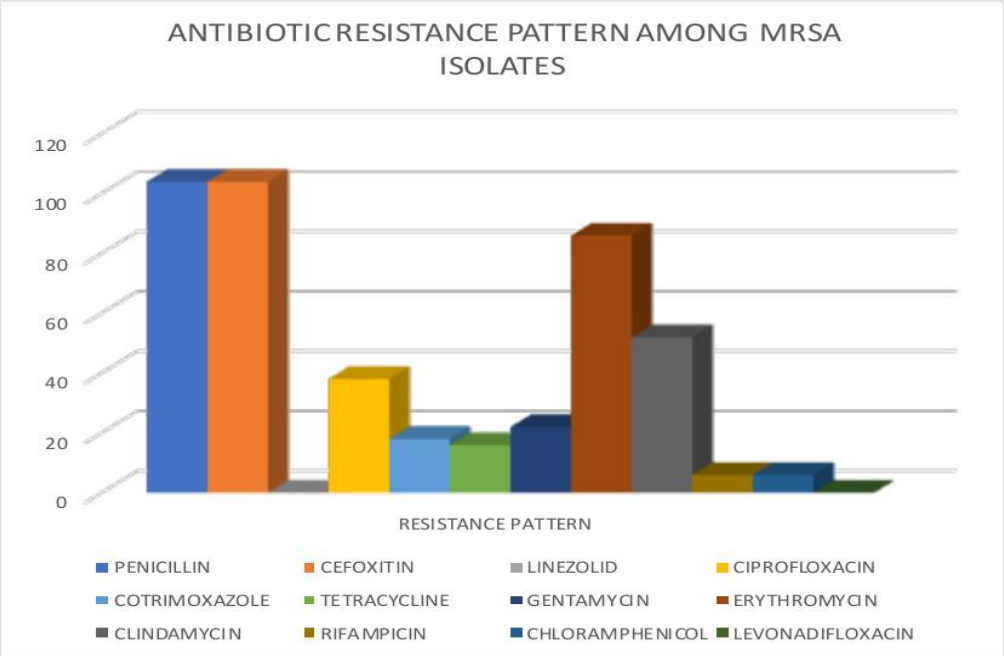
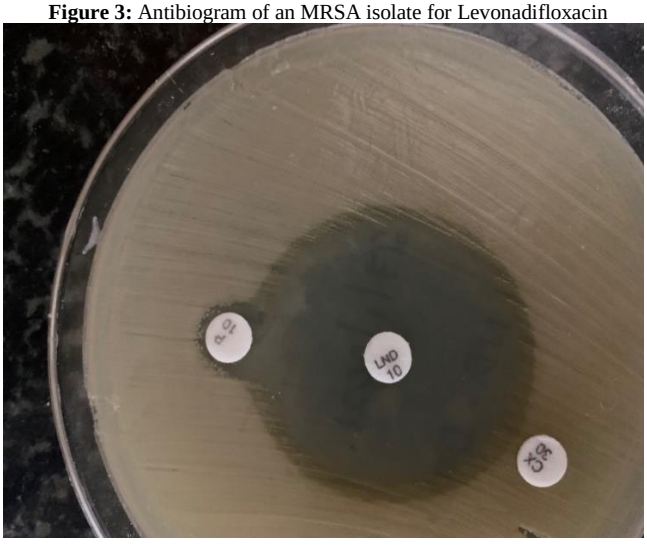


Figure 3: Antibiogram of an MRSA isolate for Levonadifloxacin



illustrated in [Table3/ Figure2]. Dissemination of male and female

DISCUSSION

In *S.aureus* methicillin resistance have been found to be due to acquisition of mecA gene, which is a part of SCC mec-cassette chromosome mec [17]. The mec A gene is responsible for penicillin binding protein (PBP2a), a peptidoglycan transpeptidase enzyme, which accords resistance to all *β*-lactam antibiotics such as penicillins, cephalosporins and carbapenems [17]. The presence of mecA gene can be studied using methicillin or oxacillin. Hence the name MRSA (methicillin resistant *S.aureus)* or ORSA (Oxacillin resistant *S.aureus)* have been termed [18].

This study was taken up to estimate the prevalence of MRSA among *S.aureus* isolates, from outpatients and inpatients admitted in a tertiary care center, Chennai. A new drug Levonadifloxacin has also been included for determining the susceptibility of the MRSA isolates. Levonadifloxacin – IV is a novel broad-spectrum antibiotics which belongs to benzo quinolizine subclass of fluoroquinolone group of drugs. Vancomycin (which is usually considered as the drug of choice) and gentamycin susceptibility were also carried out for comparison.

Levonadifloxacin has been endorsed in India for the treatment of blood stream infections and foot infections of diabetes caused due to Gram positive organisms. This drug targets the deoxyribonucleic acid gyrase enzyme which makes it more potent even against quinolone resistant *S.aureus* (QRSA). Deoxyribonucleic acid gyrase and topoisomerase IV are the two important enzymes of the bacteria which play a potential role in replication of DNA. Almost entire Quinolone group of drugs have more affinity to topoisomerase IV than DNA gyrase. Accordingly, if there is any mutation in the topoisomerase IV in the *S.aureus* isolates, the activity of quinolone drugs is highly impacted. Due to preferential affinity of Levonadifloxacin to DNA gyrase, the drug can easily overcome the ciprofloxacin and levofloxacin resistance in *S.aureus*. Levonadifloxacin has potential bactericidal effect on the biofilms of QRSA and MRSA. The other major cause of quinolone resistance in *S.aureus* is NorA efflux pump which is also inhibited by levonadifloxacin. In cases of high density cultures, this drug has an efficient cidal action [19]. The rate of prevalence of MRSA in our study is 35.1% which is in concordance with rates reported in other studies in India (32.8%) [20] 38% of the isolates showed methicillin resistance in a study by Tasneem *et al* [21]. 33.7% of MRSA has been reported in a study conducted in North India [22].

In India, it has been observed that the MRSA prevalence is not uniform. It varies in various regions of India. The range

varies from 26% to 43% across India [23,24]. Some studies have reported alarmingly high rates of MRSA infections 54.85% by Anupurba *et al* and 59.3% by Tiwari *et al* [25,26]. Further, the prevalence varies over period of time. A tertiary care center in Delhi, showed a prevalence rate of 51.6% in 2001 which further decreased to 38.44% in 2008 [26]. Reports on MRSA from various studies have shown a significant variation in the occurrence of the pathogen in different countries [27].

While comparing the gender in our study, males were more affected than females. Other studies also showed similar trends [28]. Most of the affected patients in our study belonged to elderly age group > 50 years. Similar trend was noted in many other studies in literature [22,29]. Most of isolates of MRSA were from Intensive care unit patients and surgical patients. This could be attributed to the possibility of invasion of MRSA colonization of skin during invasive procedures in Surgery department and indwelling devices in ICU. Similar results were observed in previous studies in literature [30,31].

Among the clinical samples, most of the isolates of MRSA were from pus samples (42.72%). The isolation rate was 24.25% from skin and soft tissue infections. Blood samples had isolation rate of 17.64% and Tissue fluids had 15.39% rate of isolation. The highest MRSA isolation from pus sample may be attributed to the wound being exposed to commensals of the skin like *S.aureus* which is likely to make the wound easier for infection and invasion of the organism. Identical results were found in many studies from different parts of India, Lohan et al [22] in Haryana (61.7%), Mallick and Basak in Maharashtra (61.4%)[30], Tiwari *et al* [26] in Varanasi (42%), and Rao and Srinivas *et al* [28] in Andhra Pradesh (64%).

In the current study, out of 296 *S.aureus* isolates, 104 were MRSA (35.1%) and 192 (64.8%) were MSSA. All the MRSA isolates showed 100% susceptibility to linezolid, followed by 94.23% susceptibility to Chloramphenicol and Rifampicin. In the current study, all the MRSA isolates were susceptible to Levonadifloxacin. This is consistent with a previous study done in Vellore, Tamilnadu by Bakthavatchalam *et al*, where all the 793 *S.aureus* isolates collected were found to be susceptible to levonadifloxacin [12]. In a study by Appalaraju B *et al*, Levonadifloxacin showed highly effective activity against 98.7% of the *S.aureus* isolates collected among 15 tertiary care hospitals from various regions of India [10]. 100% susceptibility to levonadifloxacin shown by MRSA isolates in the current study supports the therapeutic use of the drug in treating MRSA infections in clinical settings. The strong bactericidal action of Levonadifloxacin and its promising results seen in this study supports the usage of Levonadifloxacin clinically in managing the tough MRSA cases.

Though vancomycin and linezolid are still the recommended drugs in MRSA treatment, the use of vancomycin is commonly related with nephrotoxicity and prolonged linezolid usage can result in myelosuppression. On the other hand, levonadifloxacin can be administered in renal failure patients. Further, the availability of levonadifloxacin in oral formulation assists in easy switching of intravenous to oral route [32].

Mehta *et al*, in their study reported that Levofloxacin has been found to be safe and efficacious [33]. Since the drug is devoid of serious adverse effects like phototoxicity, nephrotoxicity and hepatotoxicity, levonadifloxacin offers a better option for the management of complicated bacterial infections. Antibiotic resistance is on the rise nowadays. Strategies to overcome the growing antibiotic resistance like selection of narrow spectrum antibiotics with low resistance properties and antibiotic resistance surveillance would be beneficial [34].

CONCLUSION

This study elicits potent activity of the drug levonadifloxacin against methicillin resistant *S.aureus* isolates. The rate of susceptibility of the MRSA isolates against levonadifloxacin is found to be 100%. Hence this study supports the therapeutic use of the drug for treating MRSA infections and also offers a better option for the management of resistant Gram positive bacterial infections.

Contribution of authors

Sharanya.k - concept, design, definition of intellectual content, literature search, data acquisition, manuscript preparation.

Lakshmi.k, vinod.k- literature search, data analysis, manuscript preparation, manuscript editing and manuscript review.

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