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In Vitro Susceptibility of Lefamulin Against Staphylococcus Aureus Isolated from Clinical Samples in a Tertiary Care Hospital

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ABSTRACT

Objective:To determine in vitro susceptibility of Lefamulin against Staphylococcus aureus isolated from respiratory samples in a tertiary care hospital in Pakistan.

Study Design: Cross sectional study.

Place and Duration of Study: Department of Microbiology Armed Forces Institute of Pathology, Rawalpindi, Pakistan from Jan to Jun 2022.

Methodology: The respiratory samples comprising of sputum, nasobronchial lavage (NBL), endobronchial washings (EBW) of patients were included in the study. The samples were processed at the Microbiology department laboratory using the standard microbiological procedures. The inoculation and incubation of specimens were done as per standard guidelines. The S. aureus isolates were identified on the basis of colony morphology, gram staining, Catalase, Coagulase and deoxyribonucleic acid-ase (DNAase) test.

Data was analysed using Statistical Package for Social Sciences (SPSS) 21.0. Frequencies and percentages were calculated for all quantitative variables.

Results: 215 specimens were evaluated, out of which 210(98.6%) were sensitive to Lefamulin. Out of these sensitive 212(98.6%) isolates 80(37.2%) belonged to EBW, 80(37.2%) from BAL, 40 (18.6%) from NBL and 15(7.0%) were sputum samples which were all sensitive.

Conclusion: This study showed that 98.6% of S. aureus were sensitive to Lefamulin antibiotic.

Keywords: Community-Acquired Bacterial Pneumonia (CABP), Lefamulin, Staphylococcus aureus.

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INTRODUCTION

Community-Acquired Bacterial Pneumonia (CABP) is a major contributor to mortality and morbidity globally.[1] Various pathogens responsible for CABP like Staphylococcus aureus (S. aureus), Haemophilus influenzae and Streptococcus pneumoniae are becoming resistant to frequently used empirical antibacterial treatments (Fluoroquinolones, Tetracyclines, B-Lactams, Macrolides).[2] The increasing bacterial resistance to antibiotics has led to the treatment failure of CABP which has led to the discovery of alternative novel bacterial agents.[3,4] In this regard, the United States Food and Drug Administration (FDA) in 2019 approved Lefamulin as a new therapeutic treatment for CABP.[5] It has proved to be clinically significant against Gram positive and gram negative pathogens with minimal toxicity and adverse reactions.[5] Thus, Lefamulin is a semi-synthetic Pleuromutilin which can be administered both intravenously and orally for treating CABP. Pleuromutilins were discovered in 1950s and are natural compounds used for encumbering the growth of S. aureus.[6]

Lefamulin has been through two phase 3 randomized controlled clinical trials; LEAP 1 and LEAP 2, i.e. Lefamulin Evaluation Against Pneumonia.[7,8] The acquired data from these successful trials indicated that no significant adverse events were reported against Lefamulin and it was well tolerated.[8] Lefamulin act in a way that it binds itself to the peptidyl transferase center of the 50S ribosome through four hydrogen bonds and several other interactions. Due to the distinctive mechanism of action of Lefamulin, limited cross resistance against frequently used antimicrobials has been evidenced.[9,10]

In Pakistan not much data is available regarding the healthcare burden of CABP and its associated novel empirical therapies due to inadequate research and difficulty in procuring Lefamulin, so this study aimed to highlight the use of this novel drug in existing treatment protocols of CABP. Particularly, this

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study evaluated the in vitro susceptibility of Lefamulin against S. aureus isolated from clinical respiratory samples in a tertiary care hospital in Pakistan.

METHODOLOGY

This cross sectional study was conducted at the Department of Microbiology Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan from January 2022 to June 2022 after taking approval from Institutional Review Board (IRB), (vide reference number FC-MIC-2/READ-IRB/21/1782). Sample size was calculated by using WHO sample size calculator which came out to be 215 with a confidence level 95%, keeping anticipated population proportion of Staphylococcus aureus isolates of 16% and an absolute precision of 5%,[11]

Inclusion Criteria: Clinical samples including sputum, naso bronchial lavage and endobronchial washings sent to microbiology lab during the study duration yielding growth of Staphylococcus Aureus were included in the study.

Exclusion criteria: Repeat samples from same patients, samples with insufficient bacterial growth and samples with mixed growth were excluded.

Non probability consecutive sampling technique was used. All the samples were processed using the standard microbiological procedures. The inoculation and incubation of specimens were done as per standard guideline.[12-13] 5% Blood agar (Oxoid, UK) plates were used for observing the colony morphology, gram staining and basic biochemical tests. The S. aureus isolates were identified on the basis of colony morphology, gram staining, Catalase, Coagulase and deoxyribonucleic acid-ase (DNAase) test.

Modified Kirby-Bauer disk diffusion method was used for determining susceptibility to Lefamulin using Mueller Hinton agar (Oxoid UK) using 20µg Lefamulin disk (Oxoid UK). Plates were incubated at 35°C±2 ˚C in ambient air and the zone of inhibition was read using reflected light after overnight incubation of 16 to 18 hours. As per CLSI guidelines a zone of ≥23mm was considered as susceptible.[14] Methicillin-Resistant S. aureus (MRSA ATCC 33591) and Methicillin-Susceptible S. aureus (MSSA ATCC 25923) were used control strains.

Statistical Package for Social Sciences (SPSS) 21.0 was used to analyze the data, frequencies and percentages were calculated for all quantitative variables. Descriptive statistics were calculated as Mean±SD and categorical data were expressed in terms of frequency and percentage. A Fisher exact test was applied between the sensitivity pattern of Lefamulin for Staphylococcus aureus. A *p*-value ≤0.05 was considered statistically significant.

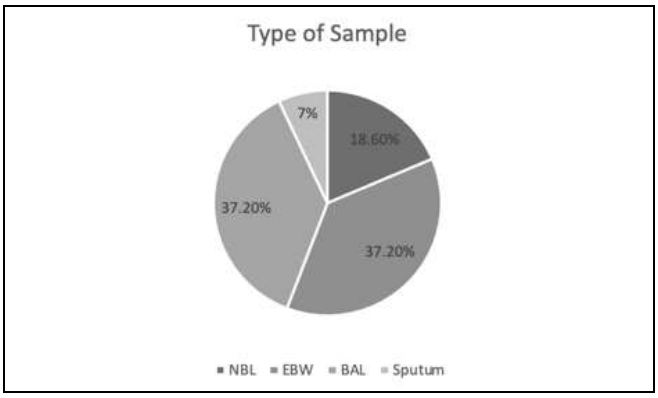

Figure-1: Distribution of the samples by biological nature (n=215)

RESULTS

Out of 215 patients were included out of which females were 71(33.0%) and males were 144(67.0%). The mean male age was 51.13±16.24 years and the mean female age was 49.79±14.73 years. The highest numbers of cases were reported among males and females in age groups 31-45 years as shown in Table-I.

Table-I: Gender Distribution across Age Groups (n=215)

Age Range (years)	Females n(%)	Males n(%)	Total n(%)
16-30	6(8.5%)	15(10.4%)	21(9.8%)
31-45	26(36.6%)	44(30.6%)	70(32.6%)
46-60	21(29.6%)	39(27.1%)	60(27.9%)
>60	18(25.4%)	46(31.9%)	64(29.8%)
Total	71(100.0%)	144(100.0%)	215(100.0%)

Figure-2 shows the distribution of the patients according to their referral location. It was noted that the highest frequency of patients were referred from the medical ward 94 (43.7%) followed by the Intensive Care Unit (ICU) 81(37.7%) and the outdoor examination 40(18.6%).

Table-II shows that there was a statistically significant difference (*p*-value <0.001) in the sensitivity pattern of Lefamulin for S. aureus. It shows that out of 215(100.0%) Lefamulin 212(98.6%) were sensitive to S. aureus, while remaining 3(1.4%) showed no behavior towards sensitivity pattern of S. aureus.

DISCUSSION

The results of our study confirmed the susceptibility of Lefamulin .Its sensitivity was found to

be 98.6% against S .aureus by using the Kirby-baur disk diffusion method. Our study showed that the novel Pleuromutilin; Lefamulin is more susceptible than generally available antibiotic regime used to treat bacterial pneumonia. Lefamulin has in vitro potency against S. aureus determined by using disk diffusion method; though post approval real world data is still not available regarding the efficacy and safety of Lefamulin. Moreover, data regarding long term use and tolerability of Lefamulin still needs further investigation worldwide and particularly in Pakistan.[15]

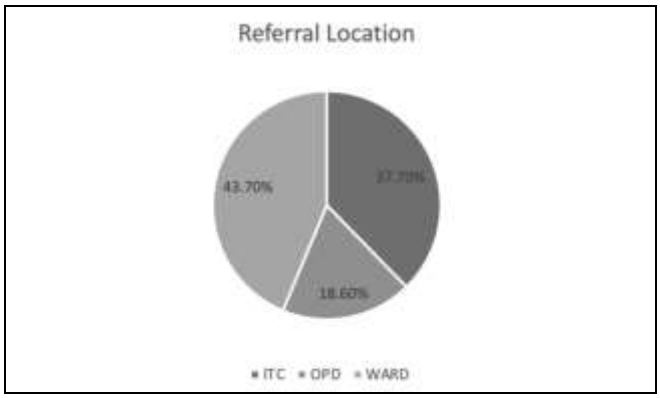

Figure-2: Distribution of the Patients by referral location (n=215)

It is expected that Lefamulin drug will soon be a novel addition to the drug list treating CABP effectively.[14]

Another study by Wu *et a*l.[20] explored the novel drug Lefamulin for treatment of CABP. This study assessed the Antibiogram of Lefamulin to find out its antimicrobial activity. The study conducted the minimum inhibitory concentrations (MICs) of Lefamulin on isolated strains from patients across China. The comparators used were micro-dilution method. The results of the study are similar to our results and showed that Lefamulin in vitro was potent against Staphylococcus and Streptococcus pneumoniae. The comparators were less active against test strains than Lefamulin. The study concluded that in vitro activity sustains the use of Lefamulin for treating CABP; and Lefamulin has broad spectrum coverage against MRSA, MSSA and Staphylococcus.[15]

In addition to this, Taylor *et al*.[21] conducted a research on the growing concern of increasing antibiotic resistance regarding Community-acquired pneumonia (CAP). Particularly, the resistance against Streptococcus pneumoniae is increasing worldwide. The study established the novel nature of Lefamulin as enhanced antibacterial agent due to scarce data available regarding its therapeutic abilities. This study also described the in vitro activity of Lefamulin

Table-II : Sensitivity pattern of Lefamulin for Staphylococcus Aureus (n=215)

Lefamulin	Sensitivity pattern of Staphylococcus aureus				Total n(%)	*p*-value
	Resistant n(%)	Sensitive n(%)	Intermediate n(%)	Not Detected n(%)		
Resistant n(%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	<0.001
Sensitive n (%)	0(0.0%)	212(98.6%)	0(0.0%)	0(0.0%)	212(98.6%)	
Intermediate n(%)	0(0.0%)	(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
Not Detected n(%)	0(0.0%)	0(0.0%)	0(0.0%)	3(1.4%)	3(1.4%)	
Total n(%)	0(0.0%)	212(98.6%)	0(0.0%)	3(100%)	215(100%)	

Sensitivity of Lefamulin against H. influenzae, S. pneumoniae, MRSA, MSSA and S. aureus have been proven by many studies,[12,16,17] however, this study only tested Lefamulin against S. aureus. A study by Kozhokar, Levien and Baker *et al*., evaluated the in vitro activity of Lefamulin against S. aureus and found out that a combination of Lefamulin with Doxycycline could be a potential empiric treatment CABP due to S. aureus.[18]

A study by Dubey *et al*.,[19] demonstrated that the cross resistance of Lefamulin with other Gram-positive antibiotic agents is low and the clinical trials of this drug have been positive. The data of Phase 3 clinical trials have proposed that Lefamulin has similar efficacy in comparison to Moxifloxacin against CABP. against isolates of CAP. Bacterial isolates of CAP were specimen of blood and lower respiratory tract. 876 isolates in total were tested against Lefamulin using MICs and micro-dilution methods. The results revealed that Lefamulin showed susceptibility to all S. pneumoniae isolates, 99% susceptibility to H. influenzae and 95.7% to MSSA. The study demonstrated that in vitro activity of Lefamulin against all respiratory pathogens was potent and this can lead to significant advancement in options for treatment of CAP.[16]

Lefamulin's efficacy against S aureus isolated from children's lower respiratory tracts, Sader, Paukner, Gelone, Arends, and Mendes examined Lefamulin's in vitro effects on S. aureus.The study

explained that due to a unique mechanism of action of Lefamulin, it has been proved potent against S. aureus. The isolates from different countries were collected and tested by broth micro-dilution method. Lefamulin showed 99% inhibition to isolates and was highly active against S. aureus collection. This novel antibiotic showed high potency against MRSA, and was resistant to Clindamycin, Levofloxacin, Gentamicin, Azithromycin. The study hence proved that the in vitro antibacterial activity of Lefamulin is highly active against S. aureus and can be used as a significant treatment option for lower respiratory tract patients.[12]

A study by Paukner, *et al.*, elucidated that the efficacy and safety of Lefamulin was evaluated after a pooled analysis which involved two phases LEAP 1 and LEAP 2 (Lefamulin Evaluation Against Pneumonia) in adults with CABP caused by atypical organisms (Chlamydia pneumoniae, Legionella pneumophila and Mycoplasma pneumoniae). The study confirmed that the clinical response of Lefamulin against atypical pathogens was high and it was well tolerated, in adults with CABP. The study further demonstrated that a short course of Lefamulin as empiric mono-therapy against CAP should be administered including oral therapy for 5 days.[16]

The results of this study put forward the current susceptibility status of this drug that lefamulin could be an important oral and intravenous mono-therapy option for empirical treatment of pneumonia. It would be a beneficial addition to the antibiotic armamentarium as it can be used as an alternative treatment to regular front-line antimicrobial agents .There is a dire need to further investigate the utility of this drug so that it can be applied on a broader scale. In this way the clinical niche of Lefamulin can be elaborated in a better way and will likely affect the extent of its future utilization and adoption.

LIMITATIONS OF STUDY

A comparative study on larger scale should be conducted in which respiratory samples from community should be included to evaluate its efficacy against community acquired bacterial pneumonia and other drug regimens.

CONCLUSION

This study concludes that Lefamulin is highly sensitive against Gram positive S. aureus in vitro and its susceptibility can be detected by using Disk diffusion technique. This study was the first of its kind to determine the susceptibility of this antibiotic.

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Authors Contribution

Following authors have made substantial contributions to the manuscript as under:

RB & SHN: Conception, study design, drafting the manuscript, approval of the final version to be published.

IAM & AI: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

RS & MMG: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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