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Original Article

EVALUATION OF MINIMAL INHIBITORY CONCENTRATIONS OF CEFTAROLINE, TEICOPLANIN AND DAPTOMYCIN FOR TREATMENT OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN A TERTIARY CARE SETTING

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ABSTRACT

Objective: To evaluate Minimal Inhibitory Concentrations of ceftaroline, teicoplanin & daptomycin for treatment of *methicillin resistant Staphylococcus aureus* in a tertiary care setting.

Study Design: Experimental study.

Place and Duration of Study: Department of Pathology, Microbiology Laboratory from Nov 2022 to Oct 2023.

Methodology: All the isolated Staph. aureus was processed and identified by colony morphology on blood agar, gram stain, and biochemical tests i.e., catalase, coagulase and DNAase test. Minimal Inhibitory Concentration was evaluated using E-strips for ceftaroline, teicoplanin & daptomycin for all the MRSA strains during the study period.

Results: A total of 924 *S.aureus* strains were processed, and 270 (29.22%) MRSA were recovered during the study period. The highest percentage 50 was observed in sputum (n=2), followed by 41.66% in endobronchial washing (n=10), pus 160 (31.25%), tissue 30 (31.25%), pus swab 50 (29.76%), high vaginal swab 2 (25%), and least in blood 16 (14.54%). MICs for all the MRSA isolates to teicoplanin were in susceptible range (≤8µg/ml). MIC of 22 (8.14%) and 14 (5.18%) MRSA isolates for Ceftaroline and daptomycin were in susceptible dose-dependent (SDD=2-4µg/ml) range.

Conclusion: The diagnostic modality, antimicrobial susceptibility testing, and MIC determination were found to be the best approach for adequate management of MRSA.

Keywords: Ceftaroline, Daptomycin, *Methicillin resistant Staphylococcus aureus (MRSA*), Minimal Inhibitory Concentration (MIC), Teicoplanin.

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INTRODUCTION

Bacterial antimicrobial resistance is progressing as an alarming challenge for patients and troublesome for health care personnel all over the globe. The bacterial resistance of to various antibiotic agents is evident and unavoidable as it depicts a general characteristic of bacterial evolution that cannot be stopped. Today, the global health priority is to devise innovative approaches to tackle antimicrobial resistance.[1]

Staph. aureus strains that are resistant to methicillin (MRSA) exhibit resistance to most of the betalactams. For years, Vancomycin remained the mainstay of opti-mal therapy against invasive MRSA infections. Misuse of vancomycin not only leads to higher MIC values of the drug but also to the development of heterogeneous, intermediary, and resistive forms, that are escalating in different regions of the world. Such resistant strains are a real threat to mankind.[2]

Ceftaroline fosamil is a parenteral advanced generation of cephalosporin with avid binding to Penicillin binding protein (PBP) 2a, showing remarkable spectrum of activity against MRSA infections causing pneumonia of community-based origin as well as skin and soft tissue infections (SSTI).[3] Minimal inhibitory concentration (MIC) needs to be assessed in cases where reduced susceptibility to ceftaroline was noted.[4]

Teicoplanin is also a parenteral glycopeptide like Vancomycin, reliably active invitro, clinically effective and recommended as first line or alternative agent against gram positive organisms. MICs of teicoplanin has reflected much better activity against clinical syndromes due to MRSA, instead of the standard dose.[5]

Daptomycin (DAP) is also a novel lipopeptide effective in treating persistent and problematic MRSA infections. The bactericidal effect of DAP reflects as immune modulator on monocytes and its ability

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to augment polymorphonuclear neutrophils efficacy through pathogen recognition, signal transduction and cytokine protein expression.[6] The aim of this study is to compare invitro motion of the organism with reference to minimal inhibitory concentrations of current available antimicrobial options i.e., ceftaroline, teicoplanin & daptomycin for management of *methicillin resistant Staphylococcus aureus* in a referral care setting.

METHODOLOGY

This experimental study was supported by the Microbiology Section, Pathology Department at Combined Military Hospital Lahore Pakistan, from November 2022 to October 2023 after approval from Research Institutional Review Board (Number 424/ 2023). After taking informed consent from all patients, their demographic data was recorded. Confidentiality was maintained and clinical samples were dealt with in accordance with the reference operative techniques for specimen processing in microbiology laboratory. All first positive cultures of *methicillin resistant Staphylococcus aureus (MRSA)* isolated from various sites of the body, re-isolation of MRSA from same patient after 7 days of previous positive culture and samples of patients of all ages and both genders will be included. Repeat specimens from same patient with same isolate within 7 days of first culture and duplicated samples from a single patient received on a single day were not included.[7]

All clinical samples were inoculated on primary media such as blood agar and MacConkey agar (prepared as per manufacturer's protocol) and aerobic incubation given at 35±2°C for 24 hours. Initial detection of *S. aureus* was based on colonial morphology on differential and selective media that include their size, shape, color, edges, surface, texture and existence or non- existence of hemolysis. On Microscopic examination, clusters of gram-positive cocci were further dealt with chemical tests of Catalase, Coagulase and DNase for the diagnostic identification of Staph. aureus.

Conventional disc diffusion (Modified Kirby Bauer) technique was employed for phenotypic detection of MRSA. A 30µg cefoxitin disc (Oxoid) was tested on all isolates of *S.aureus* on Muller Hinton agar plate following the Clinical and Laboratory Standards Institute (CLSI, 2023) guidelines. Bacterial suspension of 0.5 McFarland turbidity standards was adjusted for antibiotic sensitivity testing of all strains. Zone of inhibition was read on sensitivity plates after 24 hours incubation at 35±2°C. According to CLSI criteria (2023), an area of inhibition of ≤21 mm was considered as resistant. ATCC strains, i.e., MRSA ATCC 33591, and MSSA ATCC 25923 were used as positive and negative controls, respectively. Only resistant isolates were incorporated in the study project.

For MICs of ceftaroline, teicoplanin and daptomycin, E-test technique was applied to all the MRSA isolates.

Antimicrobial sensitivity of all isolated strains was done by disc diffusion susceptibility test, following CLSI (2023) protocols. Antimicrobial discs were applied according to the Tiers mentioned in the guidelines of CLSI, 2023. Disks of Penicillin 10U (P), Erythromycin 15 µg (E), Clindamycin 2µg (DA), Ciprofloxacin 5µg (CIP), Gentamicin 10µg (CN), Vancomycin 30µg (VAN), Linezolid 30µg (LZD), Trimethoprim /sulfamethoxazole 25µg (SXT), Doxycycline 30µg (DO), were employed. Plates were placed in incubator overnight at 35±2°C.[8]

Data was analyzed statistically using SPSS Version 24.0. Quantitative variables i.e., the specimens from which *methicillin resistant Staphylococcus aureus* was isolated, frequency and percentage were depicted in tabulated form. Statistically, *p*-value found to be ≤0.05, ≥0.05 and ≤0.001 were considered significant, insignificant, and highly significant, respectively.

RESULTS

All 924 (15.73%) *S.aureus* strains were isolated from 5872 (19.12%) culture positive samples. Phenotypic screening identified 270 (29.22%) MRSA strains. Table-I shows the frequency distribution of MRSA (n=270) strains isolated from different clinical specimens. MICs of ceftaroline and daptomycin is given in Table-II. MICs of all MRSA isolates to teicoplanin were in susceptible (≤8 µg/ml) range. Antibiotic susceptibility testing is given in Table-III. All the MSSA (n=654) and MRSA (n=270) isolates were 100% sensitive to Vancomycin, Linezolid, and Rifampicin.

Table-I: Distribution of MRSA (n=270) in different clinical samples.

Specimen	Staph. Aureus n=924	MRSA n=270 (%)
Blood	110	16 (14.54)
HVS*	8	2 (25)
Pus swab	168	50 (29.76)
Pus	512	160 (31.25)
Tissue	96	30 (31.25)
EBW**	24	10 (41.66)
Sputum	4	2 (50)
NBL***	2	-

** High vaginal swab, **Endobronchial washing, ***Nondirected-bronchial lavage value: 8.666 0.2778 (no significant difference)*

Table-II: MIC of Ceftaroline and Daptomycin in MRSA Isolates (n=270).

Specimen (n=270)	Ceftaroline (µg/ml)		Daptomycin (µg/ml)	
	S ≤1 n= (%)	SDD 2-4	S ≤1 n= (%)	SDD 2-4
Blood (16)	14 (87.50)	2	16 (100)	-
HVS (2)	2 (100)	-	2(100)	-
Pus swab (50)	44 (88)	6 (12)	46 (20.83)	4
Pus (160)	152 (95)	8 (5)	154 (96.25)	6
Tissue (30)	26 (86.66)	4 (13.33)	28 (93.33)	4
EBW (10)	8 (80)	2(20)	10 (25)	-
Sputum (2)	2 (100)	-	2 (100)	-

SDD → Susceptible-dose dependent, Chi-square value: 1.569, p-value: 0.210 (no significant difference)

Table-III: Sensitivity pattern of *Staph. aureus* and MRSA (n=924).

Antimicrobials	MSSA n=654 (%)	MRSA n=270 (%)
Penicillin	38 (5.81)	--
Erythromycin	258 (39.44)	92 (34.07)
Clindamycin	488 (74.62)	152 (56.29)
Gentamicin	430 (65.74)	142 (52.59)
Ciprofloxacin	82 (12.53)	28 (10.37)
Doxycycline	642 (98.16)	262 (97.03)
Trimethoprim/ sulfamethoxazole	558 (85.32)	218 (80.74)
Fusidic acid	636 (97.24)	260 (96.29)

DISCUSSION

The magnitude of the health threat posed by *Methicillin-resistant Staphylococcus aureus (*MRSA) has surged dramatically on a worldwide scale.[9] Findings of high MRSA prevalence in this study highlight its momentous burden within the community. The pervasive load of MRSA in our study is consistent with the previous reports from different regions. A systematic review revealed an overall elevated percentage of MRSA (37%) from our neighboring country.[10] Another study in Pakistan has documented even higher levels of MRSA i.e., 61.8% which contrasts with our research.[11] These findings indicate that MRSA is a significant fitness matter all around the globe.

Assessing the Minimum Inhibitory Concentrations (MICs) of drugs for MRSA isolates plays a crucial role in ensuring effective patient management. The findings of this study suggest that ceftaroline, teicoplanin, and daptomycin are promising antibiotics for treating MRSA infections. Notably, all MRSA isolates demonstrated susceptibility to teicoplanin, consistent with findings from a related study.[12] However, it is worth mentioning that another study reported a notably higher rate of resistance to ceftaroline, which contrasts with the outcomes seen in our study.[13]

Even though, MICs of tested drugs for most of the MRSA isolates were in the sensitive range, however, the MIC values for daptomycin and ceftaroline for few MRSA isolates recovered from pus, pus swabs and tissue samples were marginally higher in our study. Another study stated that although most of the MRSA were sensitive to daptomycin, some isolates showed a gradual increase in resistance as well.[14] This increase in MIC values even for few MRSA isolates might as a result selective antibiotic pressure generated by medication usage. It could endanger the already difficult treatment of serious MRSA infections and may be the initial step towards development of resistance.

All *S.aureus* isolates in this study were sensitive to vancomycin, and linezolid. MRSA isolates showed good sensitivity profile for doxycycline followed by fusidic acid, Trimethoprim/sulfamethoxazole, clindamycin, gentamicin, erythromycin, and ciprofloxacin. Drug susceptibility pattern of vancomycin and linezolid observed is similar with an earlier study in Pakistan.[15] Contrary, a study conducted in Nepal revealed increase resistance of MRSA strains for gentamycin, erythromycin, Trimethoprim/sulfamethoxazole while good susceptibility to vancomycin and linezolid was observed.[16] A previous study conducted in Pakistan revealed a worrisome trend of low sensitivity to ciprofloxacin, however, it exhibited a higher sensitivity compared to the ciprofloxacin sensitivity observed in MRSA isolates of our study.[17] This highlights the importance of maintaining vigilant monitoring and potentially reevaluating antibiotic choices within clinical settings.[18] The difference of susceptibility pattern of drugs for MRSA isolates among various studies may be due to the pattern of antibiotic prescription in different communities, improper use of antibiotics as empirical treatment, and the hospital or community-based origin of MRSA isolates.[19]

CONCLUSION

MIC evaluation demonstrated successful treatment rates for intermediate and high-risk infections due to MRSA. Our findings highlighted the necessity to adopt continuous surveillance strategies to effectively prevent the dissemination of existing multidrug resistance MRSA strains. Systematic surveillance guidelines must be designed to limit the advancing health, economic and environmental hazard imposed by "MRSA-Super Bug". Deployment of efficacious prevention and control measures can help to reduce resistance challenge and extend the usefulness of available antibiotics.

Conflict of Interest: None

Authors Contribution

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

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

FA, NY & NA: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

QA & AN 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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