ORIGINAL ARTICLE

In Vitro Activity of Ceftaroline against methicillin resistant *Staphylococcus aureus* (MRSA) isolates from different clinical samples: A study from a tertiary care hospital

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ABSTRACT

Background: Ceftaroline is a novel, fifth generation cephalosporin, with potent activity against methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Our study is aimed to assess the spectrum and potency of Ceftaroline against MRSA isolates from different clinical samples.
Methods: A total of 126 methicillin resistant *Staphylococcus aureus* (MRSA) strains isolated from different clinical samples from patients hospitalized in 2020 due to documented skin and soft tissue infection, intra-abdominal infection, lower respiratory tract infection, and blood stream infections were tested for susceptibility to Ceftaroline. Methicillin resistance was detected by Kirby-Bauer method using 30-µg cefoxitin disc. Ceftaroline minimum inhibitory concentrations (MIC) was detected for all the 126 isolates by using the E strips of Ceftaroline (biomerieux).
Results: During our study, of the 126 MRSA isolates tested, all were seen sensitive to Ceftaroline. The MIC values ranged from 0.25 to 4 µg/ml. Only six isolates showed the maximum MIC of 4µg/ml (Blood samples), which is in the intermediate susceptible range. The MIC50 and the MIC90 of the MRSA isolates were 0.5 µg/ml and 2µg/ml, respectively. The percentage of susceptible MRSA strains were 95.2%, while 4.8% were susceptible dose dependent (SDD). None of the MRSA isolates showed resistance to Ceftaroline.
Conclusion: This study reveals the patterns of ceftaroline susceptibility of MRSA isolates from various clinical samples from a previously unreported geographical location. Overall, ceftaroline showed excellent *in vitro* activity against MRSA isolates and should be considered as an efficient alternative for the treatment of MRSA infections.
Keywords: methicillin-resistant *Staphylococcus aureus* (MRSA), ceftaroline, antimicrobials, drug resistance.
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INTRODUCTION

The increasing incidence of anti-microbial drug resistance of Staphylococcus aureus strains is a serious challenge for clinicians. Methicillin-resistant Staphylococcus aureus (MRSA) is defined as Staphylococcus aureus with a minimum inhibitory concentration (MIC) to Oxacillin of ≥4 µg/mL, as opposed to methicillin-sensitive Staphylococcus aureus (MSSA), which is sensitive to penicillins and cephalosporins[1]. Infections caused by MRSA are a major health problem worldwide and are often associated with significantly high morbidity, mortality, lengthy hospital stays, and higher bills. Initially identified as a nosocomial pathogen, MRSA is now responsible for both hospital and community acquired infections[2]. The treatment options for MRSA include glycopeptides and combination regimens, also some newer agents like daptomycin and fifth generation cephalosporins (ceftaroline and ceftobiprole). While several antimicrobial agents remain available for treatment, resistance to a specific agent makes the successful treatment of MRSA infections challenging[3].

β-Lactam antibiotics were initially developed to inhibit penicillin-binding proteins (PBPs) which catalyze the bacterial cell wall synthesis. Nevertheless, as soon as they were introduced, their efficacy was significantly altered by Staphylococcus aureus strains capable of producing a β-lactamase enzyme or by the horizontal acquisition of mecA gene, encoding the penicillin binding protein 2a (PBP2a).

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PBP2a has very low affinity to β-lactams and therefore can catalyze DD - transpeptidation and collaborate with the PBP2 transglycosylase in peptidoglycan biosynthesis[4, 5]. Ceftaroline is a novel, fifth generation cephalosporin, capable of inhibiting PBP2a and is active against MRSA and other Gram-positive bacteria, and, to a lesser extent, against Gram-negative bacteria[6]. The anti-MRSA activity of ceftaroline shows an MIC90 of around 12 µg/ml and has been approved for the treatment of complicated skin and soft tissue infections and community acquired pneumonia[7,8]. Various studies appear to have shown decreased susceptibility of MRSA to Ceftaroline (CLSI MIC ≥ 4 µg/ml or EUCAST MIC values of > 1 µg/ml) in sporadic cases and limited to specific sequence types (9,10). More recently, high-level ceftaroline resistance (MIC > 32 µg/ml) has been observed during sustained MRSA bacteremias that were treated with ceftaroline[11]. Low level resistance to ceftaroline is seen to be associated with mutations in the PBP2a found in both the allosteric domain (N146K, E150K, N204K, E239K, G246E) or the transpeptidase domain (H351N, Y446N, E447)[4,11,12].

The pattern of ceftaroline resistance against MRSA isolates from clinical samples worldwide remains poorly defined and leaves a lot of scope to be widely studied in different geographical regions. This study aimed to assess the spectrum and potency of Ceftaroline against MRSA isolates from different clinical samples and to compare it with other antimicrobial agents routinely used for anti-microbial susceptibility testing in patients hospitalized due to infection with MRSA.

MATERIALS AND METHODS

Study design and setting

This hospital based, cross sectional study was carried out in the Bacteriology division of the Postgraduate department of Microbiology, Government Medical College, Srinagar, and Associated hospitals, a tertiary care center that caters to the majority of population in the Kashmir division. All the relevant clinical, demographic, and epidemiological information was recorded.

Inclusion criteria

In this hospital-based study, we included clinical samples of patients of all age groups. The samples were collected from different patients who were hospitalized due to, complicated skin and soft tissue infection, intra-abdominal infection, urinary tract infection, lower respiratory tract infection, and bloodstream infections.

Samples collection and processing

Clinical samples including Pus, swabs, aspirates, urine, sputum, and blood were collected according to the standard protocol and subjected to aerobic bacterial cultures in the laboratory as per the standard Microbiological procedures[13]. Once received, the clinical specimens were subjected to gram staining and subsequently inoculated on sheep blood agar and MacConkey agar plates and incubated at 37°C for 1824 hours for identification of the microorganisms. Various spot tests and detailed biochemical panels were used to identify the isolated organisms to the species level. Further, anti-microbial susceptibility testing (AST) was done on Mueller Hinton agar (MHA) by Kirby-Bauer disc diffusion method and individual zone diameters were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines, 2020[14]. Antibiotic discs considered for routine primary testing panel were chosen according to the latest CLSI 2020 guidelines. For cultures that were identified as Staphylococcus aureus, following antibiotic discs were used: Penicillin-G (P), cefoxitin (CX), vancomycin (VA), linezolid (LZ), gentamicin (GEM), erythromycin (E), clindamycin (CD), tetracycline (TE), co-trimoxazole (COT), ciprofloxacin (CIP), and rifampicin (RIF). Cefoxitin 30-µg disc was used as a surrogate marker for detecting methicillin resistance. All the isolates that were resistant to cefoxitin (Zone inhibition diameter of ≤ 21 mm) were labeled as MRSA. Ceftaroline minimum inhibitory concentrations (MIC) were detected for all the recovered MRSA isolates by using the E strips of Ceftaroline (biomerieux). The latest CLSI clinical break points were applied for interpretation of ceftaroline MIC (sensitive ≤ 1 µg/ml, 2-4 µg/ml intermediate, and ≥8 µg/ml resistant)[14]. Control strain, ATCC 29213 of Staphylococcus aureus was used for MIC detection.

Table 1: Clinical sample distribution of *Staphylococcus aureus* isolates.

Sample	*Staphylococcus aureus* isolated	MRSA	MSSA
PUS	132	**56**	77
SWABS	86	**36**	50
BLOOD	38	**16**	22
URINE	19	**08**	11
ASPIRATES	15	**06**	09
SPUTUM	10	**04**	06
Total (%)	300 (100%)	**126 (42%)**	174 (58%)

RESULTS

Of the total 300 *Staphylococcus aureus* isolates that were recovered from various clinical samples and subsequently tested for methicillin resistance, 126 (42%) isolates were confirmed as MRSA and 174 (58%) as MSSA isolates. The specimen sources of MRSA isolates included 92 from superficial and deep wound infections (73 %), 16 from bloodstream infection (12.7 %), and 08 from urinary tract infections (6.3%) (Table1). Antimicrobial susceptibility profile of the recovered MRSA isolates is given in details in Figure 1. All MRSA isolates (n=126) were resistant to Penicillins (100%) followed by Gentamycin (65%), Erythromycin (45%), Clindamycin (43%), 37% isolates were resistant to Ciprofloxacin. All the MRSA isolates (100%) were sensitive to Vancomycin, Linezolid, and Tetracyclines.

To determine ceftaroline susceptibility we further examined the 126 MRSA isolates recovered from clinical samples. According to the CLSI 2020 MIC interpretation breakpoints, the percentage of susceptible strains were >95.2% (MIC ≤ 1 µg/ml) (Image 1), and the percentage of resistant strains were 0%. 4.8% of tested isolates showed MIC values (2-4 µg/ml) falling under susceptible dose dependent (SDD) / Intermediate category (Table 2). The Ceftaroline MIC values ranged from 0.25 to 4 µg/ml. Only two isolates showed the maximum MIC of 4 µg/ml (Blood samples) (Image 2), which is in the intermediate susceptible range. The MIC50 and the MIC90 of the isolates were 0.5 µg/ml and 2 µg/ml, respectively.

Table 2: In Vitro Activity of Ceftaroline tested against Isolates of MRSA

Organism (n)	**Ceftaroline MIC (µg/ml)**			**CLSI MIC Interpretation**		
	MIC_{50}	**MIC_{90}**	**MIC range**	**% Susceptible**	**% SDD**	**% Resistant**
MRSA (126)	0.5	2	0.25 to 4	95.2	4.8	0

MIC, minimal inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute; SDD, susceptible-dose dependent.

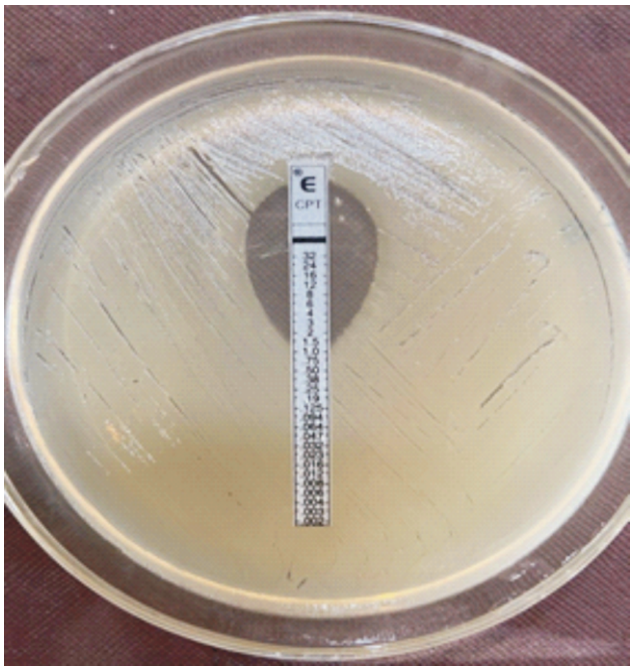


Image 1; Ceftaroline E-Strip MIC 1 µg/ml

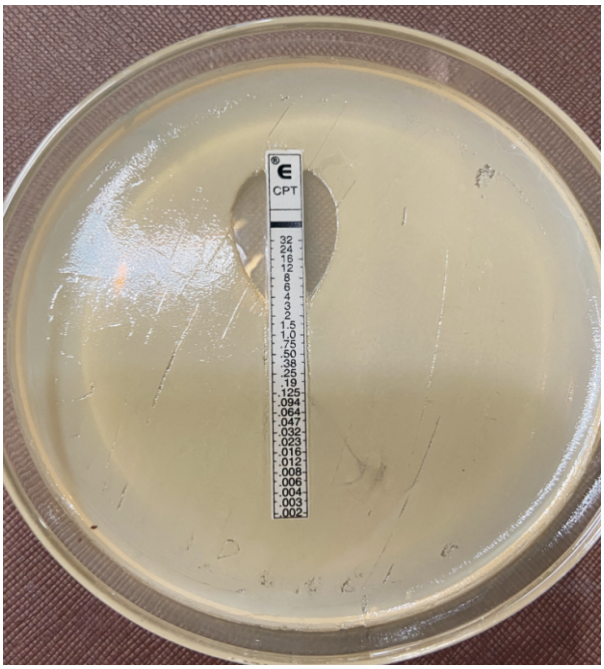
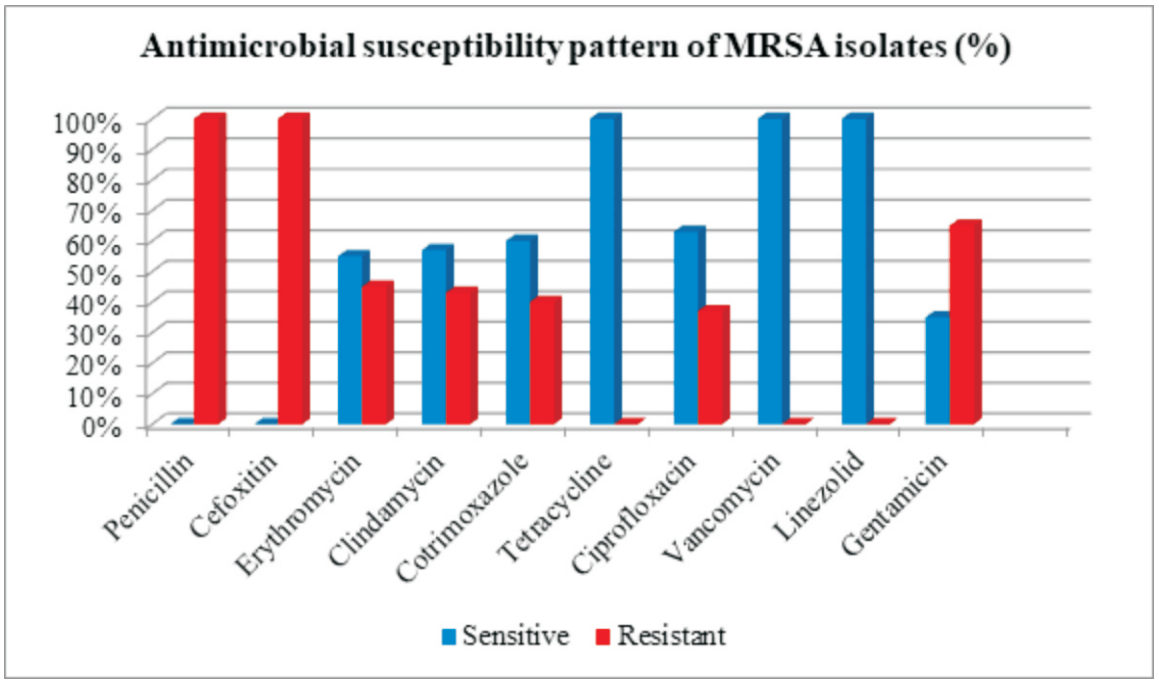


Image 2; Ceftaroline E-Strip MIC 4 µg/ml

Figure 1: Antimicrobial susceptibility pattern of Methicillin resistant *Staphylococcus aureus*



DISCUSSION

Staphylococcus aureus is among the most commonly encountered microorganism implicated in a plethora of human infections and recovered from almost all clinical samples at a fairly constant rate in our laboratory.With the emergence of antimicrobial resistance among Staphylococcus aureus isolates, particularly the MRSA strains, newer drugs are needed to test and treat these life threatening infections both at community and health care levels[2,3].Ceftaroline and other β-Lactams like, ceftobiprole and razupenem, show enhanced targeting of PBP2a,encoded by the mecA gene located on the Staphylococcal Cassette Chromosome (SCC)[15,16]. The clinical formulation of ceftaroline is a water-soluble N-phosphono prodrug (ceftaroline fosamil) that exhibits potent inhibition of PBP2a and resulting low MIC values of 0.250.5 mg/L[17]. Ceftaroline has a documented bactericidal activity against Staphylococcus aureus[18].

In our study, >95.2% (MIC ≤ 1 µg/ml) of tested MRSA isolates showed susceptibility to this drug with the MIC90 ≤ 2µg/ml (Table 2). Our findings correlate well with the surveillance studies of other geographical areas around the world. In the Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE) programme, 98.4% of the MRSA were inhibited at MIC value of 1 mg/L. The highest MIC in their study was 2 mg/L[19]. In a study by Jones et al.[20] 94.8% of the MRSA isolates from USA were inhibited at a concentration of 1 mg/L, whereas only 82.6% European isolates were inhibited at 1 mg/L and 99.5% isolates at 2 mg/L. This high degree of susceptible to ceftaroline by MRSA isolates which otherwise document high levels of resistance to commonly used β-Lactam antibiotics is a welcome sign. Ceftaroline has been sparingly used to test and treat MRSA infections in our region and hence the resistance pattern is not well documented. So, the high susceptibility may be attributed to the restricted use of this novel antibiotic.

In our study, the percentage of resistant strains were 0%, while 4.8% of tested isolates showed MIC values (2-4 µg/ml) falling under susceptible dose dependent (SDD) / Intermediate category. In a similar study by Zhang Zet al.[21] for MRSA, the MIC50 and the MIC90, and MIC range of ceftaroline were 0.5, 2, and 0.01564 µg/ml. The proportion of susceptible MRSA strains were 89.3% and resistant MRSA strains were 0.7%. In another study by Basireddy S et al[22] from Indian state of Telangana, all the 50 MRSA isolates tested against ceftaroline were sensitive, the MIC

values ranged from 0.125 to 1.5 mg/L. Only two isolates showed the maximum MIC of 1.5 mg/L, which is in the intermediate susceptible range and all the remaining isolates showed MIC ≤ 1 mg/L. The MIC50 and the MIC90 of the isolates were 0.5 mg/L and 1 mg/L, respectively. These results are in concordance with the results of our study. The SDD/ intermediate category isolates are often found responsive to ceftaroline if the dose in increased or the duration between two doses is shortened [14].

CONCLUSION

Our study, the first from this region reveals the spectrum and potency of Ceftaroline against MRSA isolates recovered from different clinical samples from a previously unreported geographical location. While we found alarming levels of β-Lactam resistance, the MRSA isolates showed 100% susceptibility to Vancomycin, Linezolid, and Tetracyclines. The overall susceptibility to ceftaroline is excellent and no MRSA strain showed resistance to this novel cephalosporin. Although there has been documented resistance reported from various geographical locations to ceftaroline, it still remains an excellent choice considering the low levels of reported resistance. To conclude, ceftaroline showed excellent in vitro activity against MRSA isolates and must be considered as an efficient alternative for the treatment of both community as well as healthcare associated MRSA infections.

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CONFLICT OF INTEREST: There is no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors have made a substantial, direct, and intellectual contribution to the research work, and approved it for publication.

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DATA AVAILABILITY: All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT: This study was approved by the ethical clearance committee.

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