DOI: 10.7860/NJLM/2021/48509:2485

Original Article

Microbiology Section

Ceftaroline In-vitro Activity against Methicillin Resistant Staphylococcal Isolates in a Rural Tertiary Healthcare Centre

VIJAYA SHIVANNA[1], SS PRAJWAL[2], D VENKATESHA[3]

ABSTRACT

Introduction: The emergence of Methicillin Resistant (MR) staphylococcal infections had led to fewer therapeutic options. Ceftaroline fosamil is the only cephalosporin approved by United States of America (USA) Food and Drug Administration (FDA) till now which has activity against MR staphylococcal isolates. Until now, studies on in-vitro activity of ceftaroline are limited.

Aim: To know the susceptibility pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Resistant Coagulase Negative Staphylococci (MRCoNS) against ceftaroline in a rural tertiary health care centre.

Materials and Methods: The present cross-sectional study was conducted in a tertiary care hospital in rural part of Karnataka between two months (July and August 2019). A total of 50 consecutive, non repetitive clinical isolates of MRSA and MRCoNS were obtained. Antibiotic susceptibility testing was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The Minimum Inhibitory Concentration (MIC) of ceftaroline was detected using E-strips (Biomerieux). The CLSI breakpoints applied for the interpretation of ceftaroline MIC-Sensitive: ≤1 µg/mL; Susceptible Dose Dependent (SDD): 2-4 µg/mL and resistant ≥8 µg/mL). *Staphylococcus aureus* American Type Culture Collection (ATCC) 29213 was used as a quality control. Statistical analysis was done using microsoft excel. Percentages were used in this study to analyse variables.

Results: Out of 50 MR staphylococcal isolates, 10 (20%) were MRSA and 40 (80%) were MRCoNS. Of the MR staphylococcal isolates tested, 49 (98%) were sensitive to ceftaroline. The MIC 50 and MIC 90 for the 50 MR staphylococci was 0.25 µg/mL and 1 µg/mL, respectively.

Conclusion: Ceftaroline demonstrated a potent in-vitro activity against MR staphylococci. So it can be used as an effective drug in the treatment of such infections.

Keywords: Antibiotic policy, Epsilometer test, Minimum inhibitory concentration, Surveillance study

INTRODUCTION

Staphylococcus spp. is a major cause of both hospital and community acquired infections worldwide [1]. With the emergence of MR strains, the only option available is glycopeptides such as vancomycin, teicoplanin and linezolid [2]. But the reports of vancomycin-intermediate resistant and vancomycin resistant staphylococci, and other limitations of vancomycin like slow bactericidal activity, poor penetration in tissues, nephrotoxicity at higher dose and narrow therapeutic index are leading to treatment failure in critically ill patients [3,4]. Even though linezolid is highly active against MR strains, it is bacteriostatic and may cause thrombocytopenia and myelosuppression [5]. Newer drugs such as ceftaroline, dalbavancin, oritavancin and tedizolid are available to treat such infections [2].

Ceftaroline Fosamil (prodrug of ceftaroline), is a fifth generation, parenteral cephalosporin with a wide spectrum of bactericidal activity against multidrug resistant *Streptococcus pneumoniae*, *Staphylococcus aureus* and some Gram negative bacteria [6]. It also acts against MRSA, Vancomycin Intermediate resistant *Staphylococcus aureus* (VISA), Vancomycin Resistant *Staphylococcus aureus* (VRSA), MR and Sensitive Coagulase Negative Staphylococci (MRCoNS and MSCoNS) [7]. The CLSI gave it a new sub class as, 'Cephalosporins with anti MR *Staphylococcus aureus* activity' [8]. It is a newer generation cephalosporin approved by USA FDA in 2010 for the treatment of Community Acquired Pneumonias (CAPs), Acute Bacterial Skin and Skin Structure Infections (ABSSSIs) [9]. The unique activity against MR strains is because of its affinity for Penicillin Binding Protein (PBP) 2a-MRSA specific protein, which distinguishes ceftaroline from other cephalosporins [10]. It causes bacterial cell wall irregularities and eventually bacterial cell death.

As ceftaroline is a newer agent with only limited studies about its in-vitro activity [1,2,10], the present study was done to know the susceptibility pattern of MRSA and MRCoNS against ceftaroline.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Microbiology, of a tertiary care rural health centre in Southern India for a period of two months (July and August 2019) as part of Indian Council of Medical Research Short Term Studentship (ICMR-STS) 2019 (Reference ID. 2019-01176). Institutional Ethical Committee clearance (AIMS/IEC/1954/2019-20) was obtained to conduct the study.

Sample size calculation:

$$\text{Sample size (n)} = \frac{Z^2 \times P\,(1-P)}{d^2}$$

Z=1.96 (For conventional confidence level of 95%)

P=Expected percentage of susceptibility to ceftaroline among MR staphylococcal isolates, based on previous studies [2,10] -95%

d=absolute error of precision -5%

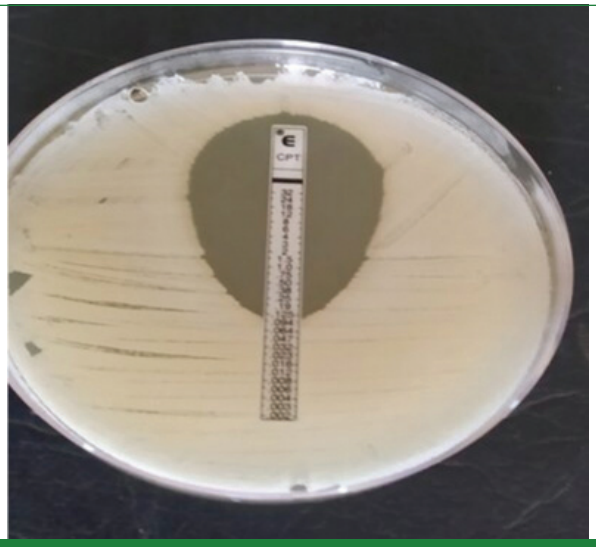
$$n = \frac{(1.96)^2 \times 0.95\,(1-0.95)}{(0.05)^2} \approx 73$$

Due to the short duration of study period (two months) and limited availability of ceftaroline E-strips, the sample size was limited to 50.

Sample collection: A total of 50 consecutive, non-repetitive, clinical isolates of MRSA and MRCoNS from various samples were included in the study. The isolates were identified as *S.aureus* and CoNS by standard laboratory techniques [11]. The pathogenic role of CoNS was established by repeated isolation of same species from two different occasions.

Antibiotic susceptibility testing: The antibiotic susceptibility testing was done by CLSI recommended Kirby-Bauer disc diffusion testing on Muller Hinton agar with antibiotic discs obtained from Himedia, Mumbai. Methicillin resistance was detected by cefoxitin disc (30 µg) along with routine sensitivity testing [12].

Detection of in-vitro activity of ceftaroline: The MIC of ceftaroline was detected for all the 50 MR staphylococcal isolates using E-strips (Biomerieux) [Table/Fig-1]. The CLSI break points was applied for the interpretations of ceftaroline MIC- Sensitive ≤1 µg/mL, {SDD- a category defined by a break point that susceptibility of an isolate for which the susceptibility testing results; either MICs or zone diameter are in the SDD category, it is necessary to use a dosage regime i.e., higher doses, more frequent doses or both. This results in higher drug exposure than that was used to establish the susceptible break point} 2-4 µg/mL and resistant ≥8 µg/mL) [12]. *Staphylococcus aureus* ATCC 29213 was used as a quality control.



[Table/Fig-1]: Detection of ceftaroline MIC in MR staphylococci using E-strips.

STATISTICAL ANALYSIS

Statistical analysis was done using Microsoft excel. The data analysis involved transcription, preliminary data inspection, content analysis and interpretation. Percentages were used in this study to analyse variables.

RESULTS

A total of 50 consecutive, non duplicate MR staphylococci isolates were included in the study. Among the 50 MR staphylococcal isolates, majority were obtained from pus (25), followed by urine (10), blood (08), high vaginal swab (03), sputum (01), corneal ulcer (01), central catheter tip (01) and dialysis catheter tip (01). Out of 50 MR staphylococcal isolates, 10 (20%) were MRSA and 40 (80%) were MRCoNS. Among the 50 MR staphylococcal isolates, 30 were from In-Patient Department (IPD) and 20 from Out-Patient Department (OPD). The age range of the patients from whom the isolates were obtained ranged from one-day-old neonate to 80-year-old man and the mean age was 38 years. The male to female sample distribution ratio was 1:1. [Table/Fig-2] shows the age and gender distribution of MRSA and MRCoNS.

Age group (in years)	MRSA		MRCoNS		Total (n=50)
	No. of males	No. of females	No. of males	No. of females	
0-20	0	1	2	7	10
21-40	1	1	6	10	18
41-60	2	2	6	2	12
61-80	3	0	5	2	10
Total (n=50)	6	4	19	21	50

[Table/Fig-2]: Age and gender distribution of MRSA and MRCoNS.

Out of 10 MRSA isolates, 8 (80%) were sensitive to chloramphenicol and tetracycline; 4 (40%) were sensitive to gentamicin and clindamycin. Least susceptibility was seen to penicillin (0%), erythromycin (0%), co-trimoxazole (10%) and ciprofloxacin (10%).

Out of 40 MRCoNS isolates, 75% were having sensitivity to tetracycline, 72.5% were sensitive to chloramphenicol and 42.5% were sensitive to gentamicin, minimal sensitivity was seen to penicillin (0%), followed by cotrimoxazole (10%), erythromycin (10%), ciprofloxacin (27.5%) and clindamycin (32.5%). [Table/Fig-3] shows the antimicrobial susceptibility pattern of MRSA and MRCoNS.

Antibiotic	MRSA (n=10)	MRCoNS (n=40)	Total (n=50)
Penicillin	00 (0%)	00 (0%)	00 (0%)
Co-trimoxazole	01 (10%)	04 (10%)	05 (10%)
Gentamicin	04 (40%)	17 (42.5%)	21 (42%)
Ciprofloxacin	01 (10%)	11 (27.5%)	12 (24%)
Tetracycline	08 (80%)	30 (75%)	38 (76%)
Erythromycin	00 (0%)	04 (10%)	04 (8%)
Clindamycin	04 (40%)	13 (32.50%)	17 (34%)
Chloramphenicol	08 (80%)	29 (72.50%)	37 (34%)

[Table/Fig-3]: Antimicrobial susceptibility pattern of MRSA and MRCoNS.

Of the 50 MR staphylococcal isolates tested, 49 (98%) were sensitive to ceftaroline. One isolate (MRCoNS, 2.50%) was in SDD category. [Table/Fig-4] shows the in-vitro activity of ceftaroline by E-test.

Variables	MIC (n=50)			Total n=50 (%)
	Sensitive n (%)	SDD* n (%)	Resistance n (%)	
MRSA (n=10)	10 (100)	0 (0)	0 (0)	10 (20)
MRCoNS (n=40)	39 (97.5)	1 (2.50)	0 (0)	40 (80)
Total (n=50)	49 (98)	1 (2)	0 (0)	50 (100)

[Table/Fig-4]: In-vitro activity of ceftaroline by E-test.
SDD*: susceptible dose dependent

Of the 40 MRCoNS isolates, 39 (97.5%) were sensitive; only one isolate was SDD with a MIC of 2 µg/mL. It was obtained from the blood of one day old neonate. The MIC 50 and MIC 90 for MRCoNS were 0.38 µg/mL and 1 µg/mL respectively. All the MRSA (n=10) isolates showed uniform susceptibility to ceftaroline with MIC ranging from 0.125 µg/mL to 0.38 µg/mL. The MIC 50 and MIC 90 for MRSA were 0.25 µg/mL and 0.38 µg/mL, respectively. [Table/Fig-5,6] shows the MIC distribution of ceftaroline in MRCoNS and MRSA, respectively.

MRCoNS (n=40)	Number of isolates inhibited at Ceftaroline MIC (µg/mL)									
	0.032	0.094	0.125	0.19	0.25	0.38	0.50	0.75	1.0	2.0
	1 (2.5%)	2 (5%)	5 (12.5%)	2 (5%)	5 (12.5%)	11 (27.5%)	3 (7.5%)	4 (10%)	6 (15%)	1 (2.5%)

[Table/Fig-5]: Ceftaroline MIC distribution in MRCoNS.

MRSA (n=10)	No. of isolates inhibited at Ceftaroline MIC (µg/mL)			
	0.125	0.19	0.25	0.38
	1 (10%)	1 (10%)	6 (60%)	2 (20%)

[Table/Fig-6]: Ceftaroline MIC distribution in MRSA.

DISCUSSION

The treatment of staphylococcal infections is of great concern to the clinicians because the infections with MR strains are associated with a poorer prognosis than methicillin sensitive staphylococcal

infections. The inappropriate initial antibiotic therapy can have a profound impact on their clinical outcome [13]. Thus, therapeutic options against these organisms need constant investigation.

In the present study, 98% MR staphylococcal isolates were sensitive to ceftaroline. This was in comparison with the worldwide surveillance studies [14-16]. Similar result was seen in the United States (US) surveillance study showing 98% susceptibility [14]. But a slightly lower sensitivity rates were found in Asia-Pacific region (86.9%), Europe (87%) and Latin America (83.6%) [15]. Hence, it can be understood from these previous studies that invitro activity of ceftaroline against MRSA can vary from one geographic region to another.

The present study demonstrated 100% susceptibility of MRSA isolates to ceftaroline. This was in concordance with the study conducted by Basireddy S et al., [10]. The results of various studies are summarised in [Table/Fig-7] [2,10,14,16-18].

Author (publication year)	Place	Ceftaroline susceptibility rate	MIC range (µg/mL)	MIC 50 (µg/mL)	MIC 90 (µg/mL)
Flamm RK et al., [14] (2012)	USA	98.4%	1-2	-	-
Jones RN et al., [16] (2010)	USA	94.8%	1-2	-	-
	Europe	82.6%	1-2	-	-
Bakthavatchalam YD et al., [17] (2016)	India	92%	0.03-4	0.25	1
Basireddy S et al., [10] (2016)	India	100%	0.125-1.5	0.5	1
Gaikwad V et al., [2] (2015)	India	93.33%	0.25-4	0.38	0.75
Sreedharan H and Pai KBA, [18] (2021)	India	100%	0.064-0.50	0.25	0.50
Present study (2021)	India	100%	0.125-0.38	0.25	0.38

[Table/Fig-7]: Comparision of in-vitro activity of ceftaroline against MRSA from various studies.

The present study showed that 97.5% of MRCoNS isolates were sensitive to Ceftaroline. This study corelates well with the surveillance studies of other geographical areas. But only very few ceftaroline surveillance studies are conducted among MRCoNS in the World [Table/Fig-8] [16,19].

Author (year)	Place	Ceftaroline sensitivity rate	MIC range (µg/mL)	MIC 50 (µg/mL)	MIC 90 (µg/mL)
Jones RN et al., [16] (2010)	USA	100%	0.3-2	0.25	0.5
	Europe	100%	0.5-4	0.25	1
Basireddy S et al., (2015) [19]	India	100%	0.25-3	1	2
Present study (2021)	India	97.5%	0.032-1	0.38	1

[Table/Fig-8]: Comparison of in-vitro activity of ceftaroline against MRCoNS from previous studies.

The interpretation of relative ceftaroline activity across regions, or species, or patient types, or any other parameters is based only on percent susceptibility and should be reviewed carefully. Since, the MIC distributions were very similar across countries, and the non-susceptible populations entirely comprised of strains with ceftaroline MICs, only 1 or 2 dilutions above the susceptibility breakpoint of 1 µg/mL. This probably can be due to substitution of PBP2a in certain lineages and less likely to be an acquisition of clear resistance mechanism [15].

Considering the underlying mechanisms behind higher ceftaroline MICs, the clustering and proximity of the MICs; 1 µg/mL (Sensitive), 2-4 µg/mL (SDD) or 8 µg/mL (Resistant) suggested only variations in setting up of interpretive breakpoints than of any emerging resistance. Hence, distinguishing its clinical relevance should be done vigilantly. Ongoing surveillance and further molecular characterisation of such isolates is necessary to understand the emerging trends.

Limitation(s)

Since it was an ICMR STS project with a limited study duration of two months, the sample size was small. Moreover, speciation of CoNS was not done. Antibiotic susceptibility of MR staphylococci to vancomycin, linezolid and teicoplanin was not determined which may be considered as a limitation of the study.

CONCLUSION(S)

Ceftaroline showed a potent in-vitro activity against all the species of MR staphylococci. So it can be used as an effective alternative for the treatment of infections caused by MR staphylococci. However, since staphylococci have a proven propensity to develop resistance to many antimicrobial agents, continuous surveillance and strict antimicrobial policies are needed as the clinical use of ceftaroline expands.

Acknowledgement

Authors would like to thank Indian Council of Medical Research (ICMR) for supporting this study as a short term research project.

REFERENCES

[1] Andrey DO, Francois P, Manzano C, Bonetti EJ, Harbarth S, Schrenzel J, et al. Antimicrobial activity of ceftaroline against Methicillin-reistant *Staphylococcus aureus* (MRSA) isolates collected in 2013-2014 at the Geneva University Hospitals. Eur J Clin Microbiol Infect Dis. 2017;36(2):343-50.
[2] Gaikwad V, Gohel T, Panikar S, Chincholkar V, Mangalkar S. Invitro activity of ceftaroline: A novel antibiotic against methicillin-reistant *Staphylococcus aureus*. Indian J Microbiol. 2016;59(4):496-98.
[3] Bhateja P, Mathur T, Pandya M, Fatma T, Rattan A. Detection of vancomycin resistant *Staphylococcus aureus*: A comparative study of three different phenotypic screening methods. Indian J Med Microbiol. 2005;23(1):52-55.
[4] Jacob JT, Diaz Granados CA. High vancomycin minimum inhibitory concentration and clinical outcomes in adults with methicillin-resistant *Staphylococcus aureus* infections: A meta-analysis. Int J Infect Dis. 2013;17(2):93-100.
[5] Vardakas KZ, Ntziora F, Falagas ME. Linezolid: Effectiveness and safety for approved and off- label indications. Expert Opin Pharmacother. 2007;8:2381-400.
[6] Laudano JB. Ceftaroline fosamil: A new broad-spectrum cephalosporin. J Antimicrob Chemother. 2011;66(3):01-08.
[7] Garcia AB, Candel FJ, Lopez L, Chiarella F, Vinuela-Prieto JM. Invitro ceftaroline combinations against methicillin resistant *Staphylococcus aureus*. J Med Microbiol. 2016;65(10):1119-22.
[8] CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 23th ed. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
[9] Teflaro, Package Insert. Forest Laboratories, December 2013. http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/200327s000lbl.pdf.
[10] Basireddy S, Ali S, Singh M, Kabra V. Invitro activity of ceftaroline against methicillin reistant *Staphylococcus aureus* isolates. Indian J Med Microbiol. 2015;33(3):464-65.
[11] Collee JG, Marr W. Culture of Bacteria In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th Ed. New York: Churchill Livingstone; 1996. Pp.113-29.
[12] Clinical and Laboratory Standards Institute (CLSI) M100-S24. Performance standards for Antimicrobial Susceptibility Testing: 29th Informational supplement. Wayne, PA: CLSI; 2019.
[13] Sader HS, Farrell DJ, Flamm RK, Jones RN. Activity of ceftaroline and comparator agents tested against *Staphylococcus aureus* from patients with blood stream infections in US medical centres (2009-13). J Antimicrob Chemother. 2015;70(7):2053-56.
[14] Flamm RK, Saer HS, Farrell DJ, Jone RN. Summary of ceftaroline activity against pathogens in the United States, 2010: Report from the Assessing worldwide Antimicrobial Resistance Evaluation (AWARE) Surveilliance program. Antimicrob Agents Chemother. 2012;56(6):2933-40.
[15] Biedenbach DJ, Alm RA, Lahiri SD, Reiszner E, Hoban DJ, Sahm DF, et al. Invitro activity of ceftaroline against *Staphylococcus aureus* isolated in 2012 from Asia-Pacific countries as part of the AWARE surveillance Program. Antimicrob Agents Chemother. 2015;60(1):343-47.
[16] Jones RN, Rae M, Sader HS. Ceftaroline activity against pathogens associated with complicated skin and skin structure infections. Results from an international surveillance study. J Antimicrob Chemotherapy. 2010;65(4):17-31.

[17] Bakthavatchalam YD, Pragasam AK, Anandan S, Joshi S, Chaudhuri BN, Chitnis DS, et al. Comparative invitro activity of ceftaroline against *Staphylococcus aureus* isolates from India. J infect Dev Ctries. 2016;10(1):109-12.
[18] Sreedharan H, Pai KB A. Susceptibility of clinical isolates of *Staphylococcus aureus* to ceftaroline. J Clin Diag Res. 2021;15(1):DC10-13.
[19] Basireddy S, Ali S, Singh M, Kabra V. Surveillance of Ceftaroline activity against clinical isolates of Methicillin resistant Coagulase negative Staphylococci. Indian J Basic Appl Med Res. 2015;4(2):494-96.

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara, Mandya Dist, Karnataka, India.
2. MBBS Student, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara, Mandya Dist, Karnataka, India.
3. Professor and Head, Department of Microbiology, Adichunchanagiri Institute of Medical Sciences, B. G. Nagara, Mandya Dist, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Vijaya Shivanna,
Adichunchanagiri Institute of Medical Sciences, B. G. Nagara,
Mandya Dist-571448, Karnataka, India.
E-mail: drvijayas@bgsaims.edu.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Jan 15, 2021
- Manual Googling: Feb 22, 2021
- iThenticate Software: Mar 30, 2021 (24%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Jan 13, 2021**
Date of Peer Review: **Feb 04, 2021**
Date of Acceptance: **Feb 23, 2021**
Date of Publishing: **Apr 01, 2021**