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Comparative Ceftaroline Activity Tested Against Staphylococcus Aureus Associated with Acute Bacterial Skin and Skin Structure Infection from A Tertiary Care Center

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ABSTRACT *Methicillin resistant Staphylococcus aureus (MRSA), commonest cause of acute bacterial skin and skin structure infection (ABSSSI) has only limited treatment options like vancomycin, Linezolid and teicoplanin. Staphylococcus aureus with reduced susceptibilities to vancomycin is developing nowadays among nosocomial infection. A newest cephalosporin – Ceftaroline has received FDA approval for the treatment of ABSSSI recently has unique activity against MRSA. The aim of the study is to know the prevalence of Staphylococcus aureus in ABSSSI patients and to evaluate the in vitro activity of Ceftaroline, in comparison with other drugs. Minimum Inhibitory Concentration for vancomycin and Ceftaroline were determined by E-test strips. Out of 235 Staphylococcus aureus isolated from ABSSSI patient 32% were MRSA. No vancomycin resistant strain isolated in our study. In vitro, Ceftaroline control the growth of MRSA very effectively at 2mg/ml itself. Ceftaroline is a most welcomed drug in the treatment of MRSA.*

INTRODUCTION:

Complicated skin and skin structure infection (CSSSIs), now known by the new US FDA as Acute bacterial skin and skin structure infections (ABSSSI) are the most common infections treated in the hospital setting [1]. The commonest organisms causing CSSSIs are Gram positive *Staphylococci, Streptococci, Anaerobes* and Gram negative bacilli like E. *coli* and *Pseudomonas.*

Staphylococcus aureus the commonest cause of ABSSSI pose a great challenge to the clinician in the treatment options for the infection caused by Methicillin resistant Staphylococcus aureus (MRSA), which include Vancomycin and newer agents such as Linezolid and Daptomycin.

Ceftaroline fosamil, the prodrug form of ceftaroline is a new parenteral, bactericidal cephalosporin. It has broad spectrum of activity against ß-lactam resistant *Staphylococcus* like MRSA, Multidrug resistant *Streptococcus pneumonia* and Gram negative bacilli [2&3]. Ceftaroline is also proved to be more bactericidal against MRSA than Vancomycin and Linezolid in In-vitro time – kill studies. [4]

Ceftaroline has received approval by FDA on 29 October 2010 as an effective option for monotherapy of acute bacterial skin and skin structure infections caused by susceptible organisms including MRSA. Against MRSA, ceftaroline exhibits noteworthy activity when compared with other ß-lactam antibiotics. [5]

The purpose of this study was to find out the distribution of the *Staphylococcal aureus* in the ABSSSI patient and the prevalence of oxacillin resistance among them. Also to assess the in vitro efficacy of ceftaroline and to compare that with other antistaphylococcal drugs like Linezolid, Vancomycin and other drugs against *Staphylococcus aureus* in a tertiary care center.

MATERIALS AND METHODS:

This study was carried out in Chettinad health city and Research Institute, a tertiary care center hospital (Chennai) between April 2013 and May 2014. Pus specimen was collected from patient suffering from ABSSSI. ABSSSI defined as deeper soft tissue infection, surgical/ traumatic wound infections, major abscesses, cellulitis and infected ulcers and burns. The samples were sent to the microbiology laboratory without delay for culture. 235 *Staphylococcus aureus* were isolated from patients with ABSSSI.

CULTURE/ IDENTIFICATION:

The pus was inoculated on 5% sheep blood agar and chocolate agar and incubated aerobically at 37˚C for 24 hours. Smears were prepared from plates showing growth and stained by Gram's stain. Gram positive cocci in clusters were identified using standard biochemical tests. Catalase positive and coagulase positive strains were *Staphylococcus aureus.* [6]

METHICILLIN RESISTANCE DETECTION:

Methicillin resistance was detected using oxacillin screening agar and cefoxitin disk diffusion test.

Oxacillin screening agar was Muller Hinton agar with 4% Nacl and 6µg/ml oxacillin. Direct colony suspension of 0.5 McFarland turbidity was spot on oxacillin screening agar area of 10 to 15 mm in diameter. Incubate at 35˚c for 18 hours and examine with transmitted light for the presence of > 1 colony than oxacillin resistant.

According to the CLSI guidelines cefoxitin disk diffusion test was performed with 30µg cefoxitin disk in Muller Hinton agar plate. Incubate at 35˚C and read with transmitted light after 24 hours. Among *Staphylococcus aureus* if zone size ≤ 21 mm than mec A positive (MRSA).

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ANTIBIOTIC SUSCEPTIBILITY TEST:
Antibiotic susceptibility test of all Staphylococcus isolates was performed by Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines[7], using Muller Hinton Agar .The following antibiotic discs, produced by Hi-media were used; Teicoplanin (30µg), Erythromycin(15µg), tetracycline (30µg), Ciprofloxacin(5µg), Cefazolin ,Penicillin (10 units) and Cotrimoxazole (25µg)

MINIMUM INHIBITORY CONCENTRATIONS:
MIC for vancomycin and Ceftaroline was determined by E-test strips (bio Merieux India Pvt. Ltd. Chennai) shown in Fig.1. 0.5 McFarland turbidity adjusted *Staphylococcus* colonies were subcultered on the Muller Hinton agar (MHA) plate. The E- Strips of Vancomycin and Ceftaroline were placed on the MHA plate and incubate for 24 hours at 35°C. The E-test MIC end point was read at complete inhibition of growth.

RESULT:
A total of 235 *Staphylococcus aureus* were isolated from the ABSSSI patients, of which (137/235) 58% were from male and (98/235) 42% from female. Most of the specimens, (142/235) 60% were from ICU, (69/235) 29 % percent from ward and (24/235) 11 % from OPD

Among 235 *Staphylococcus aureus* isolates (75/235) 32% showed resistance to methicillin. Cefoxitin disc diffusion test detected (75/75) all the methicillin resistant staphylococcus aureus (MRSA) but oxacillin screening agar detected only (70/75) 93% strains as MRSA. It was noted that oxacillin screening agar test is less specific than mec A detected method.

Anti microbial sensitivity test of all 235 Staphylococcus isolates were done. The highest drug resistance was obtained against penicillin for both (75/75) 100% MRSA and (131/160) 82% MSSA. Moderate resistance was seen in Cotrimoxazole, Erythromycin, Tetracycline and Gentamicin. Antibiotic resistance pattern against *Staphylococcus aureus* was shown in table 1.

Fig 1: Ceftaroline MIC detection by using E –test strips

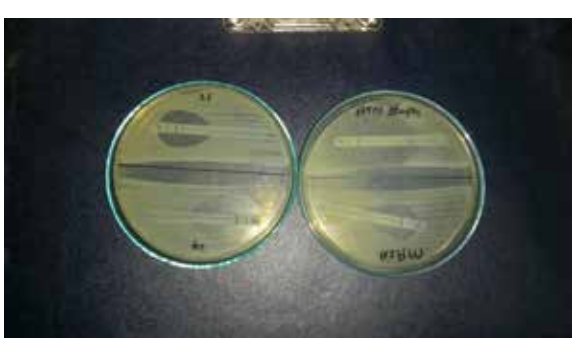

Table 1 : Antibiotic resistant pattern in Staphylococcus

ANTIBIOTIC	MRSA (n=75)	MSSA (n=160)
Ceftaroline	Nil	Nil
Vancomycin	Nil	Nil
Linezolid	Nil	Nil
Teicoplanin	Nil	Nil
Penicillin	75(100%)	131(81%)
Cefazolin	75(100%)	10(0.06%)
Ciprofloxacin	30(40%)	65(40%)
Ofloxacin	30(40%)	59 (37%)
Cotrimoxazole	75(100%)	121(76%)
Clindamycin	30 (40%)	55(34%)
Erythromycin	60(80%)	69(43%)
Gentamicin	60(80%)	56(35%)
Tetracycline	60(80%)	56(35%)

The MIC value for the ceftaroline and comparator drug-Vancomycin against MRSA and MSSA was shown in table 2 and 3.

Table 2: MIC distribution for MRSA and MSSA for Vancomycin

MIC gradient mg/ml	MRSA (75)	MSSA(160)
0.012	0	1
0.032	0	3
0.125	0	29
0.19	1	0
0.25	2	127
0.38	3	0
0.75	10	0
1.0	14	0
1.5	22	0
2.0	23	0

Table 3: MIC distribution of MRSA & MSSA for Ceftaroline

MIC gradient mg/ml	MRSA (75)	MSSA(160)
0.012	0	1
0.032	0	5
0.064	0	5
0.125	3	14
0.25	6	134
0.50	20	1
1.0	44	0
2.0	2	0

Among the MRSA, 30.6% (23/75) had vancomycin MIC 2mg/ml, 18.6% (14/75) had MIC 1mg/ml and 21.3% (16/75) had a MIC <1 mg/ml. The MIC 50 and MIC 90 value for MRSA were found to be 1.5 mg/ml and 2mg/ml. Out of 160 MSSA , 79.3 % (127/160) had vanomycin MIC 0.25mg/ml and 18.1% (29/160) had 0.125mg/ml. No vancomycin resistant was detected.

Out of 75 MRSA , 58.6 % (44/75) had ceftaroline MIC 1 mg/ml , 26.6% (20/75) had MIC 0.5mg/ml and only 0.12% (9/75) had a MIC <0.5 mg/ml. Among the MSSA, 83.7% (134/160) had ceftaroline MIC 0.25mg/ml and 15.6% (25/160) had MIC≤0.125mg/ml. All staphylococcus aureus were inhibited by 2mg/ml ceftaroline.

DISCUSSION:
In the present study the commonest organism isolated from Acute bacterial skin and skin structure infection (ABSSSI) is *Staphylococcus aureus.* Among *Staphylococcus,* MRSA (Methicillin resistant staphylococcus aureus) is a major nosocomial pathogen causing significant morbidity and mortality. [8]

Resistance to methicillin among *Staphylococcus aureus* increased from 1.5% in 1986 to 31.2 % in 2012. [9] Methicillin resistance among *Staphylococci* is widespread in India. In our study the prevalence rate of MRSA is 32%, in other studies conducted in Coimbatore [10] it is 31.1% and in Vellore it was 24% [11]. The studies conducted in Northern India such as New Delhi showed 44% to 51% [12, 13].

Nowadays vancomycin is the drug of choice of infection caused by MRSA. As the percentages of MRSA were constantly increasing the use of vancomycin is also increasing which may lead to the MRSA with reduced susceptibility or

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resistance to glycopeptides. [14, 15] In our study, the MRSA has MIC50 is 1.5mg/ml and MIC90 is 2mg/ml. No vancomycin resistant *Staphylococcus aureus* (VRSA) was found.

According to IDSA-2011 (Infection Disease Society of America) guidelines, vancomycin MIC > 2mg/ml, an alternate drug should be used for MRSA treatment and if MIC < 2 mg/ml, can continue vancomycin treatment depending upon patients clinical response [16].The efficacy of the drug vancomycin in the treatment of Staphylococcus aureus infections will decrease and clinical failures will result if vancomycin MIC> 1mg/ml [17]

Another study from North India states the shifting trends of vancomycin susceptibility patterns in staphylococcus aureus that is a gradual rise in MIC for vancomycin from 1 to 2 mg/ml from 2004-2008.[18] Due to clinical failure with increasing vancomycin MIC's needed a search for more effective new drug for MRSA treatment [19].

Ceftaroline, new cephalosporin has documented bactericidal activity against *Staphylococcus*. [20, 21] It has improved targeting PBP2a encoded by mec A gene located on the SCC. [22, 23] It is a safe and well tolerated cephalosporin.

In our study the ceftaroline MIC90 for MRSA is 0.5 - 2mg/ml, which is similar to the studies conducted in USA. [2, 3] When the MRSA MIC50 was1mg/ml and MSSA MIC50 was 0.25 mg/ml for ceftaroline, these results were compared and found MRSA MIC was 4-fold higher. The highest ceftaroline MIC for 4mg/ml for staphylococcus was observed in a European study.

Thus Ceftaroline has consistent level of activity against the principal pathogen causing ABSSSI and active against SCC mec-harbouring *Staphylococcus*. [22] The MRSA infection cure rate is proved to be 91.4% to 95.1%. [2]

Since treatment of ABSSSI with MRSA continues to be a challenge to the physician, an agent like Ceftaroline is a welcome therapeutic modality.

REFERENCE

1. FDA Guidelines for industry: acute bacterial skin and skin structure infections. Developing drugs for treatment. Draft Guidance Federal Register: August 27.2010; 75: 166. | 2. Ronald N. Jones, Rodrigo E. Mendes, Helio S.Sader. Ceftaroline activity against pathogens associated with complicated skin and skin structure infections: results from an international surveillance study. J Antimicrob Chemother 2010; 65 supple 4: iv17-31. | 3. Ronald N.Jone, David J.Farrell, Rodrigo E Mendes et al. Comparative ceftaroline activity tested against pathogens associated with community- acquired pneumoniae: results from an international surveillance study. J antimicrob chemother 2011; 66 suppl 3: iii 69- 80. | 4. Aurelia Melard, Lactitia G.Garcia, Debaditya Das et a. Activity of ceftaroline against extracellular (broth) and intracellular (THP-1 monocytes) forms of methicillin resistant staphylococcus aureus: comparison with vancomycin, linezold and daptomycin .J Antimicrob chemother 2013; 68: 648-658. | 5. Helene Moisan, Mireile Pruneau and Francois Malouin. Binding of ceftaroline to penicillin-binding proteins of Staphylococcus aureus and Streptococcus pneumonia. J Antimicrob Chemother 2010; 65: 713-716. | 6. Braid D, Staphylococcus; cluster-forming gram positive cocci. In: collee JG.Fraser AG, Marmion BP, Simmons A. editors, Mackie and McCartney practical medical microbiology , London, United kingdom ; Churchill Livingstone : 1996.P 245 – 258 | 7. Clinical and Laboratory standards Institute (CLSI), Performance Standards for antimicrobial susceptibility Document 100- S23 CLSI ;2013. | 8. Nitish kumar Sharma, Raina Garg, Shrihala baliga et al .Nosocomial infections and drug susceptibility patterns in Methicillin sensitive and Methicillin resistant staphylococcus aureus. J clinical and diagnostic research 2013; 10: 2178-2180. | 9. Cuevas O, Cercenado E, Vinde A, Guinea J et al. Evolution of the antimicrobial resistance of staphylococcus species in Spain: Five Nationwide prevalence Studies 1986 to 2002. Antimicrob Chemother 2004; 48: 4240- 4245. | 10. Mallick SK, Basak S. MRSA – too many hurdles to overcome; a study from central India. Tropical doctor 2010; 40: 108-10. | 11. Pulimood TB, Lalitha MK, Jesudason MV, Pandian R et al. The spectrum of antimicrobial resistance among methicillin resistant Staphylococcus aureus [MRSA] in a tertiary care center in India. India J med res.1996; 103:212-5. | 12. Vidhani S, Mehndiratta PL, Mathur MD. Study of Methicillin resistant Staphylococcus aureus [MRSA] isolates from high risk patients. Indian J Med Microbiol 2001;19:13-b | 13. Tyagi A, Kapil A, Singh P. Incidence of methicillin resistant Staphylococcus aureus [MRSA]in pus sample at a tertiary care hospital AIIMS ,New Delhi. JIACM 2008; 9:33-35. | 14. Abhishek mewara, Vikas Gautam, Harisimran Kaur, Pallab Ray. In vitro evaluation of antibiotics for methicillin resistant Staphylococcus aureus from North India. Indian J Med Res 2014; 139:319-322. | 15. Appelbaum PC. Reduced glycopeptides susceptibility in methicillin resistant staphylococcus aureus [MRSA]. Int J Antimicrob agents 2007; 30: 398-408. | 16. Liu C, Bayer A, Cosgrose SE, Daum RS et al. Clinical practice guidelines by the Infectious Disease Society of America for the treatment of Methicillin-resistant Staphylococcus aureus infections in adults and Children; executive summary. Clin Infection Dis 2011; 52:285-292. | 17. Sahoulas G. Moise – Broder PA , schentag J , Forrest A et al. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin resistant Staphylocoocus aureus bacteremia. J Clinical Microbiol 2004:42: 2398-2402. | 18. Dhawan B. Godepalli R, Rao C, Kapil A, Sreenivas V. Decreased susceptibility to vancomycin in methicillin- resistant Staphylococus aureus : a 5 year study in an Indian tertiary hospital. J medical Microbiol 2010 ; 59 : 375-376. | 19. Levine DP. Clinical experience with daptomycin : bacteraemia and endocarditis J Antimicrob Chemother 2008 ; 62: (supple 3) iii 35- 39. | 20. Lizawa Y, Nagai J , Ishikawa T et al. In vitro antimicrobial activity of T- 91825, a novel anti-MRSA activity of its prodrug , TAK-599. J Infect Chemother 2004;10: 146-156. | 21. Jones RN, Fritsche TR, Ge Y et al. Evaluation of PPI-0903M (TP1825),a novel cephalosporins : bactericidal activity, effects of modifying in vitro testing parameters and optimization of disc diffusion tests. J Antimicrob chemother 2005; 56: 1047-1052. | 22. Ishikawa T, Matsunga N. Tawada H et al.TALC-599, a novel N-phosphono type prodrug of anti-MRSA cephalosporin T-91825; synthesis, physiochemical and pharmacological properties. Bio org med chem. 2003; 11:2427-2437. | 23. Kanafani ZA, Cory GR. Ceftaroline: a cephalosporin with expanded Gram positive activity. Future microbiol 2009, 4:25-33. |