

Antimicrobial activity of Ceftaroline and Ceftobiprole tested against MRSA isolates from Turkey, in 2016

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Abstract: Ceftaroline and ceftobiprole are two new cephalosporin molecules on which intense studies have been made for the treatments of bacteria most of which are hospital infection factor, especially resistant gram positive cocci. Ceftaroline which has broad Gram-positive activity recently has been approved for clinical uses by the US Food and Drug Administration. Ceftobiprole has been approved for use in Canada and Switzerland. The objective of this investigation was to evaluate the activities of ceftaroline and ceftobiprole, against community-associated and hospital-associated methicillin-resistant *S. aureus* (MRSA). The activity of ceftaroline was measured in Kirby-Bauer disk diffusion method, broth microdilution method and E test for 60 MRSA isolates. The efficacy of ceftobiprole was measured in E test for 60 MRSA isolates. Ceftaroline MIC values of 51 (85%) isolates were determined as ≤ 1 $\mu\text{g/ml}$ meaning of susceptible according to the criteria of CLSI/EUCAST and MIC values of 9 (15%) isolates were found as >1 $\mu\text{g/ml}$ meaning of resistant by broth microdilution method. The range of ceftaroline MIC values of the isolates was found between 0.125-2 $\mu\text{g/ml}$. MIC₅₀ and MIC₉₀ values of all the isolates were determined as 0.50 $\mu\text{g/ml}$ and 1 $\mu\text{g/ml}$ for ceftaroline, respectively. Ceftobiprole MIC values of 60 (100%) isolates were determined as ≤ 2 $\mu\text{g/ml}$ meaning of susceptible according to the criteria of EUCAST by E test. The range of ceftobiprole MIC values of the isolates was found between 0.125-2 $\mu\text{g/ml}$. MIC₅₀ and MIC₉₀ values of all the isolates were determined as 0.50 $\mu\text{g/ml}$ and 1 $\mu\text{g/ml}$ for ceftobiprole, respectively. The in vitro activities of ceftaroline and especially ceftobiprole against MRSA indicates that they should be a good therapeutic option for the treatment of infection caused by methicillin-resistant *S. aureus*.

Key words: Ceftaroline, Ceftobiprole, MRSA

I. Introduction

The resistance problems about methicilline-resistant *Staphylococcus aureus* (MRSA), an important factor which is known to colonize in the nose and skin and which can cause severe infections resulting from society and hospital, give rise to difficulties in the treatment. The need for the new and broad spectrum antibiotics has revealed intense in vitro studies. Ceftaroline, having recently taken approval for clinical usage in the USA by Food and Drug Administration, and ceftabiprole, having taken approval for clinical usage in Canada and Switzerland, are new generation antibiotics of cephalosporin group (1-3)

The preliminary matter of ceftaroline, performed parenterally and named as the fifth generation cefalosporin, is ceftaroline fosamil (3). It displays in vitro activity on numerous Gram-positive and Gram-negative microorganisms. MRSA is the leading of them (4). Ceftobiprole medocaril has broad-spectrum activity against Gram-positive and Gram-negative pathogens causative of complicated infections (5). In this study, it was aimed to investigate in vitro activity of ceftaroline and ceftobiprole against MRSA isolates obtained from several clinic samples in Microbiology Laboratory of İzmir Menemen State Hospital.

II. Material and Method

Bacterial Isolates and Specimens:

A total of 60 MRSA isolates which have been isolated in İzmir Menemen State Hospital during the period from July 2014 to January 2016 were included in the study. Specimens included skin/wound/abscess (n=16), blood culture (n=10), respiratory tract (n=7), urine (n=25), catheter (n=2). Bacterial identification was done by colony morphology, Gram stain, catalase test, coagulase test and using Kirby-Bauer disk diffusion method with 30 μg cefoxitin disk (Bioanalyse, Turkey) according to CLSI (6).

Determination of Antibiotic Susceptibility

Ceftaroline susceptibilities of the strains were detected by disk diffusion method (Mast Diagnostic, UK), broth microdilution method and E test (BioMérieux, France). Ceftaroline fosamil powder form was obtained from the manufacturer firm Astra Zeneca. Ceftobiprole susceptibilities of the strains were detected by E test (Liofilchem, Italy).

Susceptibility test for ceftaroline was studied with three different methods. Depending on the suggestions of CLSI/European Committee on antimicrobial Susceptibility Testing (EUCAST) (6,7), the values of minimal inhibitor concentration (MIC) were determined through Standard broth microdilution method. As a medium, Mueller Hinton II Broth (MHB) with 2% NaCl (cation-adjusted) (BD, USA) was used. Primarily, 100 µl MHB was distributed to 96-well microtiter plates with sterile U base; then powder form of ceftaroline fosamil (Astra Zeneca, England) was dissolved in 100% dimetil sulphoxide (DMSO) and was diluted with 30% DMSO in 0.85% sterile serum physiologic. Afterwards, a series of dilution was made as 64 µg/ml in the first well and the twelfth column was left as a growth control. According to 0.5 McFarland turbidity standard of 24 hour cultures of bacteria, bacterium suspension was prepared and the last inoculum concentration was diluted by 1/100 with the last inoculum concentration to be 5×10^5 cfu/ml and 100 µl was distributed to all wells each. As a control strain, *S.aureus* ATCC 29213 was utilized. After all plates were incubated for 18-24 hours at 37 °C, the results were assessed. According to the criteria of EUCAST (7), the isolates the MIC values of which were ≤ 1 µg/ml were accepted to be susceptible and those >1 µg/ml were accepted to be resistant.

Based on the suggestions of EUCAST/CLSI, after bacteria suspension was prepared according to 0,5 McFarland turbidity standard from 24 hour cultures of bacteria, it plated on Mueller Hinton Agar (MHA) (Salubris, Turkey) plate and then Ceftaroline disks (Mast Diagnostic, UK), Ceftaroline E test stripes (BioMérieux, France) and Ceftobiprole E test stripes (Liofilchem, Italy) were placed on the plate. As a control strain, *S.aureus* ATCC 29213 were utilized. After all plates were incubated for 18-24 hours at 37 °C, the results were assessed. The inhibition zone formed with ceftaroline disk were accepted to be susceptible if ≥ 20 mm, and to be resistant if <20 mm according to the criteria of EUCAST (7). For ceftobiprole, the isolates the MIC values of which were ≤ 2 µg/ml were accepted to be susceptible and those with >2 µg/ml were accepted to be resistant according to the criteria of EUCAST (7).

III. Results

When all the isolates under study were assessed for ceftaroline, MIC₅₀, MIC₉₀ and MIC range was found to be 0.5 µg/ml, 1 µg/ml and 0.125-2 µg/ml respectively. MIC values of isolates are displayed in Table 1. MIC values of totally 21 isolates (85%) were determined to be ≤ 1 µg/ml and to be susceptible according to EUCAST (7) criteria. The MIC values of 9 isolates were determined to be >1 µg/ml and accepted to be resistant.

It was noted that 9 isolates found to be resistant to ceftaroline through broth microdilution showed correlation in terms of MIC values determined through E test and disk diffusion method (Table II).

When an assessment was made according to clinical sample type, a definite difference couldn't be found among sample groups in terms of isolate numbers found to be susceptible and resistant to ceftaroline.

When ceftobiprole is assessed with E test, MIC₅₀, MIC₉₀ and MIC range was found to be 0.5 µg/ml, 1 µg/ml, and 0,125-2 µg/ml. MIC values of isolates are shown in Table 1. MIC values of all isolates (n=60 100%) were determined to be ≤ 2 µg/ml and to be susceptible according to EUCAST (7) criteria.

Table I. MIC values of MRSA isolates for Ceftaroline ve Ceftobiprole

	MIC values (µg/ml)						
	MIC50(µg/ml)	MIC90(µg/ml)	≤ 0.125 n(%)	0.25 n(%)	0.5 n(%)	1 n(%)	2 n(%)
Ceftaroline	0.50	1	6(10)	18(30)	20(33)	7(12)	9(15)
Ceftobiprole	0.50	1	3(5)	14(23)	26(44)	12(20)	5(8)

Table II. MIC values with three methods of Ceftaroline resistant MRSA isolates and *S.aureus* ATCC 29213

Isolate no	Specimen	Broth microdilution (µg/ml)	E test (µg/ml)	Disk Diffusion (mm)
1	skin	1.5	1.5	18
2	catheter	1.5	1.5	16
3	respiratory tract	2	2	16
4	wound	2	2	17
5	skin	2	1.5	16
6	vaginal discharge	2	2	18
7	urine	2	1.5	19
8	catheter	2	colonies within zone	18
9	urine	2	2	19
<i>S.aureus</i> ATCC 29213		0.125	0.125	23

IV. Discussion

Today there is a need for new antibacterial agents with strong activity against resistant bacteria (8). For this purpose, a great number of studies are made over ceftaroline and ceftobiprole, new molecules of cephalosporine having recently got the approval for usage (5, 9-11).

Ceftaroline fosamil is a novel prodrug which is converted by plasma phosphatases into active ceftaroline after intravenous administration. Ceftaroline shows bactericidal effect on MRSA, vancomycin-intermediate *S.aureus* strains and macrolide-resistant *Streptococcus pyogenes*, as well as potential Gram-negative pathogens, including non extended spectrum β -lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* (3).

Ceftobiprole medocaril is converted by plasma esterases to ceftobiprole in <30 minutes (12). Ceftobiprole is a weak inducer for AmpC beta-lactamases. In this respect, ceftobiprole is similar to cefepime (13). Ceftobiprole is a broad-spectrum and potent cephalosporin. In vitro activities of ceftobiprole has been demonstrated against Gram-positive cocci, including MRSA and methicillin-resistant *Staphylococcus epidermidis*, penicillin-resistant *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and AmpC-producing *E.coli*. On the other hand, ceftobiprole is not effective against extended-spectrum beta-lactamase-producing strains (14).

Severe clinical cases such as necrotizing skin infections, necrotizing fasciitis, pyomyositis, septic thrombophlebitis of the extremities, septic arthritis, pelvic osteomyelitis, rapidly progressive necrotizing pneumonia were added among the infections for which community-associated MRSA isolates were responsible, in the last decade (15, 16). Vancomycin, linezolid, daptomycin, telavancin and tigecycline are most commonly used in complicated skin and soft tissue infections (17-18). Although it is still a golden standard in parenteral treatment, the usage of antibiotics to be alternative to vancomycin is suggested in treatment guidelines in America and Europe (19).

Ceftaroline is recently approved by the Food and Drug Administration for use in the treatment of complicated skin and skin structure infections (cSSSIs) and community-acquired bacterial pneumonia (CABP). There are a number of studies the in vitro efficacy of ceftaroline against MRSA and multidrug-resistant *S.pneumoniae* (5,10,11,20). The study of CANVAS 1 (21) was performed on a total of 702 patients with cSSSIs. In this study, 353 of these patients were treated with ceftaroline fosamil and 349 of them with vancomycin plus aztreonam. In the process of the treatment the success of clinical cure and microbiological treatment were found to be almost same in both groups. Ceftaroline fosamil produced similar results to vancomycin in terms of the incidence of side-effects.

In CANVAS 2 study (22), 694 patients with cSSSIs were taken under study and ceftaroline fosamil reached clinical cure by 92.2% in 348 patients. 92.1% of clinical cure was obtained in vancomycin plus azteonam treatment administered to the other 346 patients. Also, clinical cure rates were similar in patients with common comorbidities such as diabetes mellitus or peripheral vascular disease in those studies. However, clinical cure rates with ceftaroline fosamil for bacteremia were detected as 84.6% (23).

It is a new threat that decreased susceptibility to vancomycin, linezolid, and daptomycin. A total of 19,350 *S.aureus* isolates were tested for susceptibility against ceftaroline by broth microdilution method, in U.S. hospitals, 2008 to 2011 (24). Sader et al reported that ceftaroline was very active against *S.aureus* overall (MIC_{50/90}, 0.5/1 μ g/ml; 98.5% susceptible), including MRSA (MIC_{50/90}, 0.5/1 μ g/ml; 97.2% susceptible). These results are compliance with our data.

In-vitro susceptibility data has been collected for ceftaroline against 1,971 *S.aureus* isolates in 2012 from seven countries (26 centers) in the Asia-Pacific region as part of the Assessing Worldwide Antimicrobial Resistance and Evaluation (AWARE) program (25). The ceftaroline MIC₉₀ for all *S.aureus* isolates has been found 2 μ g/ml and 62% of the isolates were MRSA. Susceptibility rate to ceftaroline has been reported 78.8% of MRSA isolates and 100% of methicillin-sensitive *S. aureus* (MSSA) isolates.

Karlowsky et al. have obtained that ceftaroline MIC₅₀ and MIC₉₀ values were 0.5 μ g/ml of 1 μ g/ml, respectively, in CANWARD surveillance study against 232 MRSA isolates (26). In this same study, ceftobiprole MIC₅₀ and MIC₉₀ values were 1 μ g/ml of 2 μ g/ml, respectively.

Mengelöglü et al. (27) reported that ceftaroline MIC values of 181 (94.3%) isolates were determined as \leq 1 μ g/ml and MIC values of 11 (5.7%) isolates were found as 2 μ g/mL. The range of MIC values of the isolates have been found between 0.25-2 μ g/ml. This findings are compatible our results.

Several studies related to ceftobiprole have demonstrated potent *in-vivo* efficacy against infections caused by MRSA, including strains intermediately resistant to vancomycin (1,5,13). Bogdanovich et al. have demonstrated that the MICs of ceftobiprole against 303 staphylococci (1). Among 126 MRSA (including 5 VISA and 2 VRSA) strains, MIC₅₀ and MIC₉₀ values for ceftobiprole were each 2 μ g/ml. Among 151 coagulase-negative staphylococci (including 4 vancomycin intermediate resistant coagulase negative staphylococci strains), MIC₅₀ and MIC₉₀ values were 1 μ g/ml and 2 μ g/ml, respectively, against methicillin

resistant coagulase negative staphylococci strains. When we compared our results, our MRSA isolates have been determined more susceptible to ceftobiprole.

In another study, Pillar et al. were evaluated that *in-vitro* activity of ceftobiprole against 798 MRSA isolates (5). They have demonstrated that ceftobiprole MICs against MRSA (MIC₅₀ and MIC₉₀ values were 1 mg/L of 2 mg/L, respectively) were elevated relative to methicillin susceptible *S.aureus*.

In a global surveillance study (SENTRY Antimicrobial Surveillance Program) for the years 2005 to 2006, ceftobiprole have inhibited 100% of tested *S. aureus* at ≤ 4 $\mu\text{g/mL}$ (28). It was found that MIC₉₀ values for MRSA were 4-fold higher than MSSA isolates.

Farrell et al. have evaluated the *in vitro* antimicrobial activity of ceftobiprole against Gram-positive and Gram-negative pathogens in different countries (29). A total of 60,084 isolates from a wide variety of infections were collected from 33 medical centers, during 2005 through 2010. It has been shown that ceftobiprole was active against MRSA (98.3% susceptible), having a MIC₉₀ of 2 $\mu\text{g/ml}$. Our isolates were susceptible to ceftobiprole 100%.

Chan et al. (30) have shown that multiple mutations in *mecA* gene lead to high-level resistance to ceftobiprole and ceftaroline. A single amino acid change at E447 in *mecA* appears to play a key role in resistance. This mutation has been associated with low-level resistance to ceftaroline in clinical isolates (MIC 4 $\mu\text{g/ml}$).

The clinical cure success with new cephalosporine agents was shown in all performed studies. Yet, even in the countries where ceftaroline has not been started to be used in treatment, the presence of resistant MRSA isolates including different Penicilline-binding protein 2a patterns suggests the possibility that resistance can spread rapidly among *S. aureus* strains. In this context, ceftaroline fosamil may represent a useful alternative against MRSA, however, there is a need for local monitoring and surveillance studies for resistant strains.

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