Evaluation of the *in vitro* activity of ceftobiprole against clinical isolates of *Staphylococcus aureus*

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Summary

Background and aims: Ceftobiprole is a new cephalosporin characterized by a potent activity against Gram-positive and Gram-negative bacterial pathogens. It is noting that ceftobiprole has a strong affinity for penicillin binding proteins including PBP 2A, which mediates resistance to beta-lactams in methicillin (oxacillin)-resistant coagulase-negative staphylococci and *Staphylococcus aureus* (MRSA). The aim of the current study was to examine the antimicrobial activity of ceftobiprole against clinical isolates of *S. aureus* recently collected at our institution.

Materials and methods: One hundred and forty blood isolates of *S. aureus* were evaluated, including methicillin-susceptible (MSSA, n=70) and MRSA (n=70) strains. Twenty additional MRSA isolates obtained from different sites (including skin and soft tissues, blood, and lower respiratory tract) and characterized by borderline susceptibility to vancomycin were also studied to assess the ability of ceftobiprole to overcome this worrisome trait. MIC values of ceftobiprole were determined by Etest strips and results were interpreted according to EUCAST guidelines.

Results and conclusions: Study isolates were consistently susceptible to ceftobiprole, with MIC values ranging from 0.125 mg/L to 2 mg/L. Overall, MIC_{50} and MIC_{90} were 0.25 mg/L and 0.5 mg/L, respectively. Ceftobiprole showed *in vitro* activity against all *S. aureus* isolates, with small differences among groups selected on the basis of resistance to methicillin and/or reduced susceptibility to vancomycin. Thus, ceftobiprole appears a valid choice for treating infections caused by *S. aureus*, even when susceptibility results are not yet available. Additionally, ceftobiprole may be a valid option in the case of reduced susceptibility to vancomycin.

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Introduction

Staphylococcus aureus is a Gram-positive bacterium that can live as a commensal organism on the skin, in the nose and throat. Approximately 30% of healthy people are asymptomatically colonized by *S. aureus*, which permanently colonizes the anterior nares in 10-20% of the population and intermittently colonizes 30-50%; the rest of the population never becomes colonized (3,21). Importantly, this colonization is a known risk factor for infection (10,19,28,31,35). Transmission results most frequently from transient colonization through the hands of hospital staff that carry strains from one patient to another (6,23). The vast majority of people who develop infections caused by *S. aureus* are infected with their own colonizing strains; however, these infections can also be obtained from other people or environmental exposures. *S. aureus* is a prominent human pathogen that can cause a diverse range of diseases ranging from relatively minor skin infections to serious and life-threatening infections such as endocarditis, pneumonia, and sepsis (21).

Resistance to antibiotics is a well-known serious problem in medicine. *S. aureus* has a great ability to acquire resistance to antibiotics. In 1961, Patricia Jevons reported the first isolates of methicillin-resistant *S. aureus* (MRSA), only 2 years after the clinical usage of methicillin (24,33). In the following decades, MRSA isolates have spread throughout the world and can be found nowadays in many industrialized countries (6).

Resistance to methicillin is primarily the result of decreased binding affinity to its target, penicillin binding protein 2 (PBP2), owing to acquisition of an altered PBP2 (PBP2a) encoded by the *mecA* gene harboured on a mobile genetic element, the staphylococcal chromosome cassette *mec* (SCC*mec*) (16, 22). PBP2a has a low β-lactam affinity and confers resistance to most molecules of this family (including

third- and fourth-generation cephalosporins, and carbapenems). Because of its low β-lactam affinity, PBP2a can mediate cell wall assembly when the normal staphylococcal PBPs are blocked by these compounds (11). In addition to most β-lactam antibiotics, MRSA are usually resistant to many other antibiotics, such as erythromycin, gentamycin, ciprofloxacin, and fusidic acid (27). So, methicillin resistance is an indicator of resistance to a wide range of antibiotics. The continued increase of MRSA has led to a concurrent increase in reliance on non-β-lactam agents for treatment, especially vancomycin (14). Borderline susceptibility to this drug (*i.e.*, isolates having MIC values for vancomycin ranging from 1.5 to 2 mg/L) have been increasingly reported, often associated to therapeutic failures (15,32). Alternative agents that have regulatory approval for some MRSA infections include linezolid, tigecycline, and daptomycin (20). All of them have been approved for skin and soft tissue infections (SSTIs). In addition, due to their pharmacokinetic and pharmacodynamics characteristics, linezolid is also indicated especially for pneumonia, whereas daptomycin is often used in the treatment of bloodstream infections, including right-sided infective endocarditis (29,30,34). These molecules can be used for specific infections, taking into account their toxicity and the emerging increase of resistance determinants (26).

Ceftobiprole is a new broad-spectrum β-lactam antimicrobial agent belonging to cephalosporins (Figure 1), intravenously administered as a prodrug (ceftobiprole medocaril) and characterized by a potent activity against both Gram-positive and Gram-negative bacterial pathogens. Of note, it has anti-MRSA activity. Similarly to other β-lactam antibacterial agents, ceftobiprole exerts its antibacterial activity by binding to important penicillin-binding proteins (PBPs) and inhibiting their transpeptidase activity (12), which is essential for the synthesis of the peptidoglycan layer of bacterial cell walls. Ceftobiprole binds to multiple PBPs in clinically relevant pathogens, which provides its broad activity spectrum (4,5,7,12,13). The anti-MRSA activity of ceftobiprole is attributed to its rapid and tight binding to the mutant PBP2a form (encoded by the *mecA* gene) that is able to confer resistance to methicillin and most β-lactam antibiotics (12). Ceftobiprole is the first cephalosporin monotherapy approved in the EU for the treatment of both hospital- (HAP) and community-acquired pneumonia (CAP), excluding ventilator-associated pneumonia (VAP).

The aim of this study was to evaluate the activity of ceftobiprole against invasive isolates (obtained from blood cultures) of both methicillin-susceptible *S. aureus* (MSSA) and MRSA strains. In addition, MRSA isolates recovered from different sites and characterized by borderline susceptibility to vancomycin were evaluated.

Materials and Methods

A total of 160 non duplicate, clinical isolates of *S. aureus* previously collected at our Institution were evaluated against ceftobiprole and other antimicrobial agents. One hundred and forty blood isolates of *S. aureus* were evaluated, including MSSA (n=70) strains. Twenty additional MRSA isolates obtained from different sites and characterized by borderline susceptibility to vancomycin (MICs, 1.5-2 mg/L) were also studied to verify the ability of ceftobiprole to overcome this worrisome trait. These isolates were recovered from skin and soft tissues (n=7), surgical wounds (n=6), lower respiratory tract secretions (n=5), blood (n=1) and prosthetic joint infection (n=1). Bacterial identification and antimicrobial susceptibility were routinely obtained by MALDI-TOF mass spectrometry (Vitek® MS, bioMérieux, Marcy l'Étoile, France) and Vitek® 2 instrument (bioMérieux), respectively. Isolates characterized by borderline susceptibility to vancomycin were further evaluated by Etest® strips (bioMérieux) to confirm vancomycin MIC values. *S. aureus* ATCC 29213 was used as control. Susceptibility results were interpreted on the basis of EUCAST breakpoints (8). Starting from frozen isolates, collection strains were first inoculated on Columbia blood agar (bioMérieux) to resume their metabolic activity. After 18 h incubation at 36°C, a 0.5 McFarland suspension for each isolate was prepared in sterile 0.9% saline (corresponding to a cell density of 1.5×10^8 CFU/mL) and inoculated on Mueller Hinton E agar (bioMérieux). MIC values of ceftobiprole were determined by Etest (MIC range, 0.002-32 mg/L), a well-established method for antimicrobial susceptibility testing in microbiology laboratories. According to EUCAST breakpoints, isolates of *S. aureus* having MICs≤2 mg/L were interpreted as susceptible to ceftobiprole (8).

Results

Study isolates were consistently susceptible to ceftobiprole, with MIC values ranging from 0.125 mg/L to 2 mg/L. Overall, the ceftobiprole MIC_{50} and MIC_{90} values (MIC required to inhibit 50% and 90% of the isolates) were 0.25 mg/L and 0.5 mg/L, respectively, with small variations within different groups (Table 1). The overall modal MIC value was 0.5 mg/L (45 isolates; 28.1%). The MIC distribution of MSSA and MRSA groups are shown in Figure 2. In particular, MIC_{50} and MIC_{90} for the different groups were as follows: MSSA, 0.25 mg/L and 0.5 mg/L; MRSA, 1 mg/L and 1.5 mg/L. For the different groups the modal MIC was 0.5 mg/L (27 isolates; 38.6%) and 1 mg/L (32 isolates; 35.6%) for MSSA and MRSA, respectively. MSSA isolates showed low MIC values for ceftobiprole, ranging from 0.125 to 0.5 mg/L. These strains were also susceptible to most of tested antibiotics, with very low resistance rates for erythromycin (11.4%), levofloxacin (7.1%) and gentamicin (5.7%). Concerning MRSA, these strains were characterized by MIC values for ceftobiprole higher than those observed for MSSA, with a range of 0.125-2 mg/L. High resistance rates among MRSA were observed for levofloxacin (94.3%) and erythromycin (57.1%), while gentamicin and tetracycline resistance was observed in few cases (8.6%).

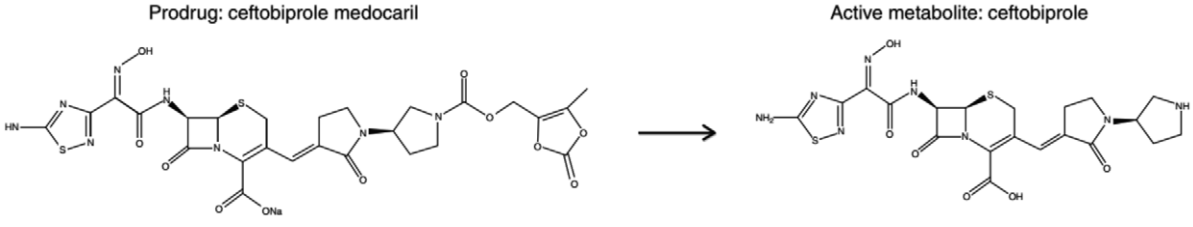

Figure 1. Chemical structure of the prodrug ceftobiprole medocaril and its active metabolite, ceftobiprole.

Regarding the vancomycin-borderline MRSA subgroup, these isolates were characterized by MIC_{50} and MIC_{90} values for ceftobiprole (0.5 mg/L and 1 mg/L, respectively) intermediate between those observed for MSSA and MRSA, with a range of 0.125-2 mg/L. High resistance rates among vancomycin-borderline MRSA were observed not only for levofloxacin (85.0%) and erythromycin (80.0%) but also for gentamicin (60.0%), teicoplanin (50.0%) and tetracycline (35.0%). *In vitro* activity of ceftobiprole and comparator agents against study isolates is summarized in Table 1. Of note, vancomycin, linezolid, and tigecyline showed 100% susceptibility independently on different groups.

Discussion and Conclusions

Ceftobiprole is a fifth-generation cephalosporin, newly approved in Europe as a single-agent therapy for HAP and CAP. Of note, it is an extended-spectrum cephalosporin that has been reported to encompass activity against both Gram-negative and Gram-positive strains, including MRSA. In our experience, focused on *S. aureus* clinical isolates, ceftobiprole showed *in vitro* activity against all *S. aureus* isolates (MIC values ≤2 mg/L; 100% susceptible), with small differences among

Table 1. Activity of ceftobiprole and comparator agents tested against 160 *Staphylococcus aureus* clinical isolates.

S. aureus groups (no. tested)	MIC (mg/L)		MIC range	% S
	MIC_{50}	MIC_{90}		
All *S. aureus* (160)				
Ceftobiprole	0.25	0.5	0.125-2	100
Vancomycin	1	2	≤0.5-2	100
Linezolid	2	4	1-4	100
Tigecycline	≤0.12	0.25	≤0.12-0.5	100
Co-trimoxazole	≤10	≤10	≤10 to ≥320	98.8
Teicoplanin	≤0.5	4	≤0.5-8	93.7
Rifampicin	≤0.03	≥4	≤0.03 to ≥4	93.1
Tetracycline	≤1	≥16	≤1 to ≥16	90.6
Gentamicin	≤0.5	≥16	≤0.5 to ≥16	86.2
Erythromycin	1	≥8	≤0.25 to ≥8	60.0
Levofloxacin	4	≥8	≤0.12 to ≥8	45.0
MSSA (70)				
Ceftobiprole	0.25	0.5	0.125-0.5	100
Vancomycin	1	1	≤0.5-1	100
Linezolid	2	2	1-2	100
Tigecycline	≤0.12	≤0.12	≤0.12-0.25	100
Co-trimoxazole	≤10	≤10	≤10 to ≥320	97.1
Teicoplanin	≤0.5	≤0.5	≤0.5	100
Rifampicin	≤0.03	≤0.03	≤0.03	100
Tetracycline	≤1	≤1	≤1 to ≥16	97.1
Gentamicin	≤0.5	≤0.5	≤0.5 to ≥16	94.3
Erythromycin	1	≥8	≤0.25 to ≥8	88.6
Levofloxacin	≤0.12	0.25	≤0.12 to ≥8	92.9
MRSA (70)				
Ceftobiprole	1	1.5	0.125-2	100
Vancomycin	≤0.5	1	≤0.5-1	100
Linezolid	2	2	1-4	100
Tigecycline	≤0.12	0.25	≤0.12-0.25	100
Co-trimoxazole	≤10	≤10	≤10	100
Teicoplanin	≤0.5	≤0.5	≤0.5	100
Rifampicin	≤0.03	≤0.03	≤0.03 to ≥4	97.1
Tetracycline	≤1	≤1	≤1 to ≥16	91.4
Gentamicin	≤0.5	≤0.5	≤0.5 to ≥16	91.4
Erythromycin	≥8	≥8	≤0.25 to ≥8	42.9
Levofloxacin	≥8	≥8	≤0.12 to ≥8	5.7
Vancomycin-borderline MRSA (20)				
Ceftobiprole	0.5	1	0.125-2	100
Vancomycin	2	2	2	100
Linezolid	2	2	2-4	100
Tigecycline	≤0.12	≤0.12	≤0.12-0.5	100
Co-trimoxazole	≤10	≤10	≤10	100
Teicoplanin	2	8	≤0.5-8	50.0
Rifampicin	≤0.03	≥4	≤0.03 to ≥4	100
Tetracycline	≤1	≥16	≤1 to ≥16	65.0
Gentamicin	≥16	≥16	≤0.5 to ≥16	40.0
Erythromycin	≥8	≥8	≤0.25 to ≥8	20.0
Levofloxacin	≥8	≥8	0.25 to ≥8	15.0

groups as selected on the basis of methicillin resistance. MIC_{50} and MIC_{90} were higher for MRSA than those observed for MSSA (MSSA, 0.25 mg/L and 0.5 mg/L; MRSA, 1 mg/L and 1.5 mg/L). Overall, our data were similar to those found in the literature (1, 2, 12, 18). Specifically, cumulative data from these studies showed that MICs of ceftobiprole for MSSA and MRSA strains ranged from 0.25 to 2 mg/L, and from 0.06 mg/L to 2 mg/L, respectively. The MIC_{90} value was 0.5 mg/L for MSSA, and 2 mg/L for MRSA (17).

As also demonstrated by our data, MSSA strains maintain *in vitro* susceptibility to several antibiotics, whereas a narrow therapeutic arsenal is available for infections caused by MRSA strains. MRSA are frequently difficult to treat because, in addition to β-lactam antibiotics, they may be resistant to many other commonly used antibiotics, such as erythromycin, gentamicin and levofloxacin (27). Furthermore, the continued increase of MRSA has led to a concurrent increase in reliance on non-β-lactam agents for treatment, especially vancomycin, a glycopeptides antibiotic with a large number of labeled indications for use (14,34). The main toxicities of vancomycin for concern in critically ill patients include hypersensitivity reactions and renal toxicity. Although vancomycin-resistant *S. aureus* (VRSA) strains are rare, isolates with increased MIC for vancomycin (MIC value ranging from 1.5 to 2 mg/L) are common in the clinical practice (15). Importantly, isolates with borderline vancomycin susceptibility are more frequently associated with treatment failures (15,32). Alternative agents that have regulatory approval for some MRSA infections include linezolid, tigecycline, and daptomycin (20).

These drugs, however, present some limitations: can be used for specific infections, are characterized by toxicity and unfortunately, decreased susceptibility and resistance to all of them has been reported (25,26). Taking into account the above limitations of alternative agents for MRSA, ceftobiprole can play an important role for related infections since it is also effective against strains with reduced susceptibility to vancomycin (1,2,12,18). In our experience, MIC distribution of ceftobiprole against vancomycin-borderline MRSA was similar to that of vancomycin-susceptible MRSA isolates, suggesting that decreased susceptibility to vancomycin has a negligible effect on ceftobiprole activity. Similarly, ceftobiprole has been demonstrated to maintain activity against resistant subset of MRSA, including those that were not susceptible to daptomycin, linezolid, or vancomycin (9).

In conclusion, the availability of antibiotics with anti-MRSA activity is critical because the prevalence of MRSA infection is increasing worldwide. Ceftobiprole is a new cephalosporin with *in vitro* broad-spectrum activity against most clinically relevant bacterial pathogens, including MRSA thanks to its strong binding to PBP2a. It appears a valid choice for treating infections caused by isolates identified as *S. aureus* even when susceptibility results are not yet available. Additionally, ceftobiprole may be a valid option in the case of reduced susceptibility to vancomycin. The use of ceftobiprole in the clinical practice, increases the therapeutic arsenal against *S. aureus* isolates and helps to reduce the selective pressure that leads to the development of resistance to traditional anti-MRSA antibiotics.

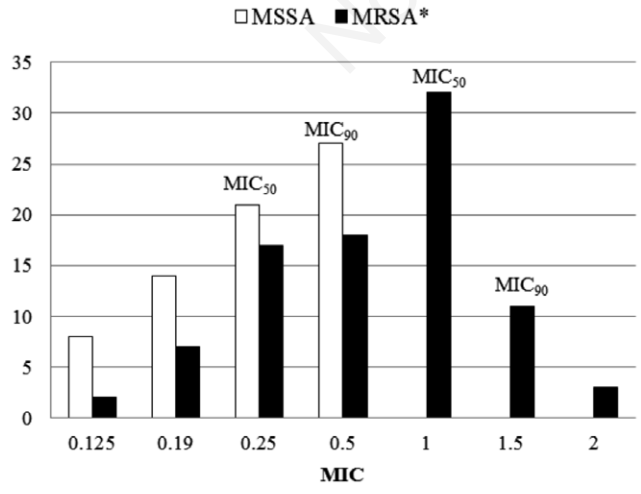

Figure 2. MIC distribution of MSSA and MRSA for ceftobiprole. Tags indicate MIC_{50} and MIC_{90} values. *MRSA group includes isolates characterized by borderline susceptibility to vancomycin.

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