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Antimicrobial Activity of Ceftobiprole (BAL9141) Tested against Staphylococcal Strains with Selected Resistance Patterns

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Amended Abstract

Background: Ceftobiprole (formerly BAL9141), the active component of prodrug ceftobiprole medocaril (formerly BAL5788), is a novel parenteral pyrrolidinone-3-ylidenemethyl cephem with activity against oxacillin-resistant (Oxa^R) staphylococci.
Methods: 518 staphylococcal strains were selected from various international surveillance programs and other specialized collections to include organisms isolated from human infections and comprising particular resistotypes towards linezolid (Lzd), quinupristin/dalfopristin (Q/D), and/or vancomycin (Van). Each strain was tested against >15 antimicrobials by NCCLS reference methods. MBC values towards ceftobiprole were determined for 53 staphylococci.
Results: Ceftobiprole activity towards staphylococci is summarized in the Table.

	ceftobiprole MIC (µg/ml)		
Organism (n)	50%	90%	Range
S. aureus Oxa^S (40)	0.5	0.5	0.03-1
S. aureus Oxa^R (181)	1	2	0.06-4
S. aureus Borderline Oxa^R (BORSA; 13)	0.5	1	0.06-1
GISA or hGISA (100)	2	2	0.5-4
S. aureus Q/D^R (10)	1	1	0.5-2
CoNS Oxa^S (20)	0.06	0.25	≤0.016-0.5
CoNS Oxa^R (80)	1	1	0.25-2
CoNS heteroresistant to Van (50)	2	4	0.06-4
CoNS Q/D^R (15)	1	2	0.12-4
Staphylococci Lzd^R (9)	2	4	0.5-4

The MBC/MIC ratio for ceftobiprole was ≤2 for 52 of 53 isolates tested (98%).
Conclusions: Although the currently accepted paradigm is that Oxa^R staphylococci are resistant to all β-lactam compounds, ceftobiprole proved highly active (MIC$_{90}$, 1-2 µg/ml) against Oxa^S staphylococci. Growth of 100% and 92.7% of isolates were inhibited by ceftobiprole at ≤4 µg/ml and ≤2 µg/ml, respectively. In a panel of pathogens chosen for their resistance profile ceftobiprole also was very active and bactericidal against staphylococcal strains with diverse resistance patterns.

Introduction

Antimicrobial resistance has continued to emerge over the past decade, with dramatic increases seen in methicillin-resistant staphylococci (MRS), vancomycin-resistant enterococci (VRE), and penicillin-resistant pneumococci (PRP). The increasing occurrence of these resistance phenotypes has limited therapeutic options and posed difficulties in the management of serious Gram-positive infections.
Ceftobiprole (formerly BAL9141), a novel parenteral pyrrolidinone-3-ylidenemethyl cephem (Figure 1) that constitutes the active component of prodrug ceftobiprole medocaril (formerly BAL5788), possesses antimicrobial activity against staphylococci (including MRS), *Enterococcus faecalis* (including VRE), and PRP, while preserving activity against Gram-negative bacteria similar to third- and fourth-generation cephalosporins (Hebeisen, 2001; Jones, 2002). Particularly noteworthy is the potent activity of ceftobiprole against oxacillin-resistant *Staphylococcus aureus* (ORSA) resulting from (*i*) its potent, long-lasting inhibition of PBP2 as well as the normal sensitive PBPs, and (*ii*) the refractoriness of ceftobiprole to hydrolysis by the penicillinases found in most staphylococcal isolates.
Here we summarize the results of testing ceftobiprole and numerous comparators against contemporary staphylococcal clinical isolates having defined resistance mechanisms, including oxacillin resistance (Oxa^R), heteroresistance to glycopeptides (hGI) or reduced susceptibility to glycopeptides (GI), quinupristin/dalfopristin non-susceptibility (Q/D^{IR}), and linezolid resistance (Lzd^R). We also evaluated the bactericidal action of ceftobiprole using broth microdilution methods against *S. aureus* and coagulase-negative staphylococci.

Materials and Methods

Bacterial strains: A panel of 518 staphylococcal clinical isolates was assembled from international surveillance programs and other specialized collections; the vast majority of strains were collected since January 2002. The panel was comprised of 344 *S. aureus* (Oxa^S [40]; Oxa^R [181]; borderline Oxa^R [BORSA, 13]; quinupristin/dalfopristin non-susceptible (Q/D^{IR}, MIC ≥2 µg/ml) [10]; hGISA and GISA (vancomycin MIC in specialized screening tests, 4 to 8 µg/ml) [100]), 165 coagulase-negative staphylococci (CoNS) (Oxa^S [20]; Oxa^R [80]; Q/D^{IR} [15]; strains with reduced susceptibility to glycopeptides [hGICoNS, vancomycin MIC = 4 µg/ml, 50]); included in the panel were 7 Lzd^R *S. aureus* and 2 Lzd^R CoNS.
Screening for BORSA: The SENTRY Antimicrobial Surveillance Program collection from North America (18,508 isolates), Latin America (8,175 isolates) and Europe (4,378 isolates) was screened for *S. aureus* strains isolated since 1998 having oxacillin MIC values of 4 µg/ml; 76 (0.2%) such organisms were identified and subjected to further *in vitro* testing. All 76 strains were evaluated by a 4-disk test screen with cefoxitin, ceftizoxime, oxacillin ± amoxicillin/clavulanic acid (NCCLS, 2004; Moriyasu, 1994; Varaldo, 1993). This eliminated strains with *mecA* phenotypes (>99% accuracy) and showed the oxacillin + clavulanate synergy of possible BORSA isolates. Eighteen screen-positive strains were retested against oxacillin by Etest® and broth microdilution methods and screened by a latex agglutination assay for the *mecA* gene product. Isolates were considered BORSA when all of the following characteristics were demonstrated:

a. Oxacillin MIC ≥4 µg/ml by consensus of broth microdilution and Etests®.
b. Disk zones ≥20 mm for cefoxitin (30 µg) and ≥20 mm for ceftizoxime (30 µg), and ≤13 mm for oxacillin (1 µg) or enhanced activity in the presence of clavulanate, and an amoxicillin/clavulanic zone diameter in the susceptible range.
c. *mecA* agglutination test result negative.

Organisms confirmed as BORSA (n = 13) were tested against ceftobiprole and comparators by the broth microdilution method.
Susceptibility testing: Staphylococci were tested by broth microdilution panels manufactured by TREK Diagnostics (Cleveland, OH). Reference quantitative methods were utilized as described by the NCCLS (2003) and interpreted by the current published criteria (M100-S14 [NCCLS, 2004]). MBC values towards ceftobiprole for a subset of 33 *S. aureus* (including 10 hGISA) and 20 CoNS also were determined.
Each isolate was tested for susceptibility against ceftobiprole using broth microdilution methods recommended by the NCCLS (2003). Starting inocula were quantified by removing a 10-µl aliquot from the positive growth control well, making a 1:1000 dilution, and duplicate-plating of 100-µl aliquots of the diluted sample onto appropriate growth media. Microtiter plates were examined visually, and all wells at and above the MIC concentration were plated onto appropriate growth media and incubated. The MBC was defined as a 99.9% reduction in colony forming units (CFUs) compared to the starting inoculum. The first ceftobiprole-containing well with fewer colonies than the cidal cutoff (≥99.9%) was defined as the MBC (NCCLS, 1999).
Antimicrobial agents. Ceftobiprole powder was provided by Basilea Pharmaceutica AG (Basel, Switzerland). All the other comparators were obtained either from their manufacturers or from commercial sources.

Results

- Ceftobiprole was the most active cephalosporin tested against staphylococci. Among Oxa^S strains ceftobiprole MIC values varied from 0.03 to 1 µg/ml (MIC$_{50}$ and MIC$_{90}$, 0.5 µg/ml), whereas among Oxa^R strains ceftobiprole MIC results ranged from 0.06 to 4 µg/ml (MIC$_{50}$, 1 µg/ml; MIC$_{90}$, 2 µg/ml) (Table 1). Only 11.1% of ORSA strains were susceptible to ceftriaxone (MIC$_{50}$, 32 µg/ml) according to the NCCLS-sanctioned susceptibility breakpoint of 8 µg/ml.
- Against BORSA strains ceftobiprole (MIC$_{50}$, 0.5 µg/ml; range, 0.06-1 µg/ml) was about as active as against Oxa^S isolates, whereas against Q/D^{IR} strains ceftobiprole activity (MIC$_{50}$, 1 µg/ml; range, 0.06-4 µg/ml) was similar to that observed against Oxa^R isolates (Table 1).
- Ceftobiprole was highly active against hGISA/GISA strains, with MICs ranging from 0.5 to 4 µg/ml (MIC$_{50}$ and MIC$_{90}$, 2 µg/ml). In contrast, 93% and 97% of hGISA/GISA isolates were resistant to ceftriaxone and ceftazidime, respectively (Table 1).
- Ceftobiprole was slightly more potent against CoNS than against *S. aureus* (Table 2). Against Oxa^S CoNS (20 strains) ceftobiprole (MIC$_{50}$ and MIC$_{90}$, 0.25 µg/ml) was 4- to 8-fold more potent than ceftriaxone (MIC$_{50}$, 2 µg/ml; MIC$_{90}$, 4 µg/ml) and 8- to 16-fold more potent than ceftazidime (MIC$_{50}$, 4 µg/ml; MIC$_{90}$, 8 µg/ml) (Table 2).
- Ceftobiprole showed excellent activity against Oxa^R CoNS (80 strains; MIC$_{50}$ and MIC$_{90}$, 1 µg/ml), but was 4-fold more active against Oxa^S CoNS (MIC$_{50}$ and MIC$_{90}$, 0.25 µg/ml).
- Ceftobiprole activity against 15 Q/D^{IR} CoNS strains (MIC$_{50}$, 1 µg/ml; MIC$_{90}$, 2 µg/ml) was similar to that demonstrated against Q/D^S Oxa^R CoNS.
- Ceftobiprole was very active (MIC$_{50}$, 2 µg/ml; MIC$_{90}$, 4 µg/ml; range, 0.06-4 µg/ml) against CoNS strains [50] having reduced susceptibility towards vancomycin (MIC, 4 µg/ml).
- Ceftobiprole MIC values ranged from 0.5 to 4 µg/ml for 9 well-characterized Lzd^R staphylococcal strains (MIC$_{50}$, 2 µg/ml).
- Most (97%) *S. aureus* strains tested had MBC values equal to, or 1 log$_2$ dilution step higher than, the corresponding MIC values (Table 3). One strain (1.9%) displayed a MBC value eight-fold higher than the MIC value. This isolate also showed a paradoxical effect (Eagle phenomenon) towards ceftobiprole during subculture of MIC panel wells.
- 18/20 (90%) CoNS strains surveyed (including 10 Oxa^R strains) had identical MIC and MBC values towards ceftobiprole.
- Some *S. aureus* isolates, hGISA strains as well as Oxa^S strains, had MBC values at lower concentrations before regrowth at higher concentrations (8-32 µg/ml). ¨Eagle-like¨ ef fects have been documented previously for ceftobiprole against staphylococci (Deshpande & Jones, 2003).

Conclusions

- Ceftobiprole was the most potent cephalosporin tested against staphylococci, especially Oxa^R strains. *Staphylococcus* spp. including Oxa^R strains appear to be particularly susceptible to ceftobiprole compared to other cephalosporins tested. Ceftobiprole was 4- to >16-fold more potent than either ceftriaxone or ceftazidime against these pathogens.
- Ceftobiprole was bactericidal towards most *S. aureus* and CoNS strains tested, with MBC values within 1 log$_2$ dilution step of the MIC.
- One *S. aureus* isolate showed ¨Eagle-like¨ ef fects with ceftobiprole typical of many earlier β-lactams.
- Ceftobiprole offers substantial advantages in terms of both potency and spectrum, compared to currently-marketed cephalosporins, against a specific group of Gram-positive bacteria that includes Oxa^R staphylococci for which resistance to vancomycin, once considered the drug of last resort, recently has been documented (CDC, 2003).

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Table 1: In vitro *activity of ceftobiprole and selected comparators against 344 isolates of* S. aureus.

	MIC (µg/ml)				
Antimicrobial agent	50%	90%	Range	% susceptible	% resistant
Oxa^S (40)					
Ceftobiprole	0.5	0.5	0.03-1	-[a]	-[a]
Ceftriaxone	4	4	1-8	100.0	0.0
Ceftazidime	8	16	4->16	87.5	2.5
Levofloxacin	0.12	0.5	0.06->4	92.5	5.0
Clindamycin	0.12	0.25	≤0.06->8	92.5	7.5
TMP/SMX	≤0.5	≤0.5	≤0.5->2	95.0	5.0
Vancomycin	1	1	1-2	100.0	0.0
Oxa^R (181)					
Ceftobiprole	1	2	0.06-4	-[a]	-[a]
Ceftriaxone	>32	>32	2->16	(11.1)[b]	(59.4)[c]
Ceftazidime	>16	>16	8->16	(2.2)[b]	(84.4)[c]
Levofloxacin	>4	>4	0.12->4	12.2	60.0
Clindamycin	>8	>8	≤0.06->8	38.3	61.7
TMP/SMX	≤0.5	>2	≤0.5->2	86.4	13.6
Vancomycin	1	2	0.5-4	100.0	0.0
BORSA (13)					
Ceftobiprole	0.5	1	0.06-1	-[a]	-[a]
Ceftriaxone	8	16	2-16	(84.6)[b]	(0.0)[c]
Ceftazidime	16	>16	8->16	(30.8)[b]	(23.1)[c]
Levofloxacin	1	4	0.12->4	60.0	10.0
Clindamycin	0.12	>8	≤0.06->8	69.2	30.8
TMP/SMX	≤0.5	>2	≤0.5->2	69.2	30.8
Vancomycin	1	1	0.5-1	100.0	0.0
Q/D^{IR} (10)					
Ceftobiprole	1	1	0.5-2	-[a]	-[a]
Ceftriaxone	>32	>32	8->32	(10.0)[b]	(79.0)[c]
Ceftazidime	>16	>16	8->16	(10.0)[b]	(90.0)[c]
Levofloxacin	4	>4	1->4	10.0	40.0
Clindamycin	>8	>8	0.5->8	20.0	80.0
TMP/SMX	>2	>2	≤0.5->2	37.5	62.5
Vancomycin	2	2	1-2	100.0	0.0
Quinupristin/Dalfopristin	8	>8	2->8	0.0	80.0
hGISA/GISA (100)					
Ceftobiprole	2	2	0.5-4	-[a]	-[a]
Ceftriaxone	>32	>32	4->32	4.0	93.0
Ceftazidime	>16	>16	16->16	0.0	97.0
Levofloxacin	>4	>4	0.12->4	3.0	73.0
Clindamycin	>8	>8	≤0.06->8	14.0	86.0
TMP/SMX	≤0.5	≤0.5	≤0.5->2	93.0	7.0
Vancomycin	2	2	0.5-8	98.0	0.0
Teicoplanin	4	8	≤2->16	91.0	2.0
Quinupristin/Dalfopristin	0.5	1	≤0.25->2	99.0	1.0
Linezolid	1	1	0.5->8	99.0	-[b]
Daptomycin	0.5	1	0.25-1	100.0	0.0

TMP/SMX, Trimethoprim/Sulfamethoxazole

a. No breakpoints have been established by the NCCLS.
b. Oxa^R S. aureus *should be considered resistant to all marketed β-lactams according to the NCCLS [2004].*
c. Includes 19 well-characterized glycopeptide-intermediate S. aureus *(GISA) strains and 81 heteroresistant GISA (hGISA) strains according to the PAP method (Wootton* et al., *J. Antimicrob. Chemother.* ***47:*** *399-403, 2001) and results reported in Howe* et al., *Antimicrob. Agents Chemother.* ***47:*** *3651-3652, 2003.*

Table 2: In vitro *activity of ceftobiprole in comparison to selected antimicrobial agents against 165 isolates of coagulase-negative staphylococci.*

	MIC (µg/ml)				
Antimicrobial agent	50%	90%	Range	% susceptible	% resistant
Oxa^S (20)					
Ceftobiprole	0.25	0.25	≤0.016-0.5	-[a]	-[a]
Ceftriaxone	2	4	0.5-4	100.0	0.0
Ceftazidime	4	8	£1-8	100.0	0.0
Levofloxacin	0.12	0.25	0.12-0.5	100.0	0.0
Clindamycin	≤0.06	0.12	≤0.06-4	95.0	5.0
TMP/SMX	≤0.5	>2	≤0.5->2	94.7	5.3
Vancomycin	1	2	0.5-2	100.0	0.0
Oxa^R (80)					
Ceftobiprole	1	1	0.25-4	-[a]	-[a]
Ceftriaxone	8	32	4->32	(55.0)[b]	(5.0)[c]
Ceftazidime	16	>16	8->16	(16.3)[b]	(25.0)[c]
Levofloxacin	4	>4	0.06->4	45.0	30.0
Clindamycin	0.12	>8	≤0.06->8	57.5	42.5
TMP/SMX	>2	>2	≤0.5->2	47.5	52.5
Vancomycin	2	2	≤0.12-2	100.0	0.0
Q/D^{IR} (15)					
Ceftobiprole	1	2	0.12-4	-[a]	-[a]
Ceftriaxone	32	>32	8->32	33.3	40.0
Ceftazidime	>16	>16	16->16	0.0	54.5
Levofloxacin	>4	>4	1->4	27.3	72.7
Clindamycin	>8	>8	1->8	0.0	93.3
TMP/SMX	>2	>2	≤0.5->2	40.0	60.0
Vancomycin	2	2	0.5-2	100.0	0.0
Quinupristin/Dalfopristin	4	>8	2->8	0.0	80.0
hGICoNS (50)					
Ceftibiprole	2	4	0.06-4	-[a]	-[a]
Ceftriaxone	>32	>32	1->32	16.0	66.0
Ceftazidime	>16	>16	8->16	10.0	80.0
Levofloxacin	4	>4	0.12->4	22.5	42.5
Clindamycin	>8	>8	≤0.06->8	38.0	60.0
TMP/SMX	>2	>2	≤0.5->2	42.0	58.0
Vancomycin	4	4	4	100.0	0.0
Teicoplanin	>16	>16	16->16	0.0	60.0
Quinupristin/Dalfopristin	0.5	1	≤0.25-1	100.0	0.0
Linezolid	1	2	0.5-2	100.0	-[a]

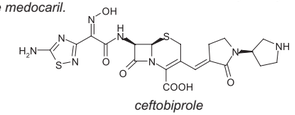
TMP/SMX, Trimethoprim/Sulfamethoxazole

a. No breakpoints have been established by the NCCLS.
b. Based on susceptibility and resistance rates for oxacillin [NCCLS, 2004].

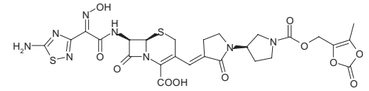
Table 3: *MBC results compared to reference (NCCLS M7-A6) MIC values for 53 contemporary isolates of staphylococci.*

	MBC/MIC ratio (%):			
Organism (n)	1	2	4	>4
S. aureus				
Oxa^S (13)	10 (77)	3 (29)	0	0
Oxa^R (10)	8 (80)	1 (10)	0	1
hGISA (10)	8 (80)	2 (20)	0	0
CoNS				
Oxa^S (10)	10 (100)	0	0	0
Oxa^R (10)	8 (80)	2 (20)	0	0

Figure 1: *Structures of ceftobiprole and its dioxolenylmethyl carbamate prodrug, ceftobiprole medocaril.*



ceftobiprole



ceftobiprole medocaril