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# *In vitro* activity profile of ceftobiprole, an anti-MRSA cephalosporin, against recent Gram-positive and Gram-negative isolates of European origin

**Chris M. Pillar, Mohana K. Aranza, Dineshchandra Shah and Daniel F. Sahm***

*Eurofins Medinet, Inc., Anti-Infective Services, 13665 Dulles Technology Drive, Herndon, VA 20171, USA*

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***Objectives*: To determine the *in vitro* activity profile of ceftobiprole, a pyrrolidinone cephalosporin with activity against methicillin-resistant *Staphylococcus aureus* (MRSA), for use as a contemporary baseline to help detect any changes in its activity profile throughout the course of clinical development and use.**

***Methods*: MICs were determined by broth microdilution testing for ceftobiprole and comparators against 6680 isolates [1201 *S. aureus*, 460 coagulase-negative staphylococci (CoNS), 526 *Streptococcus pneumoniae*, 1213 *Escherichia coli*, 854 *Klebsiella pneumoniae*, 443 *Proteus mirabilis*, 406 *Enterobacter cloacae*, 387 *Citrobacter* spp., 291 *Serratia marcescens*, 621 *Pseudomonas aeruginosa* and 278 *Acinetobacter* spp.] from 31 sites in 12 countries.**

***Results*: Ceftobiprole activity against MRSA ($MIC_{90}$ 2 mg/L) was 4-fold less than the activity against methicillin-susceptible *S. aureus* ($MIC_{90}$ 0.5 mg/L) and a similar trend was observed for methicillin-resistant CoNS ($MIC_{90}$ 2 mg/L) and methicillin-susceptible CoNS ($MIC_{90}$ 0.25 mg/L). Activity against *S. pneumoniae* ($MIC_{90}$s: penicillin-susceptible, 0.015 mg/L; -intermediate, 0.25 mg/L; -resistant, 0.5 mg/L) was comparable to that of ceftriaxone. Ceftobiprole activity against Enterobacteriaceae ($MIC_{90}$s: ceftazidime-susceptible, 0.12 mg/L; non-susceptible, >32 mg/L), *P. aeruginosa* ($MIC_{90}$s: ceftazidime-susceptible, 8 mg/L, non-susceptible, >32 mg/L) and *Acinetobacter* spp. ($MIC_{90}$: >32 mg/L for imipenem-susceptible and non-susceptible) was comparable to that of cefepime. As with cefepime, ceftobiprole activity was decreased among cephalosporin-resistant isolates of Gram-negative bacilli [extended-spectrum β-lactamase (ESBL) or non-ESBL mediated].**

***Conclusions*: Ceftobiprole demonstrated potent *in vitro* activity against MRSA and showed activity against key Gram-negative bacilli comparable to that of cefepime. Given this broad spectrum of activity, ceftobiprole appears well suited for development and use in the treatment of a variety of healthcare-associated infections.**

Keywords: susceptibility tests, MICs, microbiology

## Introduction

The prevalence of antibiotic-resistant organisms within hospitals and the severity of the infections caused by these organisms have resulted in the need for more vigilant infection control practices to diminish transmission and the need for new antimicrobial agents for treatment of these infections. A novel, investigational cephalosporin, ceftobiprole, has documented activity against a variety of resistant bacteria commonly encountered in the healthcare settings of today and has shown to be an effective antibacterial agent *in vivo*.[1–3] Ceftobiprole, formerly designated BAL9141/Ro 63-9141, is a pyrrolidinone-3-ylidene-methyl cephalosporin with demonstrated *in vitro* activity against methicillin-resistant *Staphylococcus aureus* (MRSA),[3–6] Enterobacteriaceae[3,6,7] and *Pseudomonas aeruginosa*.[6,7] Ceftobiprole was also found to be effective against ceftriaxone-resistant *Streptococcus pneumoniae* in a murine model of pneumonia,[8] against MRSA in both rat and rabbit endocarditis[1,2] and against multiple Gram-negative and Gram-positive bacteria in a murine model of septicaemia.[3] Due to these activities, ceftobiprole was selected for clinical development for the treatment of hospital-acquired pneumonia and complicated skin and skin structure infections.

*Corresponding author. Tel: +1-703-480-2500; Fax: +1-703-480-2670; E-mail: dan.sahm@eurofinsmedinet.com

Based on the clinical indications, ceftobiprole is intended for use in the hospital for the treatment of infections that frequently involve β-lactam-resistant Gram-negative and Gram-positive organisms, including MRSA. Because of its use in an environment where antibiotic resistance is already prevalent and the spread or emergence of resistance is of concern, continued surveillance throughout the development of ceftobiprole and beyond is warranted. This study was undertaken to establish the *in vitro* activity of ceftobiprole against recent European clinical isolates representative of the bacterial species and resistant-phenotypes for which ceftobiprole is intended, and to determine its activity relative to other currently utilized cephalosporins. These data will provide a baseline reference of the activity of ceftobiprole against contemporary European clinical isolates and will facilitate the detection of changes in its spectrum of activity should they occur during its clinical development or use.

## Materials and methods

### *Organism collection and identification*

Over a period of 10 months (November 2005–August 2006), a total of 6680 non-consecutive single-patient isolates were collected from 31 hospitals across the following 12 European countries: Belgium (2 hospitals; 6.8% of total isolates), the Czech Republic (2 hospitals; 6.3% of total isolates), France (5 hospitals; 17.3% of total isolates), Germany (4 hospitals; 14.2% of total isolates), Hungary (2 hospitals; 6.6% of total isolates), Italy (4 hospitals; 12.3% of total isolates), The Netherlands (1 hospital; 2.9% of total isolates), Poland (1 hospital; 3.3% of total isolates), Slovakia (1 hospital; 3.0% of total isolates), Spain (4 hospitals; 13.0% of total isolates), Sweden (1 hospitals; 2.5% of total isolates) and the UK (4 hospitals; 11.8% of total isolates). Isolates were limited to one per patient, and Amies transport swabs (Copan Diagnostics Inc., Corona, CA, USA) from overnight cultures of the bacterial isolates were shipped to a central laboratory (Eurofins Medinet, Inc., Herndon, VA, USA) for testing. Upon receipt, isolates were plated and subcultured onto appropriate media,[9] after which the reported identification of isolates were confirmed by standard clinical methods.[9] Pure cultures of the isolates were frozen and stored at −70°C. Isolates received included: 1201 *S. aureus*, 460 coagulase-negative staphylococci (CoNS), 526 *S. pneumoniae*, 1213 *Escherichia coli*, 854 *Klebsiella pneumoniae*, 443 *Proteus mirabilis*, 406 *Enterobacter cloacae*, 387 *Citrobacter* spp., 291 *Serratia marcescens*, 621 *P. aeruginosa* and 278 *Acinetobacter* spp. These included 22.6% urinary tract isolates, 23.5% respiratory tract isolates, 23.2% bloodstream isolates, 13.1% wound isolates, 6.0% skin isolates with the remainder (~11.7%) of either other or unknown origin. A designated number of each species was requested from each of the institutions enrolled in the study regardless of antibiotic resistance phenotype, with the exception of *S. aureus* where it was requested that 75% of the total isolates be MRSA.

### *Antimicrobial testing*

All isolates were tested by broth microdilution according to the guidelines set forth in standard M7-A7 by the CLSI (formerly NCCLS).[10] In short, isolates were revived from freezer stocks by streaking onto rich media and subsequent incubation overnight as appropriate. Overnight cultures grown from isolated colonies on the aforementioned plates were adjusted to the appropriate OD[11] and were used to inoculate Sensititre microdilution panels (TREK Diagnostics, Westlake, OH, USA) for susceptibility testing. The antimicrobial agents tested varied by species; each comparator tested by species is listed in Tables 1–3. MICs were interpreted as susceptible, intermediate or resistant according to CLSI M100-S17 criteria,[11] where applicable. Putative extended-spectrum β-lactamase (ESBL) isolates of *E. coli*, *K. pneumoniae* and *P. mirabilis* were identified based on the interpretive criteria set forth in CLSI M100-S17.[11] Putative derepressed AmpC isolates of *E. cloacae* and *Citrobacter* spp. were based on the screening guidelines set forth by Livermore and Brown[12] (isolates were resistant to ceftazidime, ceftriaxone and cefotaxime; the activity of ceftazidime and cefotaxime are unaffected in combination with clavulanic acid; and the isolates are susceptible to imipenem and meropenem). Results were examined to ensure that reported MICs were within acceptable standards set by the CLSI[11] based on the comparator agent used and the following ATCC quality control strains: ATCC 25922 (*E. coli*), ATCC 27853 (*P. aeruginosa*), ATCC 29212 (*Enterococcus faecalis*), ATCC 29213 (*S. aureus*), ATCC 35218 (*E. coli*), ATCC 49619 (*S. pneumoniae*) and ATCC 700603 (*K. pneumoniae*).

## Results

### *Gram-positive pathogens*

The *in vitro* activity of ceftobiprole was examined against 1201 *S. aureus*, the majority of which (66%) were MRSA. Ceftobiprole MICs ranged between ≤0.12–1 mg/L for methicillin-susceptible *S. aureus* (MSSA) and 0.25–4 mg/L for MRSA (Table 1). Against MSSA, ceftobiprole had an $MIC_{50}$ of 0.25 mg/L and an $MIC_{90}$ of 0.5 mg/L. Ceftobiprole MICs against MRSA (an $MIC_{50}$ of 1 mg/L and an $MIC_{90}$ of 2 mg/L) were elevated relative to MSSA (Table 1). Of the MRSA isolates tested, 42 isolates (5.3%) had an MIC of 4 mg/L. Based on $MIC_{50}$/$MIC_{90}$ values, the activity of ceftobiprole (0.5/2 mg/L) against the tested *S. aureus* overall was comparable to that of vancomycin (1/1 mg/L), teicoplanin (0.5/1 mg/L) and linezolid (2/2 mg/L).

Ceftobiprole was also active against both methicillin-susceptible CoNS ($MIC_{50} \leq 0.12$ mg/L, $MIC_{90} = 0.25$ mg/L) and methicillin-resistant CoNS ($MIC_{50} = 1$ mg/L, $MIC_{90} = 2$ mg/L) (Table 1). As was observed with *S. aureus*, ceftobiprole MICs against methicillin-resistant CoNS were higher than against methicillin-susceptible CoNS. However, the majority of methicillin-resistant CoNS (81.6%) had ceftobiprole MICs ≤ 1 mg/L. Among the subset of isolates (5.4%) that had MICs ≥ 4 mg/L, only one (*S. saprophyticus* with a ceftobiprole MIC of 8 mg/L) had an MIC > 4 mg/L and is currently undergoing further characterization.

Ceftobiprole MICs against *S. pneumoniae* ($MIC_{50} = 0.015$ mg/L, $MIC_{90} = 0.25$ mg/L) were lower than those of ceftriaxone ($MIC_{50} = 0.03$ mg/L, $MIC_{90} = 1$ mg/L) and cefuroxime ($MIC_{50} = 0.03$ mg/L, $MIC_{90} = 4$ mg/L) (Table 1). As with ceftriaxone and cefuroxime, ceftobiprole MICs increased with increasing resistance to penicillin($MIC_{90}$s of 0.015, 0.25 and 0.5 mg/L for penicillin-susceptible, -intermediate and -resistant isolates, respectively), but even among penicillin-resistant isolates, ceftobiprole MICs did not exceed 1 mg/L.

### *Enterobacteriaceae*

The *in vitro* activity of ceftobiprole and other cephalosporin comparators against ceftazidime-susceptible and non-susceptible

**Table 1.** *In vitro* activity of ceftobiprole and comparators against Gram-positive organisms

| Organism | Antimicrobial agent | Phenotype[a] | MIC (mg/L) range | mode | $MIC_{50}$ | $MIC_{90}$ | %S | %I | %R |
|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* | ceftobiprole | all ($n = 1201$) | ≤0.12–4 | 1 | 0.5 | 2 | —[b] | —[b] | —[b] |
| | | OXA S ($n = 403$) | ≤0.12–1 | 0.25 | 0.25 | 0.5 | —[b] | —[b] | —[b] |
| | | OXA R ($n = 798$) | 0.25–4 | 1 | 1 | 2 | —[b] | —[b] | —[b] |
| | clindamycin | all | ≤0.03 to >4 | 0.12 | 0.12 | >4 | 66.8 | 0.6 | 32.6 |
| | daptomycin | all | ≤0.12–2 | 0.25 | 0.5 | 0.5 | 99.9 | —[b] | —[b] |
| | gentamicin | all | ≤0.06 to >16 | 0.25 | 0.25 | >16 | 82.8 | 0.4 | 16.7 |
| | linezolid | all | ≤0.25–4 | 2 | 2 | 2 | 100.0 | —[b] | —[b] |
| | minocycline | all | ≤0.06 to >16 | 0.12 | 0.12 | 0.25 | 94.7 | 4.6 | 0.7 |
| | moxifloxacin | all | ≤0.015 to >16 | 2 | 2 | 4 | 38.9 | 6.9 | 54.2 |
| | teicoplanin | all | ≤0.12–4 | 0.5 | 0.5 | 1 | 100.0 | 0.0 | 0.0 |
| | tigecycline[c] | all | 0.03–1 | 0.12 | 0.12 | 0.25 | 99.9 | —[c] | —[c] |
| | trimeth/sulfa | all | ≤0.25 to >4 | ≤0.25 | ≤0.25 | ≤0.25 | 96.6 | —[b] | 3.4 |
| | vancomycin | all | 0.5–2 | 1 | 1 | 1 | 100.0 | 0.0 | 0.0 |
| CoNS[d] | ceftobiprole | all ($n = 460$) | ≤0.12–8 | 1 | 0.5 | 2 | —[b] | —[b] | —[b] |
| | | OXA S ($n = 129$) | ≤0.12–1 | ≤0.12 | ≤0.12 | 0.25 | —[b] | —[b] | —[b] |
| | | OXA R ($n = 331$) | ≤0.12–8 | 1 | 1 | 2 | —[b] | —[b] | —[b] |
| | clindamycin | all | ≤0.03 to >4 | 0.06 | 0.12 | >4 | 76.7 | 0.4 | 22.8 |
| | daptomycin | all | ≤0.12–2 | 0.5 | 0.5 | 0.5 | 99.8 | —[b] | —[b] |
| | gentamicin | all | ≤0.06 to >16 | ≤0.06 | ≤0.06 | >16 | 63.3 | 4.8 | 32.0 |
| | linezolid | all | ≤0.25–4 | 1 | 1 | 2 | 100.0 | —[b] | —[b] |
| | minocycline | all | ≤0.06–16 | 0.25 | 0.25 | 0.5 | 99.6 | 0.2 | 0.2 |
| | moxifloxacin | all | ≤0.015 to >16 | 0.06 | 0.25 | 4 | 55.9 | 13.5 | 30.7 |
| | teicoplanin | all | ≤0.12–32 | 2 | 2 | 4 | 98.9 | 0.9 | 0.2 |
| | tigecycline | all | ≤0.015–1 | 0.25 | 0.12 | 0.5 | —[b] | —[b] | —[b] |
| | trimeth/sulfa | all | ≤0.25 to >4 | ≤0.25 | ≤0.25 | >4 | 68.5 | —[b] | 31.5 |
| | vancomycin | all | 0.5–4 | 2 | 2 | 2 | 100.0 | 0.0 | 0.0 |
| *S. pneumoniae* | ceftobiprole | all ($n = 526$) | ≤0.002–1 | 0.015 | 0.015 | 0.25 | —[b] | —[b] | —[b] |
| | | PEN S ($n = 406$) | ≤0.002–0.06 | 0.015 | 0.008 | 0.015 | —[b] | —[b] | —[b] |
| | | PEN I ($n = 61$) | 0.008–0.5 | 0.25 | 0.06 | 0.25 | —[b] | —[b] | —[b] |
| | | PEN R ($n = 59$) | 0.25–1 | 0.5 | 0.5 | 0.5 | —[b] | —[b] | —[b] |
| | ceftriaxone | all | ≤0.015–4 | ≤0.015 | 0.03 | 1 | 97.9 | 1.7 | 0.4 |
| | cefuroxime sodium | all | ≤0.015 to >8 | 0.03 | 0.03 | 4 | 82.7 | 1.5 | 15.8 |
| | clindamycin | all | ≤0.015 to >2 | 0.06 | 0.06 | >2 | 80.0 | 1.0 | 19.0 |
| | levofloxacin | all | ≤0.06 to >8 | 1 | 1 | 1 | 98.5 | 0.0 | 1.5 |
| | penicillin | all | ≤0.015–4 | ≤0.015 | 0.03 | 2 | 77.2 | 11.6 | 11.2 |
| | trimeth/sulfa | all | ≤0.06–16 | 0.25 | 0.25 | 4 | 70.7 | 12.2 | 17.1 |

[a]OXA, oxacillin; PEN, penicillin; S, susceptible; I, intermediate; R, resistant.
[b]CLSI breakpoints unavailable for interpretation of susceptible, intermediate and/or resistant.
[c]FDA breakpoints were used to interpret tigecycline results: 0.5 mg/L (S), no interpretations are available for (I) and/or (R).
[d]Coagulase-negative staphylococci.

Enterobacteriaceae is presented in Table 2. Among ceftazidime-susceptible Enterobacteriaceae, the ceftobiprole $MIC_{90}$ was 0.12 mg/L, which was the same as the $MIC_{90}$ for cefepime, and two doubling dilutions lower than the $MIC_{90}$s obtained with either ceftazidime or ceftriaxone. This hierarchy of activity was generally consistent across populations of Enterobacteriaceae largely susceptible to ceftazidime, including non-ESBL *E. coli* and *K. pneumoniae* and non-derepressed AmpC *E. cloacae* and *Citrobacter* spp.

Against ceftazidime non-susceptible Enterobacteriaceae overall, MICs of ceftobiprole and comparator cephalosporins were notably increased, as the $MIC_{90}$ of each agent was >32 mg/L against this resistant population of organisms (Table 2). By $MIC_{50}$ and $MIC_{90}$, this pattern was generally maintained across all populations of enteric species studied where high levels of ceftazidime resistance are expected (ESBL screen-positive populations of *E. coli*, *K. pneumoniae* and *P mirabilis*; derepressed AmpC screen-positive populations of *E. cloacae* and *Citrobacter* spp.). Although the $MIC_{90}$ remained at >32 mg/L for the tested cephalosporins against putative derepressed AmpC isolates of *E. cloacae* and *Citrobacter* spp., the $MIC_{50}$ of ceftobiprole (8 mg/L for *E. cloacae*, 2 mg/L for *Citrobacter* spp.) and cefepime (4 mg/L for *E. cloacae*, 2 mg/L for *Citrobacter* spp.) against these isolates

**Table 2.** *In vitro* activity of ceftobiprole and comparators against Enterobacteriaceae

| Organism | Antimicrobial agent | Phenotype[a] | MIC (mg/L) range | mode | $MIC_{50}$ | $MIC_{90}$ | %S | %I | %R |
|---|---|---|---|---|---|---|---|---|---|
| All Enterobacteriaceae | ceftobiprole | all ($n = 3594$) | ≤0.015 to >32 | 0.03 | 0.06 | 32 | —[b] | —[b] | —[b] |
| | | CAZ S ($n = 3126$) | ≤0.015 to >32 | 0.03 | 0.06 | 0.12 | —[b] | —[b] | —[b] |
| | | CAZ NS ($n = 468$) | 0.06 to >32 | >32 | >32 | >32 | —[b] | —[b] | —[b] |
| | cefepime | all | ≤0.015 to >32 | 0.03 | 0.03 | 4 | 93.0 | 0.7 | 6.3 |
| | | CAZ S | ≤0.015 to >32 | 0.03 | 0.03 | 0.12 | 98.1 | 0.1 | 1.8 |
| | | CAZ NS | ≤0.015 to >32 | >32 | 8 | >32 | 58.5 | 4.9 | 36.5 |
| | ceftazidime | all | ≤0.03 to >32 | 0.12 | 0.12 | >32 | 87.0 | 1.2 | 11.8 |
| | | CAZ S | ≤0.03–8 | 0.12 | 0.12 | 0.5 | 100.0 | 0.0 | 0.0 |
| | | CAZ NS | 16 to >32 | >32 | >32 | >32 | 0.0 | 9.2 | 90.8 |
| | ceftriaxone | all | ≤0.015 to >64 | 0.03 | 0.06 | >64 | 84.3 | 2.8 | 12.9 |
| | | CAZ S | ≤0.015 to >64 | 0.03 | 0.06 | 0.5 | 96.0 | 1.1 | 2.9 |
| | | CAZ NS | ≤0.015 to >64 | >64 | >64 | >64 | 6.4 | 14.3 | 79.3 |
| *E. coli* | ceftobiprole | all ($n = 1213$) | ≤0.015 to >32 | 0.03 | 0.03 | 0.25 | —[b] | —[b] | —[b] |
| | | non-ESBL ($n = 1105$) | ≤0.015 to >32 | 0.03 | 0.03 | 0.06 | —[b] | —[b] | —[b] |
| | | ESBL ($n = 108$) | 0.25 to >32 | >32 | >32 | >32 | —[b] | —[b] | —[b] |
| | cefepime | all | ≤0.015 to >32 | 0.03 | 0.03 | 1 | 93.7 | 0.3 | 5.9 |
| | | non-ESBL | ≤0.015–16 | 0.03 | 0.03 | 0.12 | 99.9 | 0.1 | 0.0 |
| | | ESBL | 0.25 to >32 | >32 | >32 | >32 | 30.6 | 2.8 | 66.7 |
| | ceftazidime | all | ≤0.03 to >32 | 0.12 | 0.12 | 2 | 93.7 | 1.3 | 4.9 |
| | | non-ESBL | ≤0.03 to >32 | 0.12 | 0.12 | 0.25 | 99.6 | 0.1 | 0.3 |
| | | ESBL | 0.25 to >32 | >32 | 32 | >32 | 33.3 | 13.9 | 52.8 |
| | ceftriaxone | all | ≤0.015 to >64 | 0.03 | 0.03 | 1 | 91.0 | 1.1 | 7.9 |
| | | non-ESBL | ≤0.015 to >64 | 0.03 | 0.03 | 0.12 | 99.5 | 0.1 | 0.4 |
| | | ESBL | 4 to >64 | >64 | >64 | >64 | 3.7 | 11.1 | 85.2 |
| *K. pneumoniae* | ceftobiprole | all ($n = 854$) | ≤0.015 to >32 | 0.03 | 0.06 | >32 | —[b] | —[b] | —[b] |
| | | non-ESBL ($n = 687$) | ≤0.015 to >32 | 0.03 | 0.03 | 0.12 | —[b] | —[b] | —[b] |
| | | ESBL ($n = 167$) | 0.12 to >32 | >32 | >32 | >32 | —[b] | —[b] | —[b] |
| | cefepime | all | ≤0.015 to >32 | 0.03 | 0.03 | >32 | 85.4 | 0.8 | 13.8 |
| | | non-ESBL | ≤0.015 to >32 | 0.03 | 0.03 | 0.12 | 98.7 | 0.0 | 1.3 |
| | | ESBL | 1 to >32 | >32 | >32 | >32 | 30.5 | 4.2 | 65.3 |
| | ceftazidime | all | ≤0.03 to >32 | 0.12 | 0.12 | >32 | 83.3 | 2.1 | 14.6 |
| | | non-ESBL | ≤0.03 to >32 | 0.12 | 0.12 | 0.5 | 97.5 | 0.6 | 1.9 |
| | | ESBL | 1 to >32 | >32 | >32 | >32 | 24.6 | 8.4 | 67.1 |
| | ceftriaxone | all | ≤0.015 to >64 | 0.03 | 0.06 | >64 | 79.9 | 2.9 | 17.2 |
| | | non-ESBL | ≤0.015 to >64 | 0.03 | 0.06 | 0.12 | 98.3 | 0.1 | 1.6 |
| | | ESBL | ≤0.015 to >64 | >64 | >64 | >64 | 4.2 | 14.4 | 81.4 |
| *P. mirabilis* | ceftobiprole | all ($n = 443$) | ≤0.015 to >32 | 0.03 | 0.03 | 0.12 | —[b] | —[b] | —[b] |
| | | non-ESBL ($n = 427$) | ≤0.015 to >32 | 0.03 | 0.03 | 0.06 | —[b] | —[b] | —[b] |
| | | ESBL ($n = 16$) | 0.5 to >32 | >32 | >32 | >32 | —[b] | —[b] | —[b] |
| | cefepime | all | ≤0.015 to >32 | 0.03 | 0.03 | 0.12 | 97.1 | 0.2 | 2.7 |
| | | non-ESBL | ≤0.015 to >32 | 0.03 | 0.03 | 0.12 | 99.3 | 0.2 | 0.5 |
| | | ESBL | 2 to >32 | >32 | >32 | >32 | 37.5 | 0.0 | 62.5 |
| | ceftazidime | all | ≤0.03 to >32 | ≤0.03 | ≤0.03 | 0.12 | 96.8 | 0.7 | 2.5 |
| | | non-ESBL | ≤0.03 to >32 | ≤0.03 | ≤0.03 | 0.06 | 97.4 | 0.7 | 1.9 |
| | | ESBL | 1 to >32 | 1 | 2 | >32 | 81.3 | 0.0 | 18.8 |
| | ceftriaxone | all | ≤0.015 to >64 | ≤0.015 | ≤0.015 | 0.03 | 94.1 | 1.1 | 4.7 |
| | | non-ESBL | ≤0.015 to >64 | ≤0.015 | ≤0.015 | ≤0.015 | 97.7 | 0.7 | 1.6 |
| | | ESBL | 32 to >64 | >64 | >64 | >64 | 0.0 | 12.5 | 87.5 |
| *E. cloacae* | ceftobiprole | all ($n = 406$) | 0.03 to >32 | 0.06 | 0.12 | >32 | —[b] | —[b] | —[b] |
| | | non-derep. AmpC ($n = 286$) | 0.03 to >32 | 0.06 | 0.06 | 4 | —[b] | —[b] | —[b] |
| | | derep. AmpC ($n = 120$) | 0.12 to >32 | 8 | 8 | >32 | —[b] | —[b] | —[b] |
| | cefepime | all | ≤0.015 to >32 | 0.03 | 0.06 | 8 | 95.1 | 2.7 | 2.2 |
| | | non-derep. AmpC | ≤0.015 to >32 | 0.03 | 0.06 | 0.5 | 99.0 | 0.3 | 0.7 |

*Continued*

**Table 2.** *Continued*

| Organism | Antimicrobial agent | Phenotype[a] | MIC (mg/L) range | mode | $MIC_{50}$ | $MIC_{90}$ | %S | %I | %R |
|---|---|---|---|---|---|---|---|---|---|
| | | derep. AmpC | 0.25 to >32 | 4 | 4 | 16 | 85.8 | 8.3 | 5.8 |
| | ceftazidime | all | ≤0.03 to >32 | >32 | 0.5 | >32 | 61.6 | 0.5 | 37.9 |
| | | non-derep. AmpC | ≤0.03 to >32 | 0.25 | 0.25 | 32 | 87.4 | 0.7 | 11.9 |
| | | derep. AmpC | 32 to >32 | >32 | >32 | >32 | 0.0 | 0.0 | 100.0 |
| | ceftriaxone | all | ≤0.015 to >64 | >64 | 0.5 | >64 | 61.3 | 4.2 | 34.5 |
| | | non-derep. AmpC | ≤0.015 to >64 | 0.25 | 0.25 | 32 | 87.1 | 5.9 | 7.0 |
| | | derep. AmpC | 64 to >64 | >64 | >64 | >64 | 0.0 | 0.0 | 100.0 |
| *Citrobacter* spp. | ceftobiprole | all ($n = 387$) | ≤0.015 to >32 | 0.06 | 0.06 | 2 | —[b] | —[b] | —[b] |
| | | non-derep. AmpC ($n = 368$) | ≤0.015 to >32 | 0.06 | 0.06 | 1 | —[b] | —[b] | —[b] |
| | | derep. AmpC ($n = 19$) | 1 to >32 | 2 | 2 | >32 | —[b] | —[b] | —[b] |
| | cefepime | all | ≤0.015 to >32 | 0.03 | 0.03 | 1 | 97.9 | 0.5 | 1.6 |
| | | non-derep. AmpC | ≤0.015 to >32 | 0.03 | 0.03 | 0.5 | 99.2 | 0.3 | 0.5 |
| | | derep. AmpC | 0.5 to >32 | 1 | 2 | >32 | 73.7 | 5.3 | 21.1 |
| | ceftazidime | all | ≤0.03 to >32 | 0.12 | 0.25 | >32 | 84.0 | 0.8 | 15.2 |
| | | non-derep. AmpC | ≤0.03 to >32 | 0.12 | 0.25 | >32 | 88.3 | 0.8 | 10.9 |
| | | derep. AmpC | >32 to >32 | >32 | >32 | >32 | 0.0 | 0.0 | 100.0 |
| | ceftriaxone | all | ≤0.015 to >64 | 0.06 | 0.12 | 32 | 85.3 | 4.9 | 9.8 |
| | | non-derep. AmpC | ≤0.015 to >64 | 0.06 | 0.12 | 16 | 89.7 | 5.2 | 5.2 |
| | | derep. AmpC | 64 to >64 | >64 | >64 | >64 | 0.0 | 0.0 | 100.0 |
| *S. marcescens* | ceftobiprole | all ($n = 291$) | 0.03 to >32 | 0.06 | 0.12 | 2 | —[b] | —[b] | —[b] |
| | | CAZ S ($n = 274$) | 0.03 to >32 | 0.06 | 0.12 | 0.5 | —[b] | —[b] | —[b] |
| | | CAZ NS ($n = 17$) | 4 to >32 | >32 | >32 | >32 | —[b] | —[b] | —[b] |
| | cefepime | all | ≤0.015 to >32 | 0.06 | 0.06 | 1 | 96.2 | 0.3 | 3.4 |
| | | CAZ S | ≤0.015 to >32 | 0.06 | 0.06 | 0.5 | 97.4 | 0.0 | 2.6 |
| | | CAZ NS | 0.25 to >32 | 8 | 4 | >32 | 76.5 | 5.9 | 17.6 |
| | ceftazidime | all | ≤0.03 to >32 | 0.12 | 0.12 | 2 | 94.2 | 0.3 | 5.5 |
| | | CAZ S | ≤0.03–8 | 0.12 | 0.12 | 1 | 100.0 | 0.0 | 0.0 |
| | | CAZ NS | 16 to >32 | >32 | >32 | >32 | 0.0 | 5.9 | 94.1 |
| | ceftriaxone | all | 0.03 to >64 | 0.12 | 0.25 | 16 | 85.2 | 7.6 | 7.2 |
| | | CAZ S | 0.03 to >64 | 0.12 | 0.25 | 8 | 90.1 | 6.6 | 3.3 |
| | | CAZ NS | 8 to >64 | >64 | 64 | >64 | 5.9 | 23.5 | 70.6 |

[a]CAZ, ceftazidime; S, susceptible; NS; non-susceptible; ESBL, extended-spectrum β-lactamase; derep. AmpC, derepressed-AmpC β-lactamase.
[b]CLSI breakpoints unavailable for interpretation of susceptible, intermediate and/or resistant.

were lower than the other tested cephalosporins. Although the $MIC_{90}$s of each cephalosporin were >32 mg/L against ESBL screen-positive isolates, MIC distribution analysis for the tested cephalosporins against ESBL screen-positive isolates overall showed an ~4-fold advantage in the *in vitro* activity of both cefepime and ceftazidime over the lower range of concentrations relative to ceftobiprole (at 2 mg/L, 18.6% and 12.4% of these isolates were inhibited by cefepime and ceftazidime, respectively; at 8 mg/L, 30.9% of isolates were inhibited by both cefepime and ceftazidime).

### *P. aeruginosa and Acinetobacter spp.*

Both ceftobiprole and cefepime were indistinguishable by $MIC_{50}$ (4 mg/L) and $MIC_{90}$ (16 mg/L) against *P. aeruginosa* overall, and were at least 4-fold more potent than ceftazidime (>32 mg/L) by $MIC_{90}$ (Table 3). Against ceftazidime-susceptible *P. aeruginosa* isolates, ceftobiprole MICs ranged between 0.03 and >32 mg/L with an $MIC_{50}$ of 2 mg/L and an $MIC_{90}$ of 8 mg/L (Table 3). Against these isolates, the activity of ceftobiprole was similar to that of cefepime, where 59.3% and 60.3% of isolates were inhibited by ≤2 mg/L of ceftobiprole and cefepime, respectively. At ≤8 mg/L, 90.2% of isolates were inhibited by ceftobiprole relative to 95.1% of isolates inhibited by cefepime. Both cefepime and ceftobiprole inhibited fewer of these isolates at their respective $MIC_{50}$s and $MIC_{90}$s than ceftazidime (76.6% isolates inhibited at ≤2 mg/L and 100% isolates inhibited at ≤8 mg/L).

Against ceftazidime non-susceptible (MIC ≥ 16 mg/L) *P. aeruginosa*, ceftobiprole MICs were higher than against ceftazidime-susceptible isolates ($MIC_{50}$ = 16 mg/L, $MIC_{90}$ > 32 mg/L) (Table 3). Among ceftazidime-susceptible isolates, 2.2% of isolates had ceftobiprole MICs ≥ 32 mg/L, whereas among ceftazidime non-susceptible isolates, 38.5% had ceftobiprole MICs ≥ 32 mg/L. At concentrations ≤4 and ≤8 mg/L, ceftobiprole inhibited 26.9% and 44.6% of isolates, respectively. In contrast, cefepime inhibited 8.5% of isolates at ≤4 mg/L and 24.6% of isolates at ≤8 mg/L. At ≤16 mg/L, cefepime and

**Table 3.** *In vitro* activity of ceftobiprole and comparators against *P. aeruginosa* and *Acinetobacter* spp.

| Organism | Antimicrobial agent | Phenotype[a] | MIC (mg/L) range | mode | $MIC_{50}$ | $MIC_{90}$ | %S | %I | %R |
|---|---|---|---|---|---|---|---|---|---|
| *P. aeruginosa* | ceftobiprole | all ($n = 621$) | 0.03 to >32 | 2 | 4 | 16 | —[b] | —[b] | —[b] |
| | | CAZ S ($n = 491$) | 0.03 to >32 | 2 | 2 | 8 | —[b] | —[b] | —[b] |
| | | CAZ NS ($n = 130$) | 0.25 to >32 | >32 | 16 | >32 | —[b] | —[b] | —[b] |
| | cefepime | all | ≤0.015 to >32 | 1 | 4 | 16 | 80.4 | 10.8 | 8.9 |
| | ceftazidime | all | 0.06 to >32 | 2 | 2 | >32 | 79.1 | 3.7 | 17.2 |
| | imipenem | all | 0.25 to >32 | 4 | 4 | 32 | 71.8 | 8.5 | 19.6 |
| | piperacillin/tazobactam | all | 0.5 to >128 | 8 | 16 | >128 | 79.4 | 0.0 | 20.6 |
| *Acinetobacter* spp. | ceftobiprole | all ($n = 278$) | ≤0.015 to >32 | >32 | 1 | >32 | —[b] | —[b] | —[b] |
| | | IPM S ($n = 220$) | ≤0.015 to >32 | >32 | 0.5 | >32 | —[b] | —[b] | —[b] |
| | | IPM NS ($n = 58$) | 0.25 to >32 | >32 | >32 | >32 | —[b] | —[b] | —[b] |
| | cefepime | all | 0.06 to >32 | 16 | 4 | >32 | 63.3 | 19.4 | 17.3 |
| | ceftazidime | all | 0.06 to >32 | >32 | 4 | >32 | 57.9 | 7.6 | 34.5 |
| | imipenem | all | ≤0.015 to >32 | 0.5 | 0.5 | >32 | 79.1 | 3.6 | 17.3 |
| | piperacillin/tazobactam | all | ≤0.25 to >128 | >128 | 32 | >128 | 46.4 | 10.4 | 43.2 |

[a]CAZ, ceftazidime; IPM, imipenem; S, susceptible; NS, non-susceptible.
[b]CLSI breakpoints unavailable for interpretation of susceptible, intermediate and/or resistant.

ceftobiprole inhibited a similar number of isolates (63.1% and 61.5%, respectively), while only 17.7% of these isolates were inhibited by ceftazidime at the same concentration.

Ceftobiprole and the tested comparator cephalosporin $MIC_{90}$s against *Acinetobacter* spp. overall were above the highest concentration tested (>32 mg/L) (Table 3). Against imipenem-susceptible strains, ceftobiprole had an $MIC_{50}$ of 0.5 mg/L, but that increased to >32 mg/L among imipenem non-susceptible strains. Interestingly, among the tested *Acinetobacter* spp. overall, the ceftobiprole $MIC_{50}$ (1 mg/L) was lower than that of either cefepime (4 mg/L) or ceftazidime (4 mg/L).

## Discussion

Ceftobiprole was active *in vitro* against methicillin-resistant staphylococci isolated throughout Europe. Against both MRSA and methicillin-resistant CoNS, ceftobiprole had an $MIC_{90}$ of 2 mg/L, which was in agreement with previous studies where $MIC_{90}$s between 1 and 4 mg/L were observed against MRSA, and an $MIC_{90}$ of 2 mg/L was observed against methicillin-resistant CoNS.[3,4,6,13] Other studies have also shown that against staphylococci, ceftobiprole was bactericidal and was active *in vitro* against glycopeptide-non-susceptible staphylococci.[3,5,14] Based on the findings of this current study and those of others, the activity of ceftobiprole against MRSA has been clearly established. The ability of ceftobiprole to be active against methicillin-resistant staphylococci is accomplished by its ability to bind and inhibit the transpeptidase activity of PBP2a.[2,15] No clinically available cephalosporins successfully target PBP2a, although, aside from ceftobiprole, there are other β-lactams currently in development that display activity against MRSA.[16–18]

As an agent to combat MRSA, ceftobiprole is intended for use in hospitals, an environment where antibiotic resistance is well established. Usage in such an environment highlights the importance of studies regarding the development of resistance to ceftobiprole. Previous reports where both *S. aureus* and CoNS, including methicillin-resistant isolates, were serially passaged in the presence of ceftobiprole showed that resistance to ceftobiprole was slow to develop under these conditions. After passaging, the highest ceftobiprole MIC observed was 8 mg/L which represented a 4-fold increase in the initial MIC.[4] A separate study noted that after serial passage against three strains of MRSA and one strain of MSSA, ceftobiprole MICs never rose more than one doubling dilution after passage and ceftobiprole MICs remained below 4 mg/L.[3] In the same study, against one MRSA in particular, ceftobiprole MICs did not increase during serial passage, remaining between 0.5 and 1 mg/L, in contrast to imipenem where MICs increased at least 8-fold (from 4 to >32 mg/L) by the third passage.[3] Although it is impossible to know how quickly resistance will emerge to ceftobiprole in a clinical setting, the emergence of ceftobiprole resistance via chromosomal mutation was not readily observable in the laboratory. To this point, it is important to note that resistant mutants were obtained among MRSA serially passaged in the presence of an investigational anti-MRSA carbapenem and resistance was determined to arise as a result of multiple mutations within *mecA*.[19] What effect these mutations may have on the anti-MRSA activity of ceftobiprole has yet to be determined.

Regardless of penicillin-susceptibility status, ceftobiprole was highly active against *S. pneumoniae*, even though ceftobiprole's $MIC_{90}$s increased with growing resistance to penicillin. This level of activity was comparable to or slightly greater than that of ceftriaxone and, overall, these results are in agreement with those observed in previous studies.[3,6] In summary, these data demonstrate that ceftobiprole has a potency against *S. pneumoniae* that is at least comparable to that of ceftriaxone.

The activity of ceftobiprole against Enterobacteriaceae depended largely on the β-lactam resistance phenotypes of the tested isolates. Ceftobiprole was highly active against

ceftazidime-susceptible (ceftobiprole $MIC_{50}$/$MIC_{90}$: 0.06/0.12 mg/L) and non-ESBL isolates of *K. pneumoniae*, *E. coli* and *P. mirabilis* (ceftobiprole $MIC_{50}$s/$MIC_{90}$s: 0.03/0.06–0.12 mg/L), which was similar to the activities exhibited by cefepime and ceftriaxone, though ceftriaxone was the most active agent tested against non-ESBL *P. mirabilis* ($MIC_{50}$ and $MIC_{90}$ $\leq$0.015 mg/L). Against non-derepressed AmpC isolates of *E. cloacae* and *Citrobacter* spp., slightly higher ceftobiprole $MIC_{90}$s (4 mg/L for *E. cloacae*, 1 mg/L for *Citrobacter* spp.) were observed relative to cefepime (0.5 mg/L for both *E. cloacae* and *Citrobacter* spp.), though by $MIC_{50}$ these agents were of comparable activity against these isolates. These findings were in agreement with previous reports in which ceftobiprole was similar in activity to cefepime against enteric species.[3,6,7] Ceftobiprole and cefepime MICs were notably higher among both ceftazidime non-susceptible isolates, ESBL screen-positive and derepressed AmpC screen-positive isolates. Although the activities of both agents were diminished against these phenotypes, cefepime tended to be slightly more potent than ceftobiprole over the dilution range tested by MIC distribution, $MIC_{50}$ and $MIC_{90}$.

The decreased level of activity of both cefepime and ceftobiprole against ceftazidime non-susceptible and derepressed AmpC screen-positive isolates of *E. cloacae* and *Citrobacter* spp. is likely a reflection of their relative stability to class C β-lactamases.[3] Consistent with a previous study,[3] cefepime maintained slightly greater activity than ceftobiprole against ceftazidime non-susceptible isolates and derepressed AmpC screen-positive isolates. However, both ceftobiprole and cefepime were more active against these isolates than were other cephalosporins.[3] Clearly, the activity of ceftobiprole and cefepime against collections of these enteric species depends on the incidence and proportion of derepressed AmpC strains in a given population. In addition to these findings, it is of importance to note that along with the moderate stability of ceftobiprole and cefepime against AmpC enzymes,[3,20,21] both also have a lower potential for inducing chromosomal AmpC production than other β-lactams (e.g. imipenem and cefoxitin) do.[20]

As with other cephalosporins, the *in vitro* activity of ceftobiprole against ESBL screen-positive strains of *E. coli*, *K. pneumoniae* and *P. mirabilis* was notably diminished. This finding is consistent with other studies[3,6,7] and likely reflects the increased susceptibility of ceftobiprole and other cephalosporins to hydrolysis by mutated class A β-lactamases (TEM- and SHV-type enzymes) associated with the majority of ESBLs that exist among these species. Interestingly, according to the cumulative MICs obtained against the ESBL isolates, ceftazidime was found to have activity comparable to that of cefepime and superior to that of ceftobiprole and ceftriaxone. Although limited *in vitro* activity had been observed with cefepime against these isolates in previous studies,[3,6,21,22] the level of activity observed with ceftazidime was somewhat unexpected.

No molecular analysis was done to definitively identify the specific ESBLs; however, the increased activity of ceftazidime relative to ceftobiprole against ESBLs as noted in this study could be explained best by the relative prevalence of CTX-M β-lactamases among the population studied. CTX-M ESBLs are commonly encountered in Europe and Asia,[21,23–25] and most CTX-M enzymes efficiently hydrolyse ceftriaxone and cefotaxime but not ceftazidime, although a point mutation can confer activity against ceftazidime as well.[24] Therefore, the relatively high prevalence of isolates with the putative CTX-M profile (i.e. susceptible to ceftazidime, resistant to ceftriaxone and cefotaxime) within the ESBLs of European origin in this study (*E. coli*, 29%; *K. pneumoniae*, 22%; *P. mirabilis*, 44%) is the most likely explanation for the ceftazidime activity observed at concentrations below its susceptibility breakpoint of 8 mg/L against a proportion (~30%) of the ESBL isolates overall. Furthermore, high ceftobiprole MICs (>32 mg/L) were observed among the putative CTX-M isolates (data not shown), suggesting that ceftobiprole's diminished activity against ESBL isolates relative to ceftazidime is due to ceftobiprole's instability to CTX-M β-lactamases. Therefore, this suggests that the activity of ceftobiprole relative to other advanced generation cephalosporins is affected by both the prevalence and types of ESBLs that are encountered in a specific environment.

As was observed with Enterobacteriaceae, the activity of ceftobiprole and cefepime against *P. aeruginosa* and *Acinetobacter* spp. was also dependent on the expression of β-lactam resistance. The level of activity of ceftobiprole against *P. aeruginosa* overall ($MIC_{50}$/$MIC_{90}$ = 4/16 mg/L) was comparable to cefepime ($MIC_{50}$/$MIC_{90}$ = 4/16 mg/L), which was consistent with the findings of another study.[3,7] MICs of both ceftobiprole and cefepime increased significantly among ceftazidime non-susceptible *P. aeruginosa* by both $MIC_{90}$ and MIC distribution as observed in a previous study.[3]

Against *Acinetobacter* spp., ceftobiprole displayed only moderate activity which was comparable to that of both cefepime and ceftazidime. These findings were similar to those reported in a previous study.[6] Interestingly, as evidenced by ceftobiprole's $MIC_{50}$ of 1 mg/L compared with that of cefepime (4 mg/L) and ceftazidime (4 mg/L), at the lower concentrations tested, ceftobiprole inhibited a greater percentage of the *Acinetobacter* spp. isolates in this study.

In conclusion, ceftobiprole was active against clinically relevant Gram-positive and Gram-negative pathogens of European origin. Notably, ceftobiprole displayed potent activity against MRSA and was comparable to cefepime against Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* spp. The impact that the most prevalent 'local' resistance mechanisms may have on the *in vitro* profiles of agents such as ceftobiprole and other β-lactams, as observed among the Gram-negative organisms in this study, illustrates the necessity for widespread geographical surveillance. Concomitant with the intended use of ceftobiprole in hospitals against these challenging pathogens, continued surveillance of its activity is warranted to monitor for the emergence of resistance both during its development and after its approval for clinical use.

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## Transparency declarations

None to declare.

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