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Mechanisms of action and antimicrobial activity of ceftobiprole

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ABSTRACT

Ceftobiprole, a novel last generation parenteral cephalosporin, has an extended spectrum of activity, notably against methicillin-resistant *Staphylococcus aureus* (MRSA), ampicillin-susceptible enterococci, penicillin-resistant pneumococci, *Enterobacterales* and susceptible *Pseudomonas aeruginosa*. It exerts an inhibitory action on essential peptidoglycan transpeptidases, interfering with cell wall synthesis. The inhibitory action of ceftobiprole through binding to abnormal PBPs like PBP2a in methicillin-resistant staphylococci and PBP2b and PBP2x in the case of β-lactam-resistant pneumococci, ultimately leads to rapid bacterial cell death. In the case of *Enterobacterales*, ceftobiprole retains activity against narrow spectrum β-lactamases but is hydrolysed by their extended-spectrum counterparts, overexpressed Amp C, and carbapenemases. It is also affected by certain efflux pumps from *P. aeruginosa*. For anaerobic bacteria, ceftobiprole is active against Gram-positive *Clostridioides difficile* and *Peptococcus* spp. and Gram-negative *Fusobacterium nucleatum* but not against *Bacteroides* group or other anaerobic Gram-negatives. In *in vitro* studies, a low propensity to select for resistant subpopulations has been demonstrated. Currently, ceftobiprole is approved for the treatment of community-acquired pneumonia and hospital-acquired pneumonia with the exception of ventilator-associated pneumonia. Ceftobiprole's place in therapy appears to lie mainly in its combined activity against Gram-positive organisms, such as *S. aureus* and *S. pneumoniae* alongside that against Gram-negative organisms such as *P. aeruginosa*.

Key words: Ceftobiprole, methicillin-resistant *Staphylococcus aureus*; penicillin-resistant *Streptococcus pneumoniae*; *Pseudomonas aeruginosa*

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INTRODUCTION

Staphylococcus aureus is responsible for serious skin, soft tissue and bone infections as well as pneumonia, and is one of the leading causes of bloodstream infections in Europe, particularly within intensive care units [1]. However, the emergence of methicillin-resistant *S. aureus* (MRSA) in the 1960's as a result of the widespread use of penicillin stifled the use of subsequent promising molecules e.g isoxazolyl-penicillins [2].

Methicillin resistance in *S. aureus* and other staphylococci is due to the acquisition and expression of the *mecA* or less frequently, the *mecC* gene. These genes code for a PBP2a variant of the penicillin binding protein (PBP) PBP2 which exhibits low affinity for nearly all β-lactams thus preventing the inhibition of cell wall synthesis by these antimicrobials [3]. According to the 2017 report of the European Antimicrobial Resistance Surveillance Network (EARS-net, www.ecdc.europa.eu) the EU/EEA population-weighted mean MRSA percentage (in invasive isolates from blood stream and cerebrospinal fluid) was 16.9% (ranging from 1.0% to 44.4%, 25.8% in Spain). According to ECDC, this figure reaches 23.1% in ICUs in Europe [1].

The limited number of approved antimicrobials with activity against MRSA led to a strong demand for new agents to overcome this resistance. The fifth generation cephalosporins, ceftaroline and ceftobiprole, were the first β-lactams specifically designed to have activity against MRSA [4]. Ceftaroline was approved by European Medicines Agency in 2010, followed by ceftobiprole in 2013 in major European countries.

Ceftobiprole is a bactericidal cephalosporin with an extended-spectrum of activity against both Gram-positive cocci and Gram-negative bacilli. Ceftobiprole demonstrates potent binding to PBPs from Gram-positive bacteria, including those with decreased β-lactam sensitivity, such as PBP2a in MRSA and PBP2x in penicillin-resistant *Streptococcus pneumoniae* (PRSP), the latter, in contrast to ceftriaxone. In *Escherichia coli*, ceftobiprole also exhibits strong binding to the essential PBP2 and PBP3.

Unlike ceftaroline, ceftobiprole also exhibits a binding profile similar to that of cefepime and ceftazidime to PBPs in *P. aeruginosa* but with enhanced binding to PBP2. These properties explain the extended-spectrum activity of ceftobiprole and its indication in nosocomial pneumonia in which *P. aeruginosa* is a common pathogen [4-6]. In addition, in single-step and serial passage *in vitro* resistance development studies, ceftobiprole demonstrates a low propensity to select for resistance [6].

In this article we review the mechanism of action of ceftobiprole as well as its antimicrobial activity in international surveillance studies.

MECHANISM OF ACTION AND ANTIMICROBIAL PROFILE

Ceftobiprole is a parenteral pyrrolidinone-3-ylidene-methyl cephalosporin (figure 1) with an extended-spectrum of activity against MRSA, other Gram-positive bacteria (*S. pneumoniae* and *Enterococcus faecalis*) and Gram-negative bacteria (*Enterobacterales* and *P. aeruginosa*) exerted through the inhibition of essential peptidoglycan transpeptidases. Like other cephalosporins, the binding of ceftobiprole to PBPs interferes with cell wall synthesis, inhibiting cell growth and ultimately leading to bacterial cell death. Ceftobiprole exhibits a rapid bactericidal mode of action on an extended spectrum of clinically important Gram-positive and Gram-negative pathogens [5].

The bactericidal activity of ceftobiprole against MRSA sets it apart from other cephalosporins (with the exception of ceftaroline). Their efficacy as anti-MRSA is due to a successful inhibitory interaction with the extended narrow groove of the PBP2a active site coded by *mec* genes, favouring its acylation, inhibiting cell growth and, ultimately, leading to bacterial cell death. The molecular structures of first to fourth generation cephalosporins do not lead to suitable binding to PBP2a. The presence of a large hydrophobic side chain at C3 in the ceftobiprole molecule facilitates a conformational change in PBP2a leading to a stronger and energetically more favourable interaction with the PBP2a site groove and the formation of a stable acyl-enzyme complex. This interaction along with ceftobiprole's affinity for a range of other staphylococcal PBPS such as PBP1, PBP3, and PBP4 explains its high activity against staphylococci, including coagulase-negative isolates [7] Figure 2 comparatively includes the interaction of ceftobiprole and other beta-lactams with PBPs from different microorganisms [8-12].

Ceftobiprole demonstrates potent binding to PBPs in other Gram-positive bacteria, including those resistant to other β-lactam antibiotics, such as is the case of penicillin-intermediate and-resistant *S. pneumoniae* isolates. In these resistant strains, ceftobiprole exerts higher binding affinity to PBP2b and PBP2x than ceftriaxone [13].

The bactericidal activity against *E. faecalis* is a unique characteristic of ceftobiprole among the cephalosporins and is attributed to the high affinity for the enterococcal penicillin binding proteins. However, ceftobiprole does not bind to PBP5 in *E. faecium* although, in the minority of *E. faecium* isolates that are ampicillin sensitive, ceftobiprole appears to be active [7-13, 14]. This effect has been shown to be much lower with ceftaroline, being this one 4-fold less effective on *E. faecalis* versus ceftobiprole [15].

Against Gram-negative bacteria, ceftobiprole exhibits high affinity for PBPs in *Enterobacterales*. However, ceftobiprole is inactive against *Enterobacterales* expressing Ambler's Class A β-lactamases including ESBLs, overexpressed AmpC β-lactamase types, and all carbapenemases. *P. aeruginosa*, when grown in the presence of ceftobiprole, produces filamentation, suggesting that PBP3 is the site of action [9]. Ceftobiprole is ineffective against *P. aeruginosa* expressing Ambler's Class A β-lactamases including ESBLs and all carbapenemases, as class A (PSE-type, GES and others), metallo-carbapenemases (IMP and VIM) and D (OXA-10). Ceftobiprole is partially and slowly hydrolysed by AmpC and interestingly, unlike ceftazidime and cefepime, did not select AmpC derepressed mutants [16]. In a similar fashion, ceftobiprole, and ceftaroline display limited activity against *Acinetobacter* spp., *Burkholderia cepacia* and *Stenotrophomonas maltophilia* [14, 17].

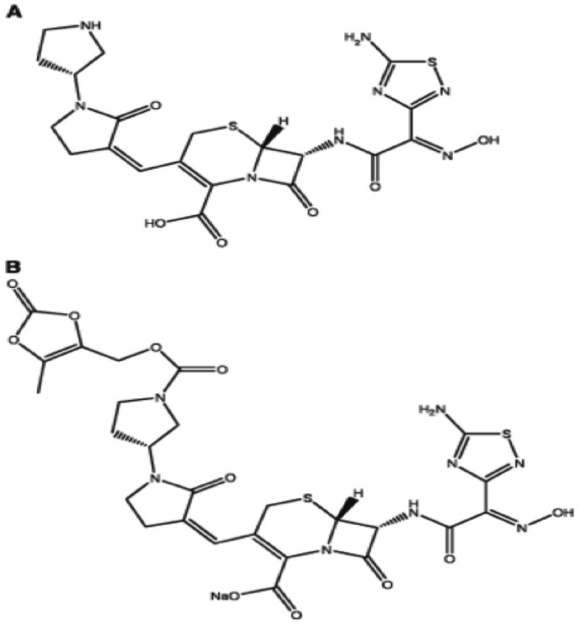

Figure 1 | **A: Ceftobiprole, the active cephalosporin. B: Ceftobiprole medocaril, the water-soluble prodrug. Substitution at position 7 of the cephem by an oxyimino aminothiazolyl confers remarkable betalactamase stability and substitution at position 3 with a vinylpyrrolidinone moiety facilitates the association of the molecule with PBP2a and hence facilitates the subsequent acylation reaction.**

Table 1 **Ceftobiprole and ceftaroline breakpoints and ECOFF values of bacterial species and groups, according to EUCAST-2019.**

Microorganism	Ceftobiprole			Ceftaroline		
	Clinical breakpoints (mg/L)		ECOFF (mg/L)	Clinical breakpoints (mg/l)		ECOFF (mg/L)
	Susceptible (≤)	Resistant (>)		Susceptible (≤)	Resistant (>)	
S. aureus (including MRSA)	2	2	1	1*	1** 2***	0.5
S. pneumoniae (including PNS[a])	0.5	0.5	0.03	0.25	0.25	0.03
E. faecalis	-[b]	-	ND[c]	-	-	ND
Enterobacterales	0.25	0.25	0.12-0.25	0.5	0.5	0.12-0.25
P. aeruginosa	IE[d]	IE	8	-	-	ND
H. influenzae	IE	IE	0.25	0.03	0.03	0.03
M. catarrhalis	IE	IE	ND	IE	IE	ND
Non-species related[e]	4[f]	4	-	0.5	0.5	-

*Including pneumonia; **Pneumonia isolates; *** Other isolates than pneumonia; [a]Penicillin-non-susceptible; [b]-: no breakpoint; [c]ND: not determined; [d]IE: insufficient evidence; [e]PK-PD breakpoints; [f]Based on PK-PD target for Gram-negative organisms.

Ceftobiprole is active against both non- and β-lactamase-producing *Haemophilus influenzae* and *Moraxella catarrhalis*, and against *Neisseria* spp.

For anaerobic bacteria, ceftobiprole is active against Gram-positive *Clostridioides difficile*, *Peptococcus* spp. and *Fusobacterium nucleatum* but not against the *Bacteroides* group and other anaerobic Gram-negatives [18]. Ceftobiprole has limited activity against Gram-negative anaerobes such as *Bacteroides fragilis* and *Bacteroides* spp. β-lactamase negative anaerobes are more susceptible to ceftobiprole than β-lactamase-positive isolates, suggesting that ceftobiprole is hydrolysed by most β-lactamases found in these bacteria. Ceftobiprole is also active against *Cutibacterium acnes*, *Peptostreptococcus* spp., *Clostridium innocuum*, *Finegoldia magna*, and many strains of *Porphyromonas* spp. It demonstrates lower MICs for *Clostridium perfringes* and *Clostridiums difficile* than other cephalosporins, and has been shown to be less active *in vitro* than ceftriaxone against isolates of *Fusobacterium* spp., *Prevotella* spp. and *Veillonella* spp. [19].

CLINICAL BREAKPOINTS AND *IN VITRO* ACTIVITY

Ceftobiprole clinical breakpoints and ECOFF values (EUCAST, 2019. www.eucast.org) for Gram-positive and Gram-negative species in comparison with those defined for ceftaroline are shown in table 1. EUCAST has not yet established ECOFF values for all the targeted species, however, where ECOFF values are defined for both for ceftobiprole and ceftaroline, they are similar. However, it should be noted that PK/PD breakpoints are higher for ceftobiprole than for ceftaroline. This situation reflects the favourable T>MIC PK/PD index for ceftobiprole associated with its administration schedule, 500 mg every 8 h with an extended 2 h IV infusion [20-24]. For ceftaroline, the regular schedule is 600 mg every 12 h with 1 h IV infusion, although recently, a higher posology of 600 mg every 8 h with an extended 2 h IV infusion has been approved for cSSTI due to *S. aureus* [25]. This higher dosing regimen might assure coverage of MRSA isolates displaying at least a ceftaroline MICs of 2 mg/l, but this posology is not approved for community acquired pneumonia by the EMA. For ceftobiprole, the higher breakpoints ascertain coverage is achieved without increasing the standard dose.

Apart from its affinity against altered PBP2a in methicillin-resistant staphylococci and PBPs involved in penicillin (PBP2b) and ceftriaxone (PBP2x) resistance in *S. pneumoniae*, the extended-spectrum of ceftobiprole activity is due to its ability to withstand hydrolysis by many β-lactamases, like PC1 from *S. aureus*, the narrow spectrum TEM and SHV β-lactamases from *Escherichia coli* and *Klebsiella pneumoniae*, respectively, among other *Enterobacterales*. However, as indicated above, ceftobiprole is susceptible to the hydrolysis by the extended-spectrum β-lactamases (ESBLs), all molecular types of carbapenemases (A, B and D) and overexpressed or derepressed AmpC β-lactamase types from both *Enterobacterales* and *P. aeruginosa*. In addition, overexpression of certain efflux pumps like MexXY from this latter organism also diminishes ceftobiprole activity [18, 26]. All of these resistance mechanisms equally affect ceftaroline.

In a recent surveillance study that included key target pathogens [27], ceftobiprole exhibited potent activity against *S. aureus* isolates (including MRSA isolates, which were 99.3% susceptible), coagulase-negative staphylococci (100% susceptible), *E. faecalis* (100% susceptible), and *S. pneumoniae* (99.7% susceptible). Likewise, ceftobiprole was highly active against enterobacterial isolates that did not exhibit an ESBL phenotype, including *E. coli* (99.8% susceptible) and *K. pneu-*

Table 2 | **Summary of activities of ceftobiprole against Gram-positive and Gram-negative species (Adapted from reference [20])**

Species	Antimicrobial	MIC (mg/L)			% Susceptibility*
		MIC_{50}	MIC_{90}	MIC range	
S. aureus	Ceftobiprole	0.5	2	≤0.03-4	99.7
	Ceftaroline	0.25	2	≤0.06-4	98.5
MRSA	Ceftobiprole	1	2	0.25-4	99.3
	Ceftaroline	0.5	1	0.25-4	96.4
CoNS[a]	Ceftobiprole	0.5	1	≤0.03-4	100.0
	Ceftaroline	0.25	0.5	≤0.06-2	-[c]
MRCoNS[b]	Ceftobiprole	1	1	0.12-4	100.0
	Ceftaroline	0.25	0.5	≤0.06-2	-[c]
S. pneumoniae	Ceftobiprole	0.015	0.5	0.002-1	99.7
	Ceftaroline	≤0.008	0.12	≤0.008-0.5	99.7
E. faecalis	Ceftobiprole	0.5	2	≤0.03-4	100.0
	Ceftaroline	2	8	≤0.06->8	-[c]
E. coli	Ceftobiprole	0.03	>16	0.015->16	82.5
	Ceftaroline	0.12	>32	≤0.015->32	78.5
K. pneumoniae	Ceftobiprole	0.03	>16	0.015->16	83.4
	Ceftaroline	0.12	>32	≤0.015->32	80.4
P. aeruginosa	Ceftobiprole	2	16	0.12->16	72.7
	Ceftaroline	16	>32	0.25->32	-[c]
H. influenzae	Ceftobiprole	0.06	0.12	0.015->1	92.0
	Ceftaroline	0.015	0.03	0.002-2	92.0
M. catarrhalis	Ceftobiprole	0.12	0.25	≤0.008->1	-[c]
	Ceftaroline	0.12	0.25	0.002-2	-[c]

***EUCAST criteria; [a]coagulase-negative staphylococci; [b]methicillin-resistant coagulase-negative staphylococci, [c]breakpoints have not been established**

moniae (99.6% susceptible) isolates. A total of 99.6% of all *H. influenzae* and *M. catarrhalis* isolates were inhibited by 1 mg/L of ceftobiprole, and 72.7% of the *P. aeruginosa* isolates were susceptible to ceftobiprole (table 2). In this study, susceptibility values were established using EUCAST breakpoints. The corresponding values for ceftaroline are also included in table 2. With the exception of *E. faecalis* and *P. aeruginosa*, in which ceftobiprole displayed a clearly higher intrinsic activity, the activity of both cephalosporins were within one-fold dilution of each other. Nevertheless, rates of ceftobiprole susceptible MRSA isolates were higher than for ceftaroline.

The high coverage of ceftobiprole in key pathogens, including *S. aureus*, *S. pneumoniae* and *P. aeruginosa* with relevant resistance mechanisms is shown in figure 3. Data were obtained for a large multicentric study in different European countries over a five-year period [28]. In the case of *S. aureus*, all methicillin susceptible isolates were susceptible to ceftaroline and only 1.7% of MRSA isolates were considered non-susceptible to ceftobiprole. Ceftobiprole displayed a high intrinsic activity against *S. pneumoniae*, although MIC increased with the decrease of penicillin susceptibility. Overall, only 0.15% of *S. pneumoniae* were considered resistant. For *P. aeruginosa* and using the EUCAST non-species-specific PK/PD breakpoints (susceptible ≤4 mg/L; resistant >4 mg/L), 78.4% of the ceftazidime-susceptible isolates were also susceptible to ceftobiprole but this percentage decrease to 22.7% in ceftazidime-resistant isolates. MIC distributions of all these isolates is summarised in figure 3.

ANTIMICROBIAL RESISTANCE

To date, ceftobiprole has demonstrated a low potential to select for resistance. Although staphylococci have a proven ability to develop resistance to most antibiotics in clinical use, results from *in vitro* studies indicate that the potential for MRSA to become resistant to ceftobiprole appears to be low [29]. Different studies using laboratory strains submitted either to serially growing concentrations or to continuous challenge with

Staphylococcus spp.					
	PBP1	PBP2	PBP2a	PBP3	PBP4
Ceftobiprole	✔	✔	✔	✔	✔
Ceftaroline	✔	✔	✔	✔	✘
Ceftriaxone	✔	✔	✘	✔	✘
Meropenem	✔	✔	✘	✔	✔
Piperacillin	✔	✔	✘	✔	✔

Escherichia coli							
	PBP1a	PBP1b	PBP2	PBP3	PBP4	PBP5	PBP6
Ceftobiprole	✔	Some	✔	✔	✔	✘	✔
Ceftazidime	✔	✔	Some	✔	✘	✘	✘
Cefepime	✔	Some	✔	✔	✘	✘	✘
Imipenem	✔	✔	✔	✘	✔	✔	✔
Piperacillin	✔	Some	✔	✔	✔	✘	✘
Ceftolozane	✘	✔	✘	✔	✘	✘	✘

Pseudomonas aeruginosa						
	PBP1a	PBP1b	PBP2	PBP3	PBP4	PBP5/6
Ceftobiprole	✔	✔	✔	✔	✔	✘
Ceftazidime	✔	✔	✘	✔	✔	✘
Cefepime	✔	✔	Some	✔	✔	✘
Imipenem	✔	✔	✔	✔	✔	✔
Piperacillin	✔	✔	✔	✔	✔	✘
Ceftolozane	✘	✔	✔	✔	✔	✘

Figure 2 | **Ceftobiprole binding to PBPs of different microorganisms in comparison with other beta-lactam compounds [7-12]**

✘: not biologically relevant; PBP, penicillin-binding protein

subinhibitory levels of the antibiotic, demonstrated that the most frequent changes leading to *in vitro* resistance are due to: i) Mutations in the *mecA* gene that result in amino acid changes within the transpeptidase domain of PBP2a together with changes in the non-penicillin-binding domain, ii) Non *mecA*-mediated mechanisms of resistance resulting from mutations in different PBPs, PBP4 (a non-essential, low-molecular weight PBP of *S. aureus*) being the most frequently involved. Mutations in PBP4 occurred in the structural coding gene and/or in its promoter region. It should also be noted that those modifications in *pbp4* gene and its promoter produce a highly crosslinked cell wall peptidoglycan, indicative of increased transpeptidase activity associated with greatly increased amounts of membrane PBP4 [30]. Moreover, additional mutations in other genes such as ClpX endopeptidase, PP2C protein phosphatase, transcription terminator Rho, and GdpP phosphodiesterase, have all been involved in fifth-generation cephalosporins resistance development [31].

At present, few studies describe the presence of ceftobiprole resistance among clinical isolates. In a study conducted in France with 440 *S. aureus* (MSSA and MRSA) isolates from bronchopulmonary infections, only one ceftobiprole resistant strain (MIC 4 mg/L) was detected among the MRSA (n=115) subpopulation [32]. This strain (clonal complex, CC8) was a PVL-negative MRSA strain isolated from a tracheal aspirate, presenting a mutation in PBP2a previously associated with low-level resistance to ceftobiprole and ceftaroline. The strain was resistant to both ceftobiprole and ceftaroline, but remained susceptible to vancomycin, daptomycin, and linezolid. The authors noted that the MRSA subpopulation displayed higher ceftobiprole MIC_{50} and MIC_{90} (1 mg/L), and interestingly, that the genetic background of *S. aureus* strains (*agr* group and CC) may slightly impact the strain susceptibility to ceftobiprole [32].

During a one-year surveillance study in an Italian Hospital, 12% of ceftobiprole resistance (12/102 isolates; MIC, 4 mg/L) among the MRSA population (only *mecA* producers) was found. After epidemiological characterization, isolates belonged to different clones, as well as substitutions in all PBPs and with a novel insertion in PBP2a [26]. It is worth mentioning that ceftobiprole became available at the hospital only one year before the study took place thus selective pressure for this situation can be excluded [33].

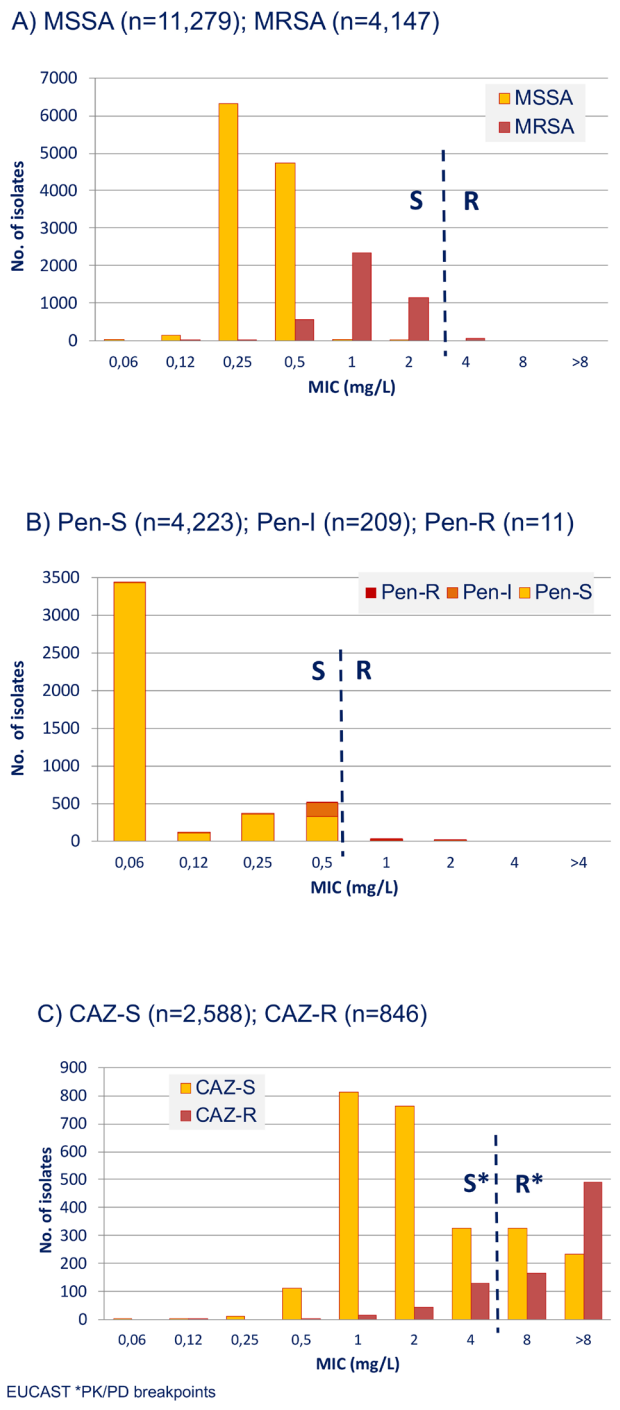

Figure 3 MIC distributions of methicillin-susceptible and resistant *S. aureus* (A), penicillin-susceptible, -intermediate and -resistant *S. pneumoniae* (B) and ceftazidime-susceptible and -resistant *P. aeruginosa* (C) isolates recovered from European surveillance studies (data obtained from reference [21])

Though the presence of resistant isolates in the clinical setting is at present scarcely observed, ceftobiprole susceptibility screening is essential to avoid therapeutic failure and the spread of resistant strains. Close microbiological monitoring of isolates should be maintained to prevent resistant strains diffusion by early detection of changes in susceptibility pattern. In a recent surveillance study monitoring ceftobiprole susceptibility performed in USA with blood isolates, only 0.3% (4 isolates over 558 tested isolates) of MRSA were non-susceptible to ceftobiprole [34].

CONCLUSIONS

Ceftobiprole is a novel parenteral extended-spectrum cephalosporin covering resistant Gram-positive and Gram-negative organisms due to its inhibition of abnormal PBP2a in MRSA and PBP2b and PBP2x in the case of β-lactam-resistant pneumococci. Moreover, it is also effective against *Enterobacterales* not producing extended-spectrum β-lactamases, AmpC overproducers or carbapenemases, and susceptible *P. aeruginosa*. This activity and results from clinical trials positions this cephalosporin for the treatment of community-acquired pneumonia and hospital-acquired pneumonia with the exception of ventilator-associated pneumonia in patients who require a broad-spectrum treatment with the highest safety due to the novel broad spectrum of coverage that has been shown as cephalosporin.

CONFLICTS OF INTERESTS

RC has participated in educational programs sponsored by Pfizer.

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