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SHORT COMMUNICATION

In vitro activity of ceftaroline against methicillin-resistant and methicillin-susceptible Staphylococcus aureus clinical isolates from a tertiary hospital in Greece

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SUMMARY

Ceftaroline is a novel cephalosporin able to bind to and inhibit PBP2a, and thus active against methicillin-resistant *Staphylococcus aureus*. In the present study we assessed the in vitro activity of ceftaroline and comparators against a large sample of methicillin-resistant and methicillin-susceptible *S. aureus* isolates collected at our hospital. Overall, both MRSA and MSSA isolates in our study were sensitive to ceftaroline, even though the MIC range was higher for MRSAs (0.12-2 mg/L against ≤0.06-0.5 mg/L for MSSAs). Our results indicate that ceftaroline may be considered a reliable alternative for the treatment of MRSA.

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Staphylococcus aureus is a commensal pathogen colonizing approximately 30% of the human skin and mucosa of healthy individuals while simultaneously able to cause mild to severe infections. The clinical manifestations of *S. aureus* infections may include relatively benign infections such as folliculitis as well as more serious ones such as erysipelas, deep-seated abscesses, osteomyelitis, pleuropulmonary, device-related infections and even bacteraemia, infective endocarditis, sepsis and toxic shock syndrome (Tong *et al*., 2015; Davis *et al.,* 1980).

Shortly after the introduction of methicillin in clinical practise, Methicillin-resistant *Staphylococcus aureus* (MRSA) was first described in 1961 and since then has spread all over the world, causing both healthcare- and community-associated infections (Lee *et al*., 2018). MRSA strains are characterized by oxacillin minimal inhibitory concentration (MIC) above 4 μg/ml. MRSA is resistant to all β-lactam agents, including penicillin and cephalosporins (Biek *et al*., 2010) with the exception of ceftaroline and ceftobiprole (Fritsche *et al*., 2008). The mechanism of methicillin resistance is mainly mediated by an altered acquired-penicillin binding protein 2A (PBP2a), which shows low affinity for β-lactams and is encoded by *mecA* gene and less frequently *mec*C gene (Lee *et al*., 2018).

According to the data reported by the European Antimicrobial Resistance Surveillance Network (EARS-Net) for 2018, notable differences in national MRSA percentages, ranging from 0% to 43%, had been observed for European countries. In Greece, the rate of MRSA for 2018 was 36.4% (ECDC 2019).

Treatment options for severe MRSA infections include vancomycin, daptomycin and linezolid, the use of which are sometimes compromised by the emergence of vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus*, vancomycin-heterogenous *S. aureus* (hVISA), and daptomycin-nonsusceptible *S. aureus* (Howden *et al*., 2010; Courvalin *et al*., 2006; Sharma *et al*., 2008).

Several new agents have been approved for the treatment of serious MRSA infections, such as ceftaroline fosfamile which, along with ceftopripole, are the first β-lactam agents with anti-MRSA activity. Ceftaroline fosamil is a 5th generation cephalosporin that inhibits cell wall synthesis of bacteria by binding to one or more penicillin-binding proteins (PBPs) and thus inducing cell death. It is active against Gram-negative and Gram-positive bacteria (Flamm *et al*., 2012), including MRSA, since it has the ability to bind with high affinity to the PBP2a in MRSAs and the PBP2x in *Streptococcus pneumoniae,* properties which are unique amongst β-lactam antibiotics (Kosowska-Shick *et al*., 2010). The European Medicines Agency (EMA) has approved its use for complicated skin

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and soft tissue infections and community-acquired pneumonia for both adults and children (CHMP 2019).

The aim of our study was to assess the in vitro activity of ceftaroline and comparators against a large number of *S. aureus* isolates collected at our hospital.

A total of 476 *S. aureus* isolates were included in the study. All isolates were recovered from patients hospitalized at AHEPA University Hospital of Thessaloniki in Greece from 5/2018 to 5/2020. The isolates included 430 clinical samples (116 blood, 50 respiratory, 77 pus, 21 central line catheters, 87 trauma and 79 other samples) and 46 nasal surveillance samples. All isolates were tested in vitro against ceftaroline, ciprofloxacin, clindamycin, daptomycin, erythromycin, gentamicin, levofloxacin, linezolid, moxifloxacin, quinopristin/dalfopristin, rifampicin, teicoplanin, tigecycline, trimethoprim/sulfamethoxazole, and vancomycin. Species identification and antimicrobial susceptibility testing were performed by the Vitek 2 automated system (bioMérieux, France). Isolates were defined as susceptible, intermediate or resistant according to the clinical breakpoints recommended by EUCAST version 8.0. MIC_{50} and MIC_{90} were calculated for all antimicrobials tested with descriptive statistics using IBM SPSS 21. Due to lack of clinical data, a strain was considered resistant to ceftaroline when the MIC was > 2 mg/L, as suggested by EUCAST for indications other than pneumonia.

During the study period, 476 *S. aureus* isolates were evaluated. Among them, 227 were methicillin-resistant (47.69%) and 249 methicillin-susceptible *S. aureus* (MSSA) (52.31%). Results are presented in detail in *Table 1*. For MSSA isolates, the MIC50, MIC90, and MIC ranges of ceftaroline were 0.25, 0.25, and 0.12-2 mg/L, respectively. For MRSA isolates, the MIC50, MIC90, and MIC ranges of ceftaroline were 0.5, 1, and ≤0.06-0.5 mg/L, respectively. According to EUCAST, MIC interpretations all MRSA and MSSA tested isolates were found susceptible to ceftaroline. Although MIC ceftaroline values were 2- to 4-fold higher among methicillin-resistant than among methicillin-susceptible staphylococci, the in vitro activity of ceftaroline was greater than that of linezolid and vancomycin, and almost the same as daptomy-

Table 1 - *MIC range, MIC_{50}, MIC_{90} and susceptibility rates of the study isolates. quin/dalf = quinupristin/dalfopristin, TMP-SMX = trimethoprim/sulfamethoxazole*

Organism	*Antimicrobial Compound*	*Number of strains tested*	*MIC range (mg/L)*	*MIC50 (mg/L)*	*MIC90 (mg/L)*	*Susceptibility (%)*
MRSA	ceftaroline	143	0.12-2	0.5	1	100
	ciprofloxacin	84	0.5-8	8	8	0
	clindamycin	227	0.12-8	4	8	27
	daptomycin	226	0.25-2	0.25	1	98
	erythromycin	139	0.25-8	8	8	19
	gentamicin	227	0.5-16	0.5	0.5	93
	levofloxacin	143	0.12-8	8	8	0
	linezolid	227	1-8	2	2	99
	moxifloxacin	84	0.25-8	4	4	25
	quin/dalf	84	0.25-1	0.25	0.5	100
	rifampicin	143	≤0.06-4	≤0.06	≤0.06	93
	teicoplanin	226	0.5-8	0.5	0.5	97
	tigecycline	227	0.12-1	0.12	0.5	94
	TMP-SMX	227	0.5-16	0.5	0.5	93
	vancomycin	226	0.5-2	1	1	100
MSSA	ceftaroline	170	≤0.06-0.5	0.25	0.25	100
	clindamycin	249	0.12-8	0.25	0.25	91
	ciprofloxacin	79	0.5-8	0.5	0.5	0
	daptomycin	249	0.12-4	0.25	1	99
	erythromycin	149	0.25-8	1	8	76
	gentamicin	249	0.5-16	0.5	0.5	98
	levofloxacin	170	0.12-8	0.25	1	0
	linezolid	249	1-4	2	2	100
	moxifloxacin	79	0.25-4	0.25	0.25	92
	quin/dalf	79	0.25-0.5	0.25	0.25	100
	rifampicin	170	≤0.06-0.5	≤0.06	≤0.06	99
	teicoplanin	249	0.5-4	0.5	0.5	99
	tigecycline	249	0.12-0.5	0.12	0.12	100
	TMP-SMX	249	0.5-16	0.5	0.5	98
	vancomycin	249	0.5-1	1	1	100

cin. Significantly, three MRSA isolates exhibited ceftaroline MICs of 2 mg/L (interpreted as susceptible, dose increase).

Ciprofloxacin, levofloxacin, moxifloxacin, clindamycin, and erythromycin exhibited the lowest activity against MRSA, with susceptibility rates of 0%, 0%, 25%, 27%, and 19%, respectively. All the antimicrobials in our study were highly active *in vitro* against MSSA isolates, with susceptibility rates ranging from 76% to 100% apart from ciprofloxacin and levofloxacin, none of which was found susceptible at standard dose, according to the latest clinical breakpoints and definitions set by EUCAST.

We have shown that ceftaroline exhibited excellent *in vitro* activity against MRSA and MSSA isolates among patients admitted to our hospital. However, although all isolates were susceptible, MRSA MICs were 2- to 4-fold higher than those of MSSA. Our findings are in agreement with the SENTRY and ATLAS studies, in which MICs also appear to be higher amongst MRSA compared to MSSA isolates, while the susceptibility rates of MRSAs to ceftaroline where 91.6% and 89.3%, respectively (Diekema *et al*., 2019; Zhang *et al*., 2019).

As expected, MSSA isolates exhibited high rates of in vitro susceptibility to all antimicrobial agents tested. On the other hand, the majority of the MRSA isolates were resistant to clindamycin, erythromycin, and quinolones, as indicated in previous former studies (Diekema *et al*., 2019; Zhang *et al*., 2019), but the susceptibility rates to the other antibiotics tested varied between 93 and 100%.

Therapeutic options for treating *S. aureus* infections, especially methicillin-resistant ones, are limited. This is because MRSA strains confer resistance not only to β-lactam antibiotics but also to other classes of antibiotics, such as aminoglycosides, tetracyclines, or fluoroquinolones, restricting the available antibacterial agents (Pantosti *et al*., 2007). Infectious Diseases Society of America (IDSA) guidelines recommend clindamycin, trimethoprim-sulfamethoxazole, tetracycline (doxycycline or minocycline), and linezolid for the treatment of skin and soft tissue infections in the community, and vancomycin, linezolid, daptomycin, telavancin, and clindamycin for hospitalized patients. Vancomycin is the first-line antibiotic for bacteremia and infective endocarditis, followed by daptomycin if the former cannot be administered. Additionally, IDSA recommends vancomycin, linezolid, or clindamycin for MRSA pneumonia (Liu *et al*., 2011). Nevertheless, the clinical efficacy of vancomycin against staphylococci is often problematic due to elevated MIC values that may approach the highest susceptible range, heteroresistance and occurrence of vancomycin-intermediate or, rarely, vancomycin-resistant staphylococci (Holmes *et al*., 2012). Linezolid, a synthetic oxazolidinone which is often used in clinical practice, with possible better clinical outcome compared to vancomycin against suspected MRSA-associated complicated skin and soft tissue infections (cSSTIs) is sometimes associated with adverse effects, including neuropathy, thrombocytopenia, or anemia and hyperlactatemia (Vinh & Rubinstein 2009). Daptomycin, a cyclic lipopeptide approved for use in cSSTIs, has shown adequate clinical activity against MRSA; nevertheless, daptomycin nonsusceptible *S. aureus* isolates have been reported (Friedman *et al*., 2006). Interestingly, ceftaroline has been shown by others to have superior bactericidal activity against vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA), heteroresistant VISA (hVISA), and daptomycin-nonsusceptible *S. aureus* (DNSSA) isolates (Saravolatz *et al*., 2010).

In our study, ceftaroline exhibited excellent in vitro activity against MRSA isolates (100%) higher than that exhibited by clindamycin (27%), daptomycin (98%), linezolid (99%), and trimethoprim-sulfamethoxazole (93%). Due to lack of sufficient data, we did not include in our study some of the recommended antibiotics against MRSA infections, as well as clinical or epidemiological information that would allow us to differentiate our isolates between community-acquired and hospital-acquired MRSA. Another limitation was the fact that we used only conventional microbiological methods, instead of molecular ones, for the identification of *S. aureus* isolates. The same applies to the characterization of the isolates as methicillin resistant, which was based on cefoxitin resistance. Considering our overall results and, especially, the very low susceptibility rates of clindamycin, one of the first-line antibiotics, ceftaroline seems to be a very promising alternative against MRSA infections.

Conflict of interest

The authors state that they have no conflicts of interest to declare. Part of the study was presented at the 2020 ECCMID Abstract book.

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