International Journal of Antimicrobial Agents 35 (2010) 527–530

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

In vitro evaluation of ceftaroline alone and in combination with tobramycin against hospital-acquired meticillin-resistant *Staphylococcus aureus* (HA-MRSA) isolates

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ARTICLE INFO

Article history:
Received 4 January 2010
Accepted 1 February 2010

Keywords:
Ceftaroline
Aminoglycoside synergy
HA-MRSA

ABSTRACT

The aim of this study was to evaluate the in vitro activity of ceftaroline and its potential for synergy with tobramycin in comparison with vancomycin against a collection of hospital-acquired meticillin-resistant *Staphylococcus aureus* (HA-MRSA), including isolates with reduced susceptibility to glycopeptides. Ceftaroline, vancomycin, daptomycin and linezolid susceptibilities were determined for 200 HA-MRSA isolates. Four randomly selected strains [including one vancomycin-intermediate *S. aureus* (VISA) and one heteroresistant VISA (hVISA)] were evaluated in time–kill experiments with ceftaroline and vancomycin alone or combined with tobramycin at 0.25 and 0.5 times the minimum inhibitory concentration (MIC). MICs for 50% and 90% of the organisms (MIC_{50} and MIC_{90}, respectively) were both 1 mg/L for ceftaroline and were 1 mg/L and 2 mg/L, respectively, for vancomycin. The same ceftaroline MIC ranges (0.25–2 mg/L) were observed for isolates recovered from respiratory tract samples, blood or skin. In time–kill experiments, no synergy was observed at 0.25× MIC against any tested isolates with either ceftaroline or vancomycin. In contrast, the combination of ceftaroline plus tobramycin at 0.5× MIC was synergistic against the two MRSA strains and the hVISA but was indifferent against the VISA isolate. In conclusion, ceftaroline demonstrated antimicrobial activity independently of the specimen source and exhibited lower MICs than vancomycin. Finally, at sub-MIC levels, ceftaroline plus tobramycin displayed significantly greater activity than vancomycin plus tobramycin against MRSA ($P < 0.01$).

1. Introduction

In the last several years, the prevalence of meticillin-resistant *Staphylococcus aureus* (MRSA) has dramatically increased, making the prevention and treatment of MRSA infections a worldwide concern [1]. Vancomycin has remained a primary empirical therapy as the emergence of resistance has been slow to appear in *S. aureus* isolates. However, its current utility has been markedly compromised by the increased dissemination of vancomycin-intermediate *S. aureus* (VISA) and heteroresistant VISA (hVISA) organisms [2]. Ceftaroline is a member of a new generation of cephalosporins exhibiting higher affinity for the penicillin-binding protein 2a of MRSA, including hVISA, VISA and vancomycin-resistant *S. aureus* [3]. Limited data are available regarding the combination of β-lactams with aminoglycosides against *S. aureus* isolates [4]. However, such antimicrobial association might have potential for synergy against selective pathogens, expanding the coverage in empirical therapy and reducing the potential for the emergence of resistance [4]. Therefore, the objective of the present study was to evaluate the in vitro activity of ceftaroline and its potential for synergy in combination with tobramycin against a collection of hospital-acquired MRSA (HA-MRSA) isolates recovered from various clinical samples and exhibiting different levels of susceptibility to vancomycin.

2. Materials and methods

2.1. Bacterial strains

In total, 200 clinical HA-MRSA isolates, including 3 VISA and 26 hVISA previously characterised by population analysis profile and Macro Etest, were selected from clinical isolates collected at the Detroit Medical Center, Detroit Receiving Hospital (Detroit, MI), from different patients between 1996 and 2009 and forming

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0924-8579/$ – see front matter © 2010 Published by Elsevier B.V. and the International Society of Chemotherapy.
doi:10.1016/j.ijantimicag.2010.02.006

part of the Anti-Infective Research Laboratory (Detroit, MI) collection.

2.2. Susceptibility testing

Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined in duplicate by broth microdilution according to the Clinical and Laboratory Standards Institute guidelines [5] for ceftaroline (lot number C1 170-7; provided by Forest Laboratories, Inc., New York, NY) as well as linezolid, daptomycin, vancomycin and tobramycin, which were commercially purchased.

2.3. In vitro time–kill curves

Time–kill experiments were performed in triplicate as previously described against four randomly selected isolates, including one hVISA (R1629), one VISA (R2303) and two glycopeptide-susceptible MRSA (R3804 and R4039) [6]. Antimicrobial regimens included ceftaroline, vancomycin and tobramycin alone, and combinations of tobramycin with ceftaroline or vancomycin at sub-MIC levels to ensure appropriate conditions for detecting synergy. Synergy, indifference and antagonism were defined as previously described [7].

2.4. Statistical analysis

Differences between regimens were analysed by *t*-test or analysis of variance (ANOVA) with Tukey's post-hoc test. A *P*-value of <0.05 was significant.

3. Results

MIC and MBC values are given in Table 1. The ceftaroline MIC distribution was narrow, with only 4.5% of the strains displaying a MIC of ≤0.25 mg/L and 1.5% with a MIC of 2 mg/L. MIC_{50} and MIC_{90} values were 1 mg/L and MBC values were equal to or one dilution higher than MICs, ranging from 0.125 mg/L to 2 mg/L. Vancomycin and linezolid MIC values ranged from 0.25 mg/L to 4 mg/L. MIC_{50} and MIC_{90} values were 1 mg/L and 2 mg/L, respectively, for vancomycin and one dilution higher for linezolid. Daptomycin MIC values ranged from 0.125 mg/L to 1 mg/L, with a MIC_{50} at 0.25 mg/L and a MIC_{90} at 0.5 mg/L. Except for linezolid, all MBC values were similar to the MICs (Table 1). Finally, 9% of the collection (18 isolates) were susceptible to tobramycin with a MIC ≤4 mg/L (Table 1).

Among the 200 isolates tested, 36% were recovered from respiratory tract samples, 17% from blood, 13.5% from skin and 2% from urine. The specimen sources for the remaining 31.5% were unknown due to a lack of clinical information. No difference in MIC values was found with regard to specimen site, except for isolates obtained from urine, which exhibited a lower ceftaroline MIC_{50} value (0.5 mg/L). However, the number of isolates from urine ($n = 4$) was not sufficient to impart statistical significance.

In time–kill experiments, none of the agents, tested alone at 0.25× MIC, affected the growth of any of the four isolates, and neither synergy nor antagonism was observed with any of the combinations (data not shown). At 0.5× MIC, neither vancomycin, ceftaroline or tobramycin alone were bactericidal against any of the tested isolates (Fig. 1). A maximum average of 1.5 $\log_{10}$ kill was observed for ceftaroline at 4 h against the two vancomycin-susceptible MRSA isolates R3804 and R4309 (Fig. 1C,D) and 3.8 $\log_{10}$ kill at 8 h for tobramycin against the hVISA R1629 (Fig. 1A). In combination at 0.5× MIC, ceftaroline plus tobramycin was synergistic against the two vancomycin-susceptible MRSA isolates and the hVISA R1629, with bactericidal

Table 1
Distribution of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of 200 hospital-acquired meticillin-resistant *Staphylococcus aureus* (HA-MRSA) isolates, and four HA-MRSA isolates [including one vancomycin-intermediate *S. aureus* (VISA) and one heteroresistant VISA (hVISA)] selected for study in time–kill experiments.

Antimicrobial agent	% of isolates														Tested isolates				
	MIC (mg/L)							MBC (mg/L)							MIC/MBC (mg/L)				
	0.06	0.125	0.25	0.5	1	2	≥4	Range	0.125	0.25	0.5	1	2	≥4	Range	R2303 (VISA)	R1629 (hVISA)	R3804	R4039
Ceftaroline	0.5	–	4	35.5	**58.5**[a]	1.5	–	0.06 to 2	0.5	0.5	21.5	61.5	**16**[a]	–	0.125 to 2	0.25/0.25	0.125/0.25	0.25/0.25	0.5/0.5
Vancomycin	–	–	1.5	12.5	61.5	**23**[a]	1.5	0.25 to 4	–	–	6	52.5	**35.5**[a]	6	0.5 to 8	4/8	2/2	0.5/1	1/1
Daptomycin	–	18.5	58.5	**21**[a]	2	–	–	0.125 to 1	9	47.5	**35.5**[a]	6	2	–	0.125 to 2	0.5/0.5	0.5/0.5	0.25/0.25	0.125/0.25
Linezolid	–	–	7.5	13	18	48.5	**13**[a]	0.25 to 4	–	–	1.5	10	11.5	**77**[a]	0.5 to 64	4/32	2/4	1/2	1/32
Tobramycin	1	–	0.5	2.5	4.5	0.5	**91**[a]	0.06 to >256	0.5	0.5	–	2	6	**91**[a]	0.125 to >256	0.125/0.5	1/1	0.5/1	1/1

[a] Concentrations represent MIC_{90} and MBC_{90} values (MIC and MBC for 90% of the organisms, respectively).