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Correlation between Broth Microdilution and Disk Diffusion Methods Results when Testing Ceftaroline against Methicillin-Resistant *Staphylococcus aureus* Using the 5-µg Ceftaroline Disk

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Introduction

- Discrepancy rates between MIC and disk zones vary according to the percentage of isolates with MIC values within +/-1 doubling dilution of the breakpoints
- Although the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates that are ceftaroline-nonsusceptible (MIC, ≥2 mg/L) is generally low, it varies substantially by geographic region
- SCC*mec* plays an import role in the antimicrobial resistance characteristics, molecular epidemiology, and evolution of MRSA, and ceftaroline potency (MIC value) against MRSA varies according to the SCC*mec* type
- Data from the SENTRY Program for isolates consecutively collected worldwide in 2016 and 2017 shows ceftaroline susceptibility rates against MRSA varying from 95.1% in the United States to 88.7% in Europe, 77.8% in the Asia-Pacific region, and 74.7% in Latin America
- We evaluated the disk-MIC correlation when testing ceftaroline against a challenge collection of MRSA

Materials and Methods

- We evaluated 158 MRSA isolates, including 106 randomly selected isolates and 52 isolates with decreased susceptibility to ceftaroline (MIC, 1–16 mg/L)
- Isolates were tested by CLSI broth microdilution (BMD) method, and disk diffusion (DD) was performed with 5-µg disks and Mueller-Hinton agar from 2 manufacturers each; thus, there were 4 DD results for each MIC result
- EUCAST breakpoints for MIC and DD were applied
- Optimal DD breakpoints were determined by the error-rate bounded method
- Selected isolates (n=51) were characterized by whole genome sequencing, including all 25 isolates with ceftaroline MIC results ≥4 mg/L, 15 of 35 isolates with ceftaroline MIC results of 2 mg/L, and 11 of 54 isolates with a ceftaroline MIC of 1 mg/L

Results

- Ceftaroline 5-µg disk inhibition zones vs MIC values for all MRSA isolates are shown in Figure 1 in a bar graph and in Figures 2 and 3 in scattergrams
- The DD breakpoints that provided the lowest error rates were ≥17/<14 mm (susceptible/resistant; Figure 2)
  - Very major (VM; false susceptible) errors: 0.0% for ≥I+2 and 0.7% for I±1, and an overall VM error rate of 0.5%
  - Major (false resistance) error rates: 0.0%
  - Minor error rates: 0.0% for ≥I+2, 25.9% for I±1, and 0.0% for ≤I-2; 17.7% overall
- Error rates for the 2018 EUCAST DD breakpoints of ≥20/<17 mm (susceptible/resistant; Figure 2) were
  - VM error rates: 0.0%
  - Major error rates: 0.0% for ≤I-2, and 3.9% for I±1; 2.7% overall
  - Minor error rates: 0.0% for ≥I+2, 50.5% for I±1, and 19.9% for ≤I-2; 40.0% overall
- Error rates for the 2018 EUCAST DD breakpoints for pneumonia (≥20/<20 mm for susceptible/resistant) and optimal breakpoints (≥18/<18 mm for susceptible/resistant) are shown in Figure 3
- Most isolates (26/39; 66.7%) exhibiting ceftaroline MIC values of ≥2 mg/L were recovered from countries in the Asia-Pacific region (Japan, South Korea, Taiwan, and Thailand; Table 1)

- The majority of isolates selected for molecular characterization belonged to clonal complex (CC) 5 (43/51; 84.3%), and the majority of CC5 isolates (39/43; 90.7%) showed ceftaroline MIC values of ≥2 mg/L (Tables 1 and 2)
- The most common SCC*mec* type observed among the 51 characterized isolates was type II (n= 34; 66.7%), followed by types I (n=9; 17.6%), IV (n=7; 13.7%), and III (n=1; 2.0%; Table 2)
- Ceftaroline-nonsusceptible (MIC, ≥2 mg/L) isolates were mainly SCC*mec* type II (30 of 40; 75.0%) and I (9 of 40; 22.5%); whereas ceftaroline-susceptible isolates were mainly SCC*mec* type IV (6 of 11; 54.5%) and II (4 of 11 tested; 36.4%; Table 2)
- Among isolates having a wild-type sequence for PBP2a, the ceftaroline MIC results varied between 1 mg/L and 4 mg/L with a modal MIC value at 1 mg/L (Table 3)
- Among isolates showing a wild-type sequence for PBP2a, only 35.7% of results were categorized as susceptible by disk diffusion, whereas 37.5% and 26.7% of results were categorized as intermediate and resistant, respectively (Table 3)
- Among isolates having PBP2a alterations in the allosteric site, 77.5% and 22.5% of disk diffusion results were categorized as resistant and intermediate, respectively, and there was no result in the susceptible range among these organisms (Table 3)
- The vast majority of disk diffusion results (95.5%) for the isolates carrying PBP2a alterations at both allosteric/transpeptidase sites were categorized as resistant, whereas 70.8%, 20.8%, and 8.3% of disk diffusion results for the isolates carrying PBP2a alterations at transpeptidase site were categorized as resistant, intermediate, and susceptible, respectively (Table 3)

Table 1 Distribution of methicillin-resistant *S. aureus* (MRSA) lineages and country of origin according to the ceftaroline MIC value

Clonal complex[a] Countries	No. of isolates at each ceftaroline MIC (mg/L): 1	2	4	8	16
CC5					
Argentina		1			
Chile		2			
Hungary	1				
Italy		2			
Japan	1	1	4		
South Korea		3	10	4	1
Mexico		1			
Peru		2	2		
Slovenia	1				
Spain	1				
Taiwan		1			
Thailand			1	1	
United States		1	2		
CC8					
Russia	2				
CC22					
Australia	1				
Ireland	1				
Italy		1			
New Zealand	1				
CC45					
Belgium	1				
CC239					
Australia	1				
Total	**11**	**15**	**19**	**5**	**1**

[a] Clonal complex (CC) 5 represented by ST5 (29 isolates), ST105 (2), ST125 (1), ST228 (3), ST518 (1), ST764 (5), ST1110 (1) and ST2883 (1); CC8, ST8; CC22, ST22; CC45, ST45; and CC239, ST239.

Table 2 Correlation of ceftaroline MIC values, clonal complex, and SCC*mec* type among 51 methicillin-resistant *S. aureus* (MRSA) strains submitted to whole genome sequencing

Clonal complex (CC) / SCC*mec* type	No. of isolates at ceftaroline MIC of: 1	2	4	8	16	Total
CC5	**4**	**14**	**19**	**5**	**1**	**43**
I		5	3	1		9
II	3	9	16	4	1	33
IV	1					1
CC8	**2**					**2**
IV	2					2
CC22	**3**	**1**				**4**
II	1					1
IV	2	1				3
CC45	**1**					**1**
IV	1					1
CC239	**1**					**1**
III	1					1
All clonal complexes	**11**	**15**	**19**	**5**	**1**	**51**
I		5	3	1		9
II	4	9	16	4	1	34
III	1					1
IV	6	1				7

Figure 1 Ceftaroline 5-µg disk inhibition zones vs MIC values for all methicillin-resistant *S. aureus* isolates

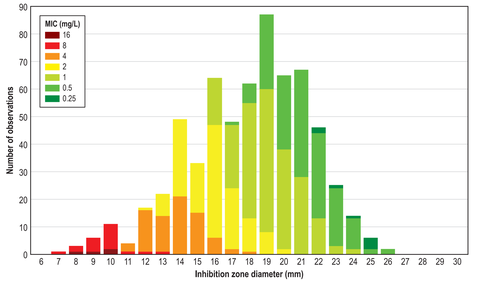
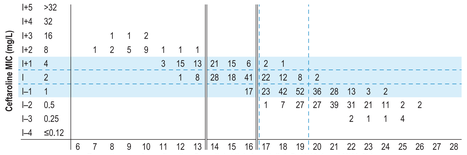


Figure 2 Scattergram of disk inhibition zones vs MIC values and table of error rates of ceftaroline MIC vs ceftaroline 5-µg disk for all MRSA isolates when current EUCAST breakpoints (broken lines; S at ≥20 mm and R at <17 mm for disk; non-pneumonia indications) and optimal disk breakpoints (double vertical lines; S at ≥17 mm and R at <14 mm) were applied



EUCAST, European Committee on Antimicrobial Susceptibility Testing; MRSA, methicillin-resistant *S. aureus*

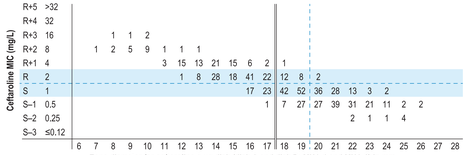
Error rates for current EUCAST breakpoints of ≥20 mm (S) and <17 mm (R)

MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥I+2	24	0	N/A	0
I+1 to I–1	432	0	17 (3.94)	218 (50.46)
≤I–1	176	N/A	0	35 (19.89)
Total	632	0	17 (2.69)	253 (40.03)

Error rates for the optimal calculated disk breakpoints of ≥17 mm (S) and <14 mm (R)

MIC range	Number	Very major (%)	Major (%)	Minor (%)
≥I+2	24	0	N/A	0
I+1 to I–1	432	3 (0.69)	0	112 (25.93)
≤I–1	176	N/A	0	0
Total	632	3 (0.47)	0	112 (17.72)

Figure 3 Scattergram of disk inhibition zones vs MIC values and table of error rates of ceftaroline MIC vs ceftaroline 5-µg disk for all MRSA isolates when current EUCAST breakpoints (broken lines; S at ≥20 mm and R at <20 mm for disk; pneumonia) and optimal disk breakpoints (double vertical lines; S at ≥18 mm and R at <18 mm) were applied



EUCAST, European Committee on Antimicrobial Susceptibility Testing; MRSA, methicillin-resistant *S. aureus*

Error rates for current EUCAST breakpoints (pneumonia) of ≥20 mm (S) and <20 mm (R)

MIC range	Number	Very major (%)	Major (%)
≥R+1	100	0	N/A
S+R	356	2 (0.56)	134 (37.64)
≤S–1	176	N/A	35 (19.89)
Total	632	2 (0.32)	169 (26.74)

Error rates for the optimal breakpoints (no intermediate) of ≥18 mm (S) and <18 mm (R)

MIC range	Number	Very major (%)	Major (%)
≥R+1	100	1 (1.0)	N/A
S+R	356	22 (6.18)	40 (11.24)
≤S–1	176	N/A	1 (0.57)
Total	632	23 (3.64)	41 (6.49)

Table 3 Correlation of ceftaroline MIC, disk zone results (5-µg) and with PBP2a alterations detected among isolates selected for further molecular characterization

PBP2a status / Ceftaroline MIC (mg/L)	Disk zone diameters (mm) 7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	%S	%I	%R
Wild type																					
1										3	2	5	3	7	5	2	3	2			
2							1	3	1	3	5	1	5	1					35.7	37.5	26.7
4						1	1	1	1												
Allosteric site[a]																					
1										4		3	1								
2							3	5	3	12	2	2	1						0.0	22.5	77.5
4								2	1	1											
Allosteric and transpeptidase sites[a,b]																					
2						1	2	2	3	1	1		1	1							
4					3	13	7	14	5	2									1.5	3.0	95.5
8	1	2	5	9	1	1	1														
16		1	1	2																	
Transpeptidase sites[b]																					
1													2		2						
4							4	4	6	3	2	1							8.3	20.8	70.8

This table depicts 4 zone diameter results for each MIC value (2 manufacturers for ceftaroline disks and 2 manufacturers for Mueller-Hinton agar). Ceftaroline breakpoints of ≤1/≥4 mg/L (susceptible/resistant) for MIC and ≥24 mm (light blue cells; susceptible)/≤20 mm (dark blue cells; resistant) for disk diffusion were applied as established by EUCAST.

[a]Allosteric site represented by amino acid residues 27–326
[b]Transpeptidase domain represented by residues 327–668.

Conclusions

- Elevated discrepancy rates were observed between DD and BMD, with a clear tendency of isolates that were intermediate by BMD being categorized as resistant by DD when current EUCAST breakpoints were applied
- DD breakpoints (non-pneumonia) should be moved 3 mm downward to provide the lowest intermethod error rates
- Isolates having a wild-type PBP2a exhibited ceftaroline MIC results (1–4 mg/L) overlapped with those carrying single (1–4 mg/L) or multiple mutations (2–16 mg/L)
- DD was not able to satisfactorily separate the group of MRSA isolates with no alterations from those with alterations or between those having alteration(s) at different regions of PBP2a

Acknowledgements

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