

A Multicenter Ceftaroline 5 µg Disk Study of *Staphylococcus aureus*

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INTRODUCTION: Ceftaroline, the active metabolite of the pro-drug ceftaroline fosamil, has in vitro activity against *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA). Susceptibility testing by disk diffusion (DD) is commonly used for routine testing in European laboratories and clinical breakpoints have been set by EUCAST for DD testing of 5 µg ceftaroline disk. (MIC, disk breakpoints: ≤1 mg/L, ≥20 mm susceptible and >1 mg/L, <20 mm resistant). As with other methods, numerous variables can impact the reproducibility of results and in particular, interpretive results can vary when testing isolates with zone diameter results near the susceptible breakpoint. The objective of this study was to compare the performance of the DD test against challenge (selected to include 49% with MICs of 1 and 2 mg/L) and recent clinical *S. aureus* isolates using multiple media across 5 sites.

METHODS: The challenge isolates consisted of 41 *S. aureus* (31 MRSA, 10 MSSA) reporting reference ceftaroline MICs of 0.12-4 mg/L (11 inhibited by 1 mg/L, 9 by 2 mg/L and 1 by 4 mg/L of ceftaroline and *S. aureus* ATCC 29213). One site tested each isolate by ISO/CLSI broth micro-dilution (BMD) method to confirm reference MIC values. In addition to the challenge isolates, 30 *S. aureus* (prospectively collected consecutive isolates from each of 3 European sites which included 12, 12 and 3 MRSA from Germany, Spain and Austria, respectively) and a retro-spective collection of 30 *S. aureus* with high prevalence of MRSA (28/30) from Italy were tested once by EUCAST disk diffusion with a 5 µg ceftaroline disk (Mast, Bootle UK) on 2 lots of Mueller Hinton agar (MHA; sources shown in Figure 2) at 5 laboratories (Austria, Germany, Italy, Spain and USA).

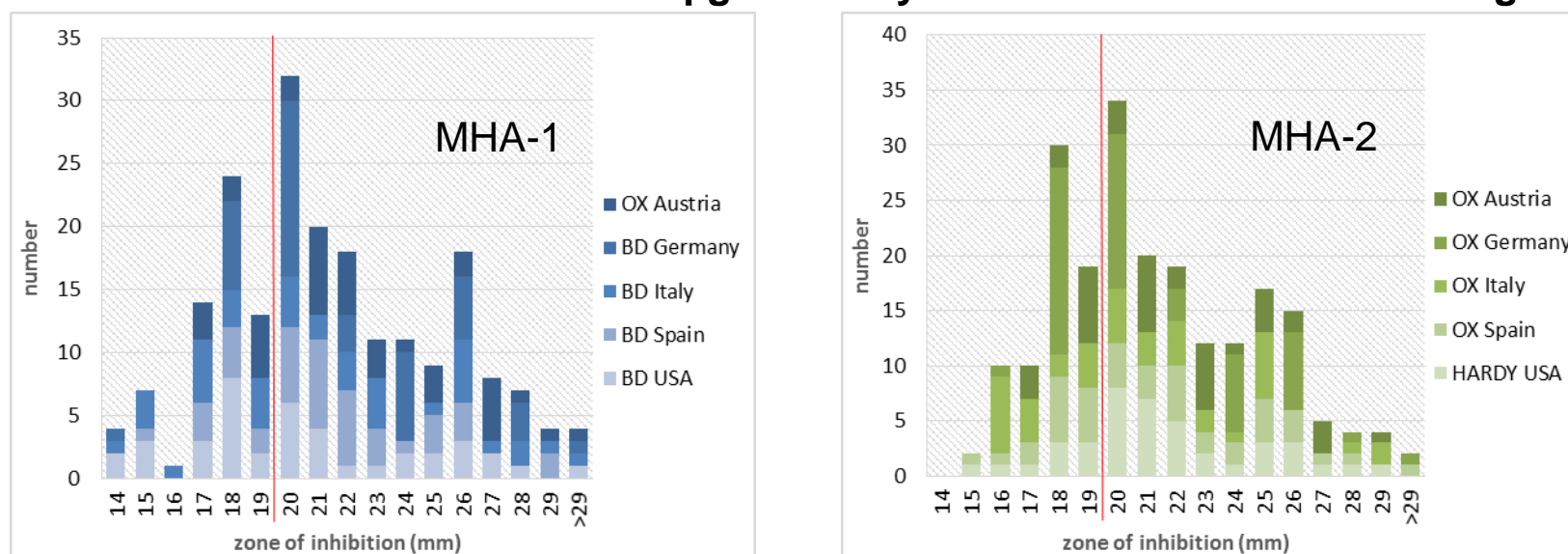
Disclosures: This study was supported by a grant from AstraZeneca; Laboratory Specialists, Inc., Westlake, also received a fee for service in relation to preparation of the abstract and poster. Jane Ambler is a former employee of AstraZeneca.

References: International Standards Organisation, ISO 20776-1 (2006) Clinical and Laboratory Standards Institute (2012). M07-A9 EUCAST: http://www.euca.org/ast_of_bacteria/disk_diffusion_methodology

RESULTS 1:

CHALLENGE SET: Ceftaroline zones for all sites and media were within a 3-6 mm range for 92.7% of results (Figure 1) and zone distributions were similar (Figure 2). Considering BMD MIC results as a reference, the category agreement (CA) rate of all site consolidated disk results was 84.9% and major error (ME) and very major error (VME) rates were 10.5% and 4.6%, respectively. CA rates by site ranged from 80.5-90.2% (Figure 3). The least number of total categorical errors occurred at the Spain and USA sites on MHA 2 (4.9% ME, 2.4% VME and 2.4% ME and 4.9% VME, respectively) and the highest number at the Italy site on MHA 2 (19.5% ME, 2.4% VME). The highest VME rates were from the Germany site on both media (9.8%) and from the Austria site on both media (7.3%). Highest ME rates were from the USA site on MHA 1 (19.5%) and from the Italy site on MHA 1 and MHA 2 (17.1% and 19.5%, respectively). For one isolate with a BMD MIC of 4 mg/L, the range of ceftaroline zones was 14-18 mm (resistant)

Figure 2: Distribution of ceftaroline 5 µg zones by media and site for 41 challenge isolates



OX: Oxoid (Basingstoke, UK), BD: Becton Dickinson (sourced locally), HARDY: Hardy Diagnostics (Santa Maria, CA); red line is EUCAST susceptible breakpoint

Table 1: Summary of ceftaroline 5 µg results for 30 site specific *S. aureus*

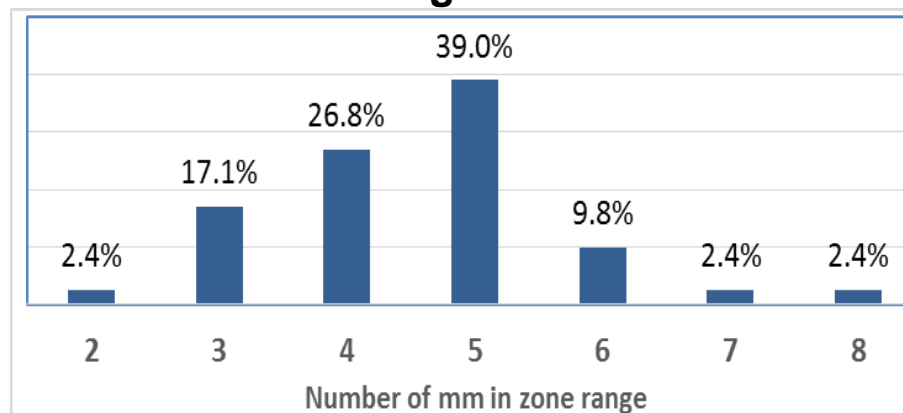
	Germany MHA-1	Germany MHA-2	Austria MHA-1	Austria MHA-2	Italy MHA-1	Italy MHA-2	Spain MHA-1	Spain MHA-2
Mean zone (mm)	24.2	24.2	27.5	27.7	20.8	20.1	24.6	24.8
Minimum zone (mm)	20	20	20	20	14	14	20	20
Maximum zone (mm)	30	30	31	32	28	26	32	30
No. Susceptible	30	30	30	30	20	19	30	30
No. Resistant	0	0	0	0	10	11	0	0
% Susceptible	100.0%	100.0%	100.0%	100.0%	66.7%	63.3%	100%	100%
% Resistant	0.0%	0.0%	0.0%	0.0%	33.3%	36.7%	0%	0%

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RESULTS 2:

SITE SPECIFIC: All site specific isolates from 3 European sites (Germany, Italy, Spain) were considered susceptible and 66.7% (MHA 1) and 63.3% (MHA 2) of Italy isolates were susceptible. (Table 1)
QC, ATCC 29213: Ceftaroline 5 µg disk results were within the EUCAST expected range of 24-30 mm for all site results with exception of one result of 23 mm from Spain on MHA-1. With the exception of this one result, zones of inhibition were in the range of 25-28 mm and mean zone was 27 mm.

Figure 1: Percentage of isolates that fall within mm zone range



CONCLUSIONS:

Although zone inhibition diameters were similar between sites and media, category agreement rates in comparison to BMD were relatively low for the set of challenge isolates. All category discrepancies were attributed to isolates with ceftaroline MICs of 1-2 mg/L, with exception of one isolate with a BMD MIC of 0.5 mg/L.

Figure 3: Comparison of ceftaroline 5 µg disk to BMD MIC for 41 challenge isolates by site (number of results at each disk/MIC; disk results x 2 lots of MHA)

Austria		Disk Diffusion Zone of Inhibition (mm)																					
BMD MIC		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	>29	CA	ME	VME		
4						2													80.5%				
2				4	4	3		2	3	2									11.0%				
1							7	3	10	2									8.5%				
0.5							2		1	2	8	2	3										
0.25											1	1		3	4	8	1						
0.12														1			1	1					
Germany		Disk Diffusion Zone of Inhibition (mm)																					
BMD MIC		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	>29	CA	ME	VME		
4								2											90.2%				
2				2		10		6											2.4%				
1							2	20											7.3%				
0.5								2		4		12											
0.25										2	2			10		4							
0.12														2			2						
Italy		Disk Diffusion Zone of Inhibition (mm)																					
BMD MIC		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	>29	CA	ME	VME		
4		1			1														80.5%				
2				3	5	2	4	3	1										18.3%				
1					2	6	1	4	7	2									1.2%				
0.5							1	1		3	7	4		1	1								
0.25									1			2	1	6	4	1	2	1					
0.12																	1	2	1				
Spain		Disk Diffusion Zone of Inhibition (mm)																					
BMD MIC		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	>29	CA	ME	VME		
4				2															86.6%				
2					1	5	6	3	2		1								9.8%				
1							3	4	8	2	4	1							3.7%				
0.5								1		6	6	3	2										
0.25										2		1	1	7	6	1							
0.12																	1	2	1				
USA		Disk Diffusion Zone of Inhibition (mm)																					
BMD MIC		14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	>29	CA	ME	VME		
4		1																	86.6%				
2			1	4			4	5	2	2									11.0%				
1							6	2	10	4									2.4%				
0.5								1	2	6	5	2	1	1									
0.25										1	1	1	2	4	6	2	1						
0.12																	1	1	1	1			

CA=Category Agreement, ME=Major Error (susceptible by BMD, resistant by Disk), VME=Very Major Error (resistant by BMD, susceptible by disk). Red lines are EUCAST MIC and disk susceptible breakpoints.