Susceptibility testing challenges with ceftaroline, MRSA, and a 1-mg/L breakpoint

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Abstract.

Background. A 1 mg/L susceptibility breakpoint for ceftaroline and staphylococci is universally agreed; EUCAST counts MIC >1 mg/L as resistant; CLSI and FDA count 2 mg/L as intermediate and >2 mg/L resistant. We investigated whether routine diagnostic tests reliably distinguish MICs of 1 *versus* 2 mg/L. **Methods**. Thirty-five UK laboratories collected *Staphylococcus aureus* isolates and performed tests with 5 μg (as EUCAST) or 30 μg (as CLSI) discs and either confluent growth on Mueller-Hinton agar (as EUCAST and CLSI) or semi-confluent growth on IsoSensitest agar (as BSAC). They also ran Etests for MRSA. Reference MICs were determined centrally by CLSI and BSAC agar dilution. **Results**. 1607 *S. aureus* (33% MRSA) had paired local disc and central MIC results. EUCAST's zone breakpoint recognised 56% of isolates found resistant in MIC tests, but the positive predictive value (PPV) for resistance was 11.0%; corresponding proportions by CLSI testing were 28.0% and 13.4%. The BSAC disc method detected 25% of resistant isolates, with a PPV of 18.2%. Agreement, ±1 dilution, of local Etests and central agar MICs was >95%, but only 20% of the isolates found non-susceptible by agar dilution were found non-susceptible by Etest, and *vice-versa*. Review for isolates with the modal MIC (0.25 mg/L) indicated that the same laboratories reported large or small zones irrespective of disc and method, implying systematic bias. **Conclusion**. MRSA with ceftaroline MICs of 1 and 2 mg/L were poorly discriminated by routine methods. Solutions lie in greater standardisation, automation, or dosages justifying a higher breakpoint.

Introduction

Ceftaroline is a recently-licensed cephalosporin that binds and inactivates PBP2' (PBP2a), which determines meticillin resistance in staphylococci.[1] Phase III trials in skin and skin structure infection (SSSI) indicated non-inferiority to vancomycin and equivalent efficacy against meticillin-resistant and -susceptible *Staphylococcus aureus* (MRSA and MSSA).[2] Case reports suggest anti-MRSA efficacy in various off-label settings, where use deserved more formal investigation, including diabetic foot infections[3] and endocarditis.[4]

MICs for most MRSA are 0.25-1 mg/L, compared with 0.12-0.25 mg/L for MSSA.[1] MICs of 2 mg/L are found for *c.* 5% of MRSA in most trials and surveys[5-7] though for 19.4% of isolates in one study in the Far East.[8] MICs exceeding 2 mg/L are extremely rare, but values of 4 mg/L were found for four MRSA from Greece. These had diverse mutations to PBP2',[9] as did isolates with MICs 2-4 mg/L from Germany.[10] A cystic fibrosis isolate with an MIC >32 mg/L also had a modified PBP2'.[11] EUCAST has set breakpoints of S $\leq$1 mg/L, R >1 mg/L,[12] whereas the US Food and Drug Administration (FDA) and CLSI both have values of S $\leq$1, I=2 and R >2. EUCAST, CLSI and BSAC have set zone breakpoints corresponding to these values,[12-15] but it is uncertain whether diagnostic laboratories can reliably distinguish the minorities of isolates with reduced susceptibility under 'real-life' conditions.[10] To test this, we recruited a panel of UK laboratories to test consecutive *S. aureus* isolates by disc and Etest methods and to refer the results, along with the isolates themselves, which then had MICs determined centrally. Results of the local and central testing were compared.

Materials and methods

SSSI survey

The SSSI survey, in which isolates were collected, has been described elsewhere.[16] Briefly we recruited 40 UK diagnostic microbiology laboratories, and asked each to collect 60 consecutive clinically-significant SSSI isolates from hospitalised patients, also a subsequent 15 MRSA from SSSIs. Collection ran from August 2012 to December 2013 and 35 of the 40 laboratories contributed isolates (See Acknowledgements): 29 sites were in England, three in Scotland, two in Wales and one in Northern Ireland. *S. aureus* dominated the collection, and is the only species considered here.

Local susceptibility testing

The laboratories were asked to perform susceptibility tests with 5 and 30 μg discs on their *S. aureus* isolates following: (i) British Society for Antimicrobial Chemotherapy (BSAC) methodology with semi-confluent growth and IsoSensitest agar,[17] and (ii) EUCAST/CLSI protocols with Mueller-Hinton agar and confluent growth.[18] The 5 and 30 μg discs were from Oxoid-Thermofisher, Basingstoke, UK and Mast Diagnostics, Merseyside, UK, respectively; they were from single batches and were supplied centrally. Laboratories were also asked to determine ceftaroline MICs for their MRSA (not MSSA) isolates using Etests (bioMerieux, Basingstoke, UK, again from a single batch), following the manufacturer's protocol, with Mueller-Hinton agar and confluent growth.

Except for discs and Etests, all other materials, including agars and diluents were sourced locally by the laboratories, as in routine practice. All sites held UK Clinical Pathology Accreditation.

Central laboratory testing

Isolates collected by the participating laboratories were also sent to the Antimicrobial Resistance and Healthcare Associated Infections Reference Unit (AMRHAI) where

they were confirmed as *S. aureus* with Chromagar *Staph aureus* (Chromagar, Paris, France), with PCR to seek *mecA*.[19] MICs of ceftaroline (AstraZeneca, Macclesfield, UK) were determined by BSAC agar dilution[20] on IsoSensitest agar (Oxoid-Thermofisher, Basingstoke, UK) and by CLSI agar dilution[21] on Mueller Hinton agar (Oxoid).

Results

Paired local disc tests and central MIC results were obtained for 1076 MSSA and for 531 MRSA, with local Etest results available for 525 of the MRSA. These totals differ minimally from the SSSI survey report,[16] owing to inclusion of a few isolates received as 'supplementary MRSA' that proved to be MSSA on reference laboratory testing. Numbers of *S. aureus* per site ranged from six to 47, with a mean of 28.

Agreement within central susceptibility testing at AMRHAI

MIC tests by the two agar dilution methods were run in parallel at AMRHAI. With 11 (0.7%) exceptions among the 1607 isolates, MICs by CLSI agar dilution equalled those by BSAC agar dilution or were two-fold higher (Table 1). MICs of 2 mg/L were found for 25 MRSA isolates by CLSI methodology (considered intermediate) and for eight of these (and no others) by BSAC methodology (considered resistant). No MICs exceeded 2 mg/L by either method (i.e. none of the isolates were considered resistant by CLSI criteria).

Agreement of local disc and central MIC testing

A relationship existed between the zones found locally and the MICs found centrally, such that mean zone diameters reduced as MICs rose (Table 2). Nevertheless correlation coefficients for zone *versus* log MIC were unimpressive, at 0.56 and 0.47 for 5 and 30 μg discs, respectively, on IsoSensitest agar with semi-confluent growth and 0.63 and 0.54, respectively for the same discs on Mueller-Hinton agar with confluent growth. Irrespective of the combination of method and disc type, there was considerable overlap of the zone sizes for isolates with MICs of 2 mg/L and those for isolates with lower MICs.

Disc-based categorisation with EUCAST and CLSI criteria

EUCAST specifies interpretive criteria of R <20 mm, S ≥20 mm using a 5 μg ceftaroline disc on Mueller-Hinton agar with confluent growth. This recognised as resistant only 14/25 (56%) isolates with MICs >1 mg/L (by the CLSI method; Table 2, panel 1). However 113 susceptible isolates were mis-categorised as resistant, giving a false resistance rate of 7.1% and leading to a 'resistant' result having a positive predictive value (PPV) of just 11.0%. CLSI has interpretive criteria of R ≤20 mm; I, 21-23 mm and S ≥24 mm for a 30 μg disc, again on Mueller-Hinton agar with confluent growth. No isolates counted as resistant using CLSI's MIC criteria (>2 mg/L) and, among the 25 that scored as intermediate (MIC 2 mg/L) the disc method correctly recognised only seven (28%; Table 2, panel 2). Five fully-susceptible isolates gave zones of ≤20 mm, equating to a false resistance (major error) rate of 0.3%, and 40 gave zones of 21-23 mm. The PPV of a non-susceptible zone result was 13.4%.

The BSAC did not have zone breakpoints for ceftaroline at the time of this study, but has since published values of R ≤19 mm, S ≥20 mm for 5 μg discs, which are slightly more liberal those of EUCAST, despite the less rich medium and lighter inoculum. These values correctly categorised only two of the eight isolates with MICs

of 2 mg/L as resistant (25%); nine susceptible isolates (0.5%) were mis-classified as resistant and the PPV of a resistant result was 18.2%. A breakpoint of R ≤23 mm correctly discriminated all eight isolates with MICs of 2 mg/L, but led to 207/1599 susceptible isolates being mis-categorised as resistant, meaning that the false resistance rate rose to 12.9% with a PPV for a resistant result of only 3.7%. Discrimination with 30 μg discs was even poorer.

Agreement of local Etest to central MIC tests

After rounding to match the normal doubling dilution scale, 500/525 (95.3%) of locally-determined Etest MICs were in essential agreement (i.e. ±1 doubling dilution) with the values found centrally by CLSI agar dilution, taken as a reference (Table 3). Nevertheless, discrimination between MICs of 1 and 2 mg/L remained poor: Among the 25 isolates with MICs of 2 mg/L by CLSI agar dilution, just five (20%) were found non-susceptible by Etest, with MICs of 1.5-2 mg/L. Counterwise, among 24 isolates with MICs of 1.5-4 mg/L by Etest, just five were found non-susceptible by agar dilution MICs. These data indicate 20% sensitivity for detection of MIC 2 mg/L, a false resistance/non-susceptibility rate of 4% and a PPV, for a 'non-susceptible' result predicting true resistance of 20%.

Differences in zone results among sites

The generally poor agreement between local and central results led us to consider cross-method agreement within sites. We reviewed laboratories' zone data for all isolates where the central laboratory had found an MIC of 0.25 mg/L. This was the modal MIC by each method, found for 967 isolates by the BSAC method and 1011 by the CLSI technique; it remained the modal MIC for all batches of isolates tested

centrally, indicating testing consistency. We then calculated, for each laboratory and test type, the mean zone diameters for isolates with this MIC (Table 4).

The mean zones for these isolates on Mueller-Hinton agar varied among laboratories by 7.4 mm with 30 μg discs and 6.5 mm with 5 μg discs. Corresponding variations on IsoSensitest agar were 5.8 and 4.6 mm, respectively. There was extremely strong correlation between the mean zones found for the two disc contents within each method (r= 0.93 within Mueller-Hinton/confluent growth and 0.91 within IsoSensitest/semi-confluent) and weaker correlation between results for the same disc type between the two different methods (r= 0.61 for 30 μg discs and 0.62 for 5 μg discs). These relationships are unsurprising because, within each method, the two discs would ordinarily be tested on the same plate with the same depth and inoculum, whereas the media and inocula differ between the methods. Nevertheless correlation coefficients of 0.61-0.62 suggest a relationship, and inspection shows that the laboratories which found the largest and smallest zone diameters for one combination of method and disc did so also for other combinations (Table 4).

Discussion

The FDA, EUCAST and CLSI all agree a susceptible breakpoint of 1 mg/L for ceftaroline, based on the licensed 600 mg every 12 hours regimen, pharmacodynamic analysis and Monte Carlo simulation.[14] This breakpoint divides around 5% of UK MRSA as resistant; the proportion may be higher or lower elsewhere according to locally prevalent strains, though MICs >2 mg/L seem to be rare everywhere. Unlike EUCAST, CLSI and FDA categorise MICs of 2 mg/L as intermediate. Rationales for the intermediate category have not been published: some pharmacodynamic analyses do support a 2 mg/L breakpoint based on the 600 mg every 12 hours regimen;[22] alternatively CLSI (unlike EUCAST) sometimes includes an intermediate category as

206 a buffer zone to minimise “major” and “very major” errors. In Phase III SSSI clinical
207 trials ceftaroline achieved cures in 142/152 MRSA cases,[2] but the proportion of isolates
208 with MICs of 2 mg/L was tiny. The present study sought to establish whether
209 diagnostic laboratories could reliably use these breakpoints -and corresponding zone
210 values- to distinguish isolates with MICs of 1 *versus* 2 mg/L.

211 We found that, in routine use, none of the combinations of disc and method
212 achieved a satisfactory balance of sensitivity and PPV for resistance detection.
213 EUCAST’s ≤20 mm breakpoint with 5 μg discs detected 56% of resistant (on EUCAST
214 criteria) isolates, but at the price of categorising many susceptible isolates as resistant,
215 so that the PPV for a resistant zone result was only 11.0%. CLSI’s
216 susceptible/intermediate breakpoint of 23 mm for 30 μg discs recognised just 28% of
217 the isolates with MICs 2 mg/L as non-susceptible, with a PPV of 13.4%. A breakpoint
218 (this study) of 23 mm for the BSAC method with 5 μg discs recognised all resistant
219 isolates, but had a derisory PPV of 3.7%; the BSAC’s subsequently-published R ≤19
220 mm[16] value detected only two of eight resistant isolates, with a PPV of 18.2%. Zone
221 breakpoints could be adjusted to improve detection sensitivity for resistance or to
222 increase the PPV, but these two criteria are counterpoised, meaning that any change
223 to improve one will worsen the other. Gradient strips are widely advocated when disc
224 tests are unreliable but, although essential agreement to agar dilution MICs was
225 excellent at 95%, minor disagreements again meant that the detection sensitivity for
226 isolates with an MIC of 2 mg/L was only 20%, with a PPV of 20%. Like others[11] we
227 found that MICs by Etest were commonly one doubling dilution below those by dilution
228 methodology.

229 The present analysis is disappointing compared with EUCAST’s published
230 study,[12] which found 92% categorical agreement between ceftaroline disc and MIC
231 tests for MRSA. The likely explanation is that the EUCAST study included a tight quality

control specifically for ceftaroline whereas we took the view that laboratories had general quality control for susceptibility testing and wished to assess likely variability in real-life conditions.

To further investigate inter-site variation we reviewed zones for all isolates where the central laboratory found an MIC of 0.25 mg/L and, irrespective of disc and method, found substantial variation in different laboratories' mean zones for these 'homogeneous' isolates. Variation was greater by CLSI/EUCAST methodology than by BSAC (Table 4) perhaps because standardisation to the BSAC method was more familiar to UK laboratories, or because it is inherently easier, with a single manufacturer of the base medium (IsoSensitest agar) and with semi-confluent growth that can easily be judged as adequate or not by eye. Strikingly, the same laboratories obtained the largest and smallest zones irrespective of the disc content or method. This implies that differences in inocula, agar depth or how zones are read, are the major arbiter of variation, not variation in media or disc quality (which was a source of recent comment and concern[23]).

Three solutions might be proposed. First, disc testing and reading might be made more precise. Secondly, automated systems –which do not depend on the human eye to judge a zone edge or the end of growth on a gradient strip– might replace disc testing. Thirdly, ceftaroline might be dosed at levels to justify a higher breakpoint. EUCAST stresses that zones for control strains should fall in the middle of the published quality control ranges and that laboratories consistently obtaining results at the extremes of ranges or >1 mm either side of the expected value, should review their performance;[24] the International Standards Organisation is also taking an interest in the improved standardisation of disc testing. Both these organisations can do much to encourage improved quality, precision and reproducibility of disc testing, nevertheless zone scatter of $\pm$3 mm per doubling dilution between tests and laboratories is not uncommon, meaning that MICs of 1 and 2 mg/L seem certain ot

remain hard to discriminate by diffusion testing. With regard to dosage, a trial of ceftaroline at 600 every 8 hours instead of every 12 hours has recently been completed in bacteraemic SSSI (Clinical Trials Identifier NCT01499277 https://clinicaltrials.gov) and may justify a 2 mg/L breakpoint.

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Table 1. Agreement of MICs by BSAC and CLSI methods, as found centrally

	MIC by BSAC method								Total MSSA	Total MRSA	Grand total
MIC by CLSI method	0.015	0.03	0.06	0.125	0.25	0.5	1	2			
0.06	1	1	5						7		7
0.125			2	41	2				45		45
0.25			1	95	909	6			1004	7	1011
0.5					56	126	1		20	163	183
1						195	141			336	336
2							17	8		25	25
Total MSSA	1	1	8	135	919	12					
Total MRSA				1	48	315	159	8			
Grand Total	1	1	8	136	967	327	159	8	1076	531	1607

Table 2. Agreement between locally determined inhibition zones (mm) and centrally determined MICs for ceftaroline

Panel 1. Mueller-Hinton agar, confluent growth 5 μg disc, as EUCAST (shaded: zones resistant on EUCAST criteria) *versus* MICs by CLSI agar dilution

MIC, mg/L	6	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	≥36	Mean zone +SD
2		2			1	1	6	1	3	4	2	1				1	1	2									19.4+4.3
1		2	1	3	2	4	6	31	43	70	65	42	27	9	14	8	4	2	1	1	1						20.8+2.6
0.5		1			1	1		4	7	22	34	35	20	14	21	8	6	3		5	1						22.6+2.8
0.25	2							1	3	5	20	64	87	122	142	131	117	91	81	67	35	18	12	6	3	4	26.2+3.1
0.125												1			1	5	5	7	9	5	4	4	3	1			29.0+2.5
0.06									1							1	1		2			2					27.7+4.5
Total	2	5	1	3	4	6	12	37	57	101	121	143	134	145	178	154	134	105	93	78	41	24	15	7	3	4	

Panel 2: Mueller-Hinton agar, confluent growth 30 μg disc as CLSI (dark grey, resistant by CLSI zone criteria; light grey, intermediate) *versus* MICs by CLSI agar dilution

MIC, mg/L	6	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	≥42	Mean zone +SD
2				2	1	4	1	5	4	3	1	1	3													25.5+2.6
1		1	2	5	6	11	34	68	69	49	42	16	19	11	2	1										26.3+2.4
0.5			1	2	5	9	14	17	24	28	25	19	20	10	3	2	2	1		1						27.2+2.8
0.25	1				3	4	8	23	52	78	123	114	145	109	106	69	67	49	27	19	7	2	2		3	30.4+3.2
0.125												3	5	2	9	5	5	10	3		2	1				33.2+2.5
0.06							1					1			2	1				2						32.0+4.5
Total	1	1	3	9	15	28	58	113	149	158	191	154	192	132	122	78	74	60	30	22	9	3	2		3	

Panel 3: IsoSensitest, semi-confluent growth, 5 μg disc (shaded: zones resistant on BSAC criteria[16]) *versus* MICs by BSAC agar dilution

MIC, mg/L	6	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	≥37	Mean zone +SD
2				1	1			3	2	1															20.6±2.1
1				1	2	1	5	15	23	24	26	32	14	9	5	1	1								23.7±2.2
0.5	1		1			1	5	15	34	45	61	67	42	32	16	3	2	1	1						24.4±2.3
0.25								1	9	22	55	151	133	126	128	72	101	69	36	25	21	9	6	3	27.8±2.9
0.125	1	1									3	2	4	8	11	11	28	15	17	16	10	5	4		30.3±3.6
0.06											2		1	1			2	1					1		28.5±4.1
0.03																								1	
0.015																								1	
Total	2	1	1	2	3	2	10	34	68	92	147	252	194	176	160	87	134	86	54	41	31	14	11	5	

Panel 4: IsoSensitest, semi-confluent growth, 30 μg disc *versus* MICs by BSAC agar dilution

MIC (mg/L)	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	≥42	Mean zone ±SD
2										1	2	2	2	1													28.0±1.3
1				1	1	1	2	1	5	7	15	28	19	32	19	17	6	4			1						29.2±.2.6
0.5	1				2		4	3	8	16	20	37	59	79	31	32	21	7	3	3		1					29.6+2.6
0.25						1			7	7	23	42	81	165	119	133	81	85	78	51	43	22	10	13	1	5	32.2±3.1
0.125											3	1	1	4	5	19	11	14	21	17	14	8	7	6	2	3	34.9±3.2
0.06													1	1	2		1	1	1					1			32.9±3.5
0.03																									1		
0.015																										1	
Total	1			1	3	2	6	4	20	31	63	110	163	282	176	201	120	111	103	71	58	31	17	20	4	9	

Table 3. Local Etest results *versus* central MIC result on Mueller-Hinton agar for MRSA isolates (n=531)

	Count of isolates with indicated MIC (mg/L) by Etest											
MIC (mg/L) by CLSI agar dilution	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3	4	Grand Total
2				1	4	4	11	3	2			25
1			8	45	95	122	50	8	1	1	3	333
0.5	1	4	15	56	51	26	1		2	2	2	160
0.25			3	2	1	1						7
Grand Total	1	4	26	103	151	153	62	11	5	3	5	525

Shaded results are in essential agreement, after routing Etest values to the normal doubling dilution scale

Table 4. Mean zones, as found at participating laboratories for isolates with ceftaroline MICs of 0.25 mg/L found centrally

Lab	No isolates with MIC 0.25 mg/L found centrally by CLSI method	CLSI/EUCAST method				No isolates with MIC 0.25 mg/L centrally by BSAC method	BSAC Method			
		30-μg disc		5-μg disc			30-μg disc		5-μg disc	
		Mean zone (mm)	SD (mm)	Mean zone (mm)	SD (mm)		Mean zone (mm)	SD (mm)	Mean zone (mm)	SD (mm)
CC	35	27.1	2.5	24	2.3	34	30.1	2.6	26.2	2.5
CH	33	27.9	2.4	24	2.9	33	31.1	2.2	27.0	2.1
CZ	32	28.3	2.3	23.8	2.3	35	32.9	3.5	27.5	3.1
DJ	32	29	2.2	26	2.1	31	31.2	2.8	28.0	2.7
DK	19	29.2	2.6	24.9	2.6	19	34.1	2.6	29.6	2.8
DM	25	29.2	2.7	25.2	2.9	26	31.7	4.8	26.5	3.8
CW	34	29.5	2.0	24.9	2	35	32.1	2.5	27.2	2.7
DB	43	29.5	2.1	25.2	2.4	35	29.1	2.7	25.3	2.2
CF	21	29.7	2.1	25.6	2.3	22	32.1	2.2	27.5	2.4
CJ	28	29.8	2.5	24.6	2.3	26	31.4	2.2	26.2	1.9
CK	22	29.8	2.4	26.1	2.3	20	32.0	2.7	27.5	2.2
CM	41	29.8	2.5	26.2	2.7	37	32.1	2.7	27.6	2.5
CP	29	29.8	2.9	25.8	2.7	27	32.7	4.0	28.6	3.5
CG	23	29.9	2.8	25.7	2.3	22	31.3	3.2	27.3	3.1
DC	32	29.9	2.9	25.3	2.7	30	31.6	3.1	27.2	3.1
CX	21	30.3	3.1	25.8	2.9	19	30.9	2.9	26.0	2.4
DI	28	30.3	2.5	25.9	2.6	26	32.4	1.9	28.2	1.8
DL	36	30.3	2.4	26.4	2.4	27	32	2.7	28.5	2.7
CO	37	30.4	2.9	26.6	2.6	38	31.6	3.3	27.7	3.3
DF	16	30.4	3.6	26.3	3.5	15	34.1	3.6	29.3	3.4
CE	31	30.6	2.4	26.8	2.6	31	33.6	3	29.3	2.8

CQ	35	30.9	3.4	26.4	3	35	32.3	2.9	28.3	2.8
CT	34	30.9	3.0	26.8	3.2	31	31.4	2.2	27.4	2.6
DD	37	31.1	3.3	26.4	3.3	39	32.5	2.9	27.1	2.5
CS	11	31.2	2.8	25.1	6.7	12	32.1	1.8	27.7	2.1
CI	37	31.5	6.4	27.3	5.8	38	32.5	2.9	27.9	2.6
CL	18	31.5	2.1	27.2	2	19	34.2	1.9	29.3	2
CN	39	31.5	3.1	26.2	3	39	33.7	3	28.8	2.8
CR	20	31.7	2.6	27	2.1	20	32.2	2.8	27.6	2.1
DO	6	31.7	1.5	27.7	2.7	6	32.7	2.7	27.7	2.9
CB	48	31.8	2.8	27.1	2	47	32.2	3.1	27.9	2.6
CV	32	32.4	2.8	27.8	2.5	28	32.6	3	28.3	3
CY	35	33.3	2.8	29	2.6	33	34.1	3.3	29.6	3.2
CU	20	33.7	2.1	29.3	2	13	34.9	3.1	29.9	2.8
DH	21	34.5	2.7	30.3	2.8	19	34.1	3	29.5	2.7

The five lowest values are shaded grey and the five highest shown as white type on a black background; more than five values are highlighted in the event of 'ties'.