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Comparison of Mupirocin 5 and 20 μg Disk Results to Microdilution and Etest MICs against Susceptible Isolates and a Resistant Challenge Set of *Staphylococcus aureus*

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Revised Abstract

Background: A 5 μg mupirocin disk currently available for testing has no definable zone category for differentiating between low-level-resistant (LLR; MICs 8–256 μg/mL) and high-level-resistant (HLR; MICs ≥512 μg/mL) isolates. A 20 μg mupirocin disk has recently become commercially available (Mast, Bootle, UK) for testing according to British Society for Antimicrobial Chemotherapy (BSAC) disk method, and has tentative interpretive criteria for LLR and HLR categories. The purpose of this study was to assess the BSAC method by comparing zone diameters for 5 and 20 μg disks with Clinical Laboratory Standards Institute (CLSI) microbroth and Etest MICs for 60 *Staphylococcus aureus* in order to determine the performance of both disks in detecting LLR and HLR isolates. BSAC and CLSI disk methods were also compared. **Methods:** Twenty-one susceptible (MICs ≤4 μg/mL), 26 LLR (MICs 16, 32, 128 μg/mL) and 13 HLR *S. aureus* (MICs >512 μg/mL) were tested by BSAC disk method with a 5 and 20 μg mupirocin disk using two lots of IsoSensitest agar (ISA) and for comparison purposes, one lot of Mueller Hinton agar (MHA) using both CLSI and BSAC methods. The same isolates were also concurrently tested by microbroth dilution (CLSI) and Etest. **Results:** 96.2% of LLR isolates were accurately categorized using the 20 μg disk. All HLR isolates were accurately categorized using the 5 μg disk. The 20 μg disk tested on two lots of ISA detected 84.6% and 92.3% of HLR isolates. The 5 μg disk tested on two lots of ISA detected 84.6% and 92.3% of LLR isolates. MHA zones were less defined and more difficult to read than ISA and were 2 mm lower by CLSI compared with BSAC. Of the 28 isolates with on-scale MICs, 100% of the microbroth dilution and Etest results were within ±1 doubling dilution; of these 20 (71.4%) were identical. **Conclusions:** There was good correlation of mupirocin Etest and microdilution MICs for all isolates tested. The use of the 20 μg mupirocin disk on ISA according to the BSAC procedure is an acceptable method for detection of mupirocin susceptible, LLR and HLR *S. aureus*.

Introduction

The current British Society for Antimicrobial Chemotherapy (BSAC) standardized disk susceptibility method includes both a 5 μg and a 20 μg mupirocin disk for testing of *Staphylococcus aureus*. There is no intermediate zone range in the BSAC method for detection of LLR isotypes using the 5 μg disk. The 20 μg disk was recently added to the BSAC disk method and does provide an intermediate zone range for detection of LLR isolates. The purpose of this study was to compare zone diameters for both the 5 and 20 μg disks (using the BSAC methodology with IsoSensitest agar [ISA] and with Mueller Hinton agar [MHA]) with Clinical Laboratory Standards Institute (CLSI) broth microdilution and Etest MIC results for a challenge set of organisms in order to determine the capability of the disk method to detect low- and high-level mupirocin resistance in isolates of *S. aureus*. BSAC and CLSI disk methods, which use different culture media and inoculum preparations, were also compared.

Methods

Microorganisms

- Sixty *S. aureus* isolates provided by IHMA (International Health Management Associates, Inc., Schaumburg, IL, USA), selected according to mupirocin susceptibility; 21 susceptible (slight variation in total number of susceptible isolates with ISA was due to unreadable zones as a result of plate contamination), 26 LLR, and 13 HLR isolates.
- Quality control strains: *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, and *S. aureus* NCTC 6571.

Media

- MHA – Becton Dickinson prepared plates, Sparks, MD, USA, Lot #5335405.
- ISA – Oxoid, LSI prepared plates, Hampshire, UK, Lot #324391 and #335431.

Testing Methodology

Disk

- ISA (two lots) – BSAC inoculum (1:10 dilution of a 0.5 McFarland)
- MHA (one lot) – BSAC inoculum (1:10 dilution of a 0.5 McFarland)
  – CLSI inoculum (0.5 McFarland).[2]

The current BSAC breakpoints are:

- 20 μg (BSAC): HLR (resistant) ≤6 mm, LLR (intermediate) 7–26 mm, susceptible ≥27 mm
- 5 μg (BSAC): resistant ≤21 mm, susceptible ≥22 mm.*

  *As there are no existing BSAC breakpoints for the 5 μg disk, following breakpoints were assigned for the purpose of this study: HLR ≤6 mm, LLR 7–21 mm.

MIC

- CLSI broth microdilution[3]
- Etest
- BSAC MIC breakpoints: resistant >256, intermediate = 8–256; susceptible ≤4.[1]

Antimicrobial Agents

- Mupirocin powder (Lot #WRS46) – GlaxoSmithKline, Collegeville, PA, USA. A 5120 μg/mL stock solution was made using water as a dilutant and trays made according to CLSI guidelines.
- Mupirocin 20 μg disks (Lot #190308) – Mast Group Ltd, Bootle, UK.
- Mupirocin 5 μg disks (Lot #3245025) – Becton Dickinson, Sparks, MD, USA.

Data Analysis

- Category agreement was calculated based on comparison of zone interpretation results to MIC interpretation results.

Results

Disk Diffusion using ISA (BSAC Inoculum)

20 μg disk (Table 1, Figures 1 and 2)

- Detection of susceptible isolates
  – Lot #324391 = 78.9% agreement (21.1% were wrongly classified as LLR)
  – Lot #335431 = 95% agreement (5% were wrongly classified as LLR).
- Detection of LLR
  – Lot #324391 = 96.2% agreement (3.8% were wrongly classified as HLR)
  – Lot #335431 = 96.2% agreement (3.8% were wrongly classified as HLR).
- Detection of HLR
  – Lot #324391 = 92.3% agreement (7.7% were wrongly classified as LLR)
  – Lot #335431 = 84.6% agreement (15.4% were wrongly classified as LLR).

5 μg disk (Table 1, Figure 3)

- Detection of susceptible isolates
  – Lot #324391 = 94.7% agreement (5.3% were wrongly classified as resistant or LLR)
  – Lot #335431 = 95% agreement (5% were wrongly classified as resistant or LLR).
- Detection of LLR
  – Lot #324391 = 84.6% (15.4% were wrongly classified as HLR)
  – Lot #335431 = 92.3% (7.7% were wrongly classified as HLR).
- Detection of HLR
  – Lot #324391 = 100% agreement
  – Lot #335431 = 100% agreement.

Disk diffusion using BSAC inoculum on MHA or CLSI method

Deviations from BSAC method, such as using MHA with BSAC recommended inoculum (**Table 2**) or CLSI recommended inoculum (**Table 3**) with the 20 μg disk resulted in very high (76.2–90.5%) error rate in erroneously calling susceptible isolates as LLR. Similarly, deviations from BSAC method using the 5 μg disk resulted in marked errors (33.3–71.4%) in calling susceptible isolates as resistant. When utilizing existing BSAC breakpoints, variations to BSAC method, including use of MHA and inoculum, will result in significant categorical errors (**Table 2, Figures 4** and **5**).

Etest

There was good correlation of mupirocin Etest and microdilution MICs. There were 28 isolates with on-scale MICs (MICs that fall within the range of MIC values tested); 20 of these were identical and eight were half or one doubling dilution different. All other isolates had MICs less than or equal to the lowest concentration or greater than the highest concentration tested and were similar by both methods.

Table 1. Correlation of Mupirocin 5 and 20 μg Disk (BSAC Method ISA) Results to Susceptible, LLR and HLR Categorical Results as Determined by MIC (CLSI Method)

			MIC susceptibility category, n (%)		
20 μg disk (ISA Lot #324391 and #335431)					
		mm	Susceptible[a]	LLR	HLR
Disk diffusion susceptibility category (current BSAC breakpoints)	Susceptible	≥27	34 (87.2)	0	0
	LLR	7–26	5 (12.8)	50 (96.2)	3 (11.5)
	HLR	≤6	0	2 (3.8)	23 (88.5)
5 μg disk (ISA Lot #324391 and #335431)					
		mm	Susceptible[a]	Resistant	
Disk diffusion susceptibility category	Susceptible	≥22	37 (94.9)	0	
	Resistant	<21	2 (5.1)	78 (100)	
				LLR	HLR
Suggested LLR and HLR breakpoints	LLR	7–21	2 (5.1)	46 (88.5)	0
	HLR	≤6	0	6 (11.5)	26 (100)

[a]Slight variation in total number of isolates was due to plate contamination

HLR by MIC, LLR by disk; LLR by MIC, HLR by disk; Susceptible by MIC, LLR by disk

Figure 1. Scatterplot of MIC by CLSI Broth Microdilution Method *versus* 20 μg Disk by BSAC Method (ISA; Lot #324391 and #335431)

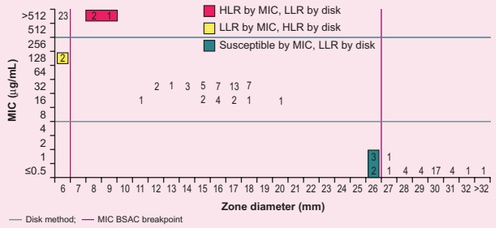


Figure 2. Correlation Between Zone Diameters Obtained by 20 μg Mupirocin Disk (Two ISA Lots) and BSAC Disk Breakpoint Interpretive Criteria

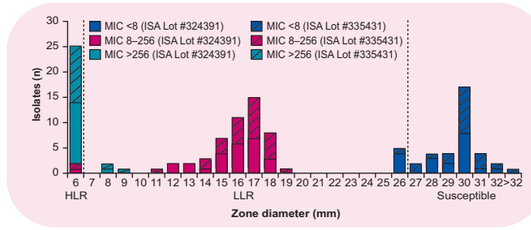


Figure 3. Correlation Between Zone Diameters Obtained by 5 μg Mupirocin Disk (Two ISA Lots) and BSAC Disk Breakpoint Interpretive Criteria

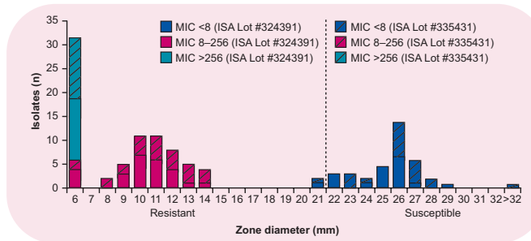


Table 2. Correlation of Mupirocin 5 and 20 μg disk (BSAC Inoculum on MHA) Results to Susceptible, LLR and HLR Categorical Results as Determined by MIC (CLSI Method)

			MIC susceptibility category, n (%)		
20 μg disk (MHA Lot #5335405)					
		mm	Susceptible	LLR	HLR
Disk diffusion susceptibility category	Susceptible	≥27[a]	5 (23.8)	0	0
		≥21[b]	21 (100)	0	0
	LLR	7–26[a]	16 (76.2)	25 (96.2)	0
		7–20[b]	0	25 (96.2)	0
	HLR	≤6[a,b]	0	1 (3.8)	13 (100)
5 μg disk (MHA Lot #5335405)					
		mm	Susceptible	Resistant	
Disk diffusion susceptibility category	Susceptible	≥22[a]	14 (66.6)	0	
		≥17[b]	21 (100)	0	
	Resistant	≤21[a]	7 (33.3)	13 (100)	
		≤16[b]	0	13 (100)	
				LLR	HLR
Suggested LLR and HLR breakpoints	LLR	7–21[a]	7 (33.3)	2 (7.7)	0
		7–16[b]	0	2 (7.7)	0
	HLR	≤6[a,b]	0	24 (92.3)	13 (100)

[a]BSAC recommended breakpoint; [b]Adjusted/suggested breakpoint

HLR by MIC, LLR by disk; LLR by MIC, HLR by disk; Susceptible by MIC, LLR by disk

Table 3. Correlation of Mupirocin 5 and 20 μg Disk (CLSI Inoculum on MHA) Results to Susceptible, LLR and HLR Categorical Results as Determined by MIC (CLSI Method)

			MIC susceptibility category, n (%)		
20 μg disk (MHA Lot #5335405)					
		mm	Susceptible	LLR	HLR
Disk diffusion susceptibility category	Susceptible	≥27[a]	2 (9.5)	0	0
		≥17[b]	21 (100)	0	0
	LLR	7–26[a]	19 (90.5)	25 (96.2)	0
		7–16[b]	0	25 (96.2)	0
	HLR	≤6[a,b]	0	1 (3.8)	13 (100)
5 μg disk (MHA Lot #5335405)					
		mm	Susceptible	Resistant	
Disk diffusion susceptibility category	Susceptible	≥22[a]	6 (28.6)	0	
		≥14	21 (100)	0	
	Resistant	≤21[a]	15 (71.4)	13 (100)	
		≤13	0	39 (100)	
				LLR	HLR
Suggested LLR and HLR breakpoints	LLR	7–21[a]	7 (33.3)	2 (7.7)	0
		7–13	0	2 (7.7)	0
	HLR	≤6[a,b]	0	24 (92.3)	13 (100)

[a]BSAC recommended breakpoint; [b]Adjusted/suggested breakpoint

HLR by MIC, LLR by disk; LLR by MIC, HLR by disk; Susceptible by MIC, LLR by disk

Figure 4. Correlation Between Zone Diameters Obtained by 20 μg Mupirocin Disk (One MHA Lot) and BSAC Disk Breakpoint Interpretive Criteria

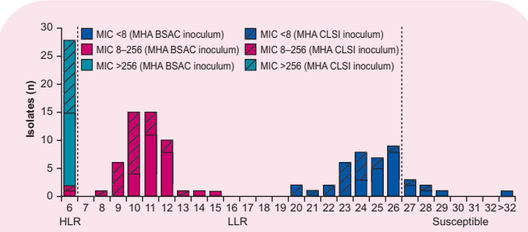
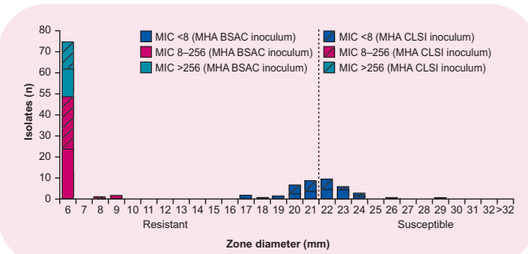


Figure 5. Correlation Between Zone Diameters Obtained by 5 μg Mupirocin Disk (One MHA Lot) and BSAC Disk Breakpoint Interpretive Criteria



Discussion

- With both disks there were susceptible isolates categorized as LLR isolates. All susceptible isolates could be detected using the 20 or 5 μg mupirocin disk on ISA according to the BSAC procedure, if the susceptible breakpoint was moved to 24 mm for the 20 μg disk and 19 mm for the 5 μg disk.
- There were some HLR isolates that were categorized as LLR using the 20 μg disk; this did not occur with the 5 μg disk. The 5 μg disk may be considered slightly more effective than the 20 μg disk in detecting HLR isolates based on the breakpoints that were chosen for the purpose of this study.

Conclusions

- The use of the 20 μg mupirocin disk on ISA according to the BSAC procedure and breakpoints is suitable for the detection of susceptible, low-level and high-level mupirocin-resistant *S. aureus*.
- Categorical errors will occur if modifications to the BSAC method (i.e. media and inoculum) are made and existing BSAC breakpoints are utilized.
- The use of the 20 μg disk on MHA using either BSAC or CLSI inoculum with adjusted breakpoints can reliably detect LLR and HLR *S. aureus* (see **Tables 2** and **3**).
- The use of the 5 μg disk on ISA according to the BSAC procedure was suitable for the detection of susceptible, resistant, and low- and high-level mupirocin-resistant *S. aureus* when applying the suggested breakpoints.
- The 5 μg mupirocin disk on MHA (using the BSAC or CLSI inoculum) did not differentiate between LLR and HLR.
- As this study included relatively small numbers of isolates and no isolates with mupirocin MICs of 8, 256 or 512 μg/mL, further investigation is warranted to confirm the suggested breakpoints.
- There was good correlation of Etest and broth microdilution MICs.

Acknowledgement

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