Evaluation of the MicroScan MICroSTREP Plus Antimicrobial Panel for Testing ß-Hemolytic Streptococci and Viridans Group Streptococci

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Background: In order to determine the clinical usefulness of the MicroScan (Siemens Healthcare Diagnostics, USA) MICroSTREP plus antimicrobial panel (MICroSTREP) for testing antimicrobial susceptibility of ß-hemolytic streptococci (BHS) and viridans group streptococci (VGS), we compared the accuracy of MICroSTREP with that of the CLSI reference method.
Methods: Seventy-five BHS and 59 VGS isolates were tested for antimicrobial susceptibility to ampicillin, penicillin, cefotaxime, meropenem, erythromycin, clindamycin, levofloxacin, and vancomycin by using MICroSTREP and the CLSI agar dilution method.
Results: The overall essential agreement with regard to minimum inhibitory concentrations (MICs) (within ±1 double dilution) between MICroSTREP and the CLSI reference method was 98.2%, and categorical agreement (CA) was 96.9%. For the BHS isolates, the CA for erythromycin was 96.0%, whereas that for cefotaxime, meropenem, levofloxacin, and vancomycin (for ampicillin, penicillin, and clindamycin; 98.7%) was 100%. For the VGS isolates, the CA for penicillin was 84.7% and that for erythromycin, clindamycin, and vancomycin (for meropenem, 86.5%; for ampicillin, 88.1%; and for cefotaxime and levofloxacin, 96.6%) was 100%. All categorical errors of penicillin and ampicillin in the VGS isolates were minor.
Conclusions: The accuracy of MICroSTREP is comparable to that of the CLSI reference method, suggesting that this panel can be effective for testing antimicrobial susceptibility of BHS and VGS.

Key Words: Antimicrobial susceptibility test, *Streptococcus*, MICroSTREP, MicroScan

INTRODUCTION

β-Hemolytic streptococcal isolates obtained from humans can be subdivided into large-colony and small-colony (<0.5 mm in diameter) formers. Large colony formers include *Streptococcus pyogenes* (Lancefield group A antigen), *Streptococcus agalactiae* (Lancefield group B antigen), and *Streptococcus dysgalactiae* subsp. *equisimilis* (Lancefield group C and G antigens) [1]. The small-colony-forming β-hemolytic strains with Lancefield group A, C, F, or G antigens are considered part of the viridans group streptococci

(VGS). VGS also include *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus sanguinis*, and *Streptococcus bovis* [1]. Although penicillin remains the drug of choice in the treatment of infections caused by large-colony-forming β-hemolytic streptococci (BHS), drug tolerance and clinical therapeutic failures have been reported [2]. Macrolides and lincosamides have been frequently used to prevent β-lactam allergies in patients. These agents are also used in empiric and preventive therapies for the treatment of BHS infections [3, 4]. However, recent studies have shown considerable changes in the susceptibility of BHS to erythromycin and clindamycin, although different resistance rates to these agents owing to geographical variation and investigators have been reported [5-7]. β-Lactam agents have been the treatment of choice for VGS infections; however, increase in the incidence of VGS with multidrug-resistance to penicillin and other agents, such as cephalosporins, macrolides, lincosamides, tetracycline, quinupristin-dalfopristin, and quinolones, has been reported [7, 8]. Moreover, CLSI has recommended that VGS isolated from normally sterile body sites should be tested for penicillin susceptibility by using a min-

imum inhibitory concentration (MIC) method and interpretive criteria [9]. Accurate susceptibility testing for BHS and VGS is required in order to guide appropriate antimicrobial therapy and to monitor further spread of resistant pathogens. Rising drug resistance of BHS and VGS has increased the need for accurate determination of antimicrobial susceptibility in a timely manner in clinical microbiology laboratories. Rapid reporting of the results of an antimicrobial susceptibility test (AST) has been shown to improve patient outcomes and reduce hospital costs [10, 11]. Because there are significant differences in the susceptibility of BHS and VGS to β-lactam agents, there are separate interpretive criteria for the susceptibility of the 2 groups of organisms to ampicillin, penicillin, cefotaxime, ceftriaxone, and cefepime [9].

Automated commercial susceptibility test systems for streptococci offer reliable AST results for MIC measurement and help accurately determine the antimicrobial susceptibility profile according to the *Streptococcus* group. However, most studies are focused on evaluating the AST performance of *Streptococcus pneumoniae.* To the best of our knowledge, no study has been conducted to evaluate the accuracy of the automated MicroScan (Siemens Healthcare Diagnostics, Sacramento, CA, USA) AST system for susceptibility testing of BHS and VGS. Therefore, this study was designed to evaluate the clinical usefulness of the MicroScan MICroSTREP plus antimicrobial panel (MICroSTREP) as a susceptibility testing system for BHS and VGS.

MATERIALS AND METHODS

1. Bacterial isolates

A total of 134 isolates of BHS (75 isolates) and VGS (59 isolates) were stocked from various clinical specimens obtained from January to December 2009 at Wonju Christian Hospital, Korea. Multiple isolates from the same patient were avoided. The predominant specimen sources were wounds (60, 44.8%), urinary tract (27, 20.1%), blood (13, 9.7%), and respiratory tract (11, 8.2%). The isolates were identified by on the basis of hemolytic patterns on 5% sheep blood agar, colony morphology, Gram staining, catalase reaction, and findings obtained by using the VITEK-2 GP identification system (bioMérieux, Marcy l'Étoile, France). The strains were stored in thioglycolate broth containing 20% glycerol at -70°C until analysis. Thereafter, the frozen isolates were thawed, inoculated onto a 5% sheep blood agar plate, and incubated at 35°C overnight. Pure isolates from 3 consecutive subcultures were tested for susceptibility. All 134 strains were tested using MICroSTREP and the

CLSI reference method. Of the 134 isolates, BHS were *S. agalactiae* (61 isolates), *S. pyogenes* (9), and *S. dysgalactiae* (5), and VGS were *S. mitis* (34), *Streptococcus anginosus* (18), *S. salivarius* (3), *S. sanguinis* (2), and *S. mutans* (2).

2. Reference method

Susceptibility to penicillin, ampicillin, cefotaxime, erythromycin, clindamycin, levofloxacin (Sigma Chemical Co, St. Louis, MO, USA), meropenem (Yuhan, Seoul, Korea), and vancomycin (Daewoong Lilly, Seoul, Korea) was tested using the agar dilution method according to the recommendations of the CLSI [9]. Mueller-Hinton agar with 5% defibrinated sheep blood was used for the agar dilution test. Inocula were prepared by suspending colonies in tryptic soy broth to obtain approximately 10^4 colonies on inoculation using a Steers replicator (Craft Machine Inc, Chester, PA, USA). MIC was determined after 24 hr of incubation at 35°C. *S. pneumoniae* (ATCC 49619) was used as a control in the MIC determination. MIC was defined as the lowest concentration of an agent that yielded no growth or a marked change in the appearance of the growth plate as compared to the growth control plate. The AST results obtained for the reference strains were consistently within the acceptable MIC range.

3. MicroScan MICroSTREP test

In the MicroScan MICroSTREP system, Renok hydrator/inoculator was used to deliver 115 μL of Mueller-Hinton broth with 3% lysed horse blood to each well. After inoculation with a 0.5 McFarland standard bacterial suspension, the panels were incubated at 35°C in ambient air for 20-24 hr and read using the MicroScan WalkAway System (Siemens Healthcare Diagnostics).

4. Comparison of results

We analyzed essential agreement (EA) and categorical agreement (CA) between MICroSTREP and the CLSI reference method for each antibiotic tested. EA was defined as MIC of MICroSTREP and the CLSI reference method was within ±1 double dilution. CA was defined as interpretive category of MICroSTREP and the CLSI reference method was same. AST error rates were calculated and reported as follows: a very major error (VME) was recorded if an isolate was found to be susceptible on using MICroSTREP and resistant on employing the reference method; a major error (ME) was recorded if an isolate was found to be resistant on using MICroSTREP and susceptible on employing the reference method; and a minor error (MIE) was recorded if an isolate was judged intermediate on using MICroSTREP or

the reference method and susceptible or resistant on employing the other method.

RESULTS

Using the agar dilution method, the VGS non-susceptibility rates were 57.6% for ampicillin (MIC ≥0.5 mg/L), 59.3% for penicillin (MIC ≥0.25 mg/L), 22.0% for cefotaxime (MIC ≥2 mg/L), 28.8% for meropenem (MIC >0.5 mg/L), 39.0% for erythromycin (MIC ≥0.5 mg/L), 22.0% for clindamycin (MIC ≥0.5 mg/L), and 13.6% for levofloxacin (MIC ≥4 mg/L). All BHS isolates were susceptible to ampicillin, penicillin, cefotaxime, and meropenem. Among the BHS isolates, the non-susceptibility rates for erythromycin, clindamycin, and levofloxacin were 28.0%, 37.3%, and 6.7%, respectively (Table 1).

The overall EAs and CAs determined for MICroSTREP and the CLSI reference method were 98.2% and 96.9%, respectively (Tables 2 and 3). For the BHS isolates, the EAs for individual antimicrobial agents ranged from 94.7% (erythromycin) to 100% (penicillin, cefotaxime, meropenem, and levofloxacin), while the EA for ampicillin, clindamycin, and vancomycin was 98.7%. The CA for erythromycin was 96.0%; for cefotaxime, meropenem, levofloxacin, and vanco-

mycin, 100%; and for ampicillin, penicillin, and clindamycin, 98.7%. For the VGS isolates, the EAs ranged from 91.5% (penicillin) to 100% (cefotaxime, erythromycin, clindamycin, and levofloxacin), and the EA for ampicillin and vancomycin was 96.6%. The CAs ranged from 84.7% (penicillin) to 100% (erythromycin, clindamycin, and vancomycin). The CAs for ampicillin and meropenem were 88.1% and 86.5%, respectively, and the CA for cefotaxime and levofloxacin was 96.6%.

Of the total 134 isolates, all categorical errors were MIEs (Table 3). In the cases of 14 isolates for which MIEs were obtained for penicillin and/or ampicillin, MIEs for penicillin and ampicillin were obtained with 4 isolates (3 *S. mitis* isolates, 1 *S. anginosus* isolate); MIE only for penicillin was obtained with 6 isolates (4 *S. mitis* isolates, 1 *S. anginosus*

Table 1. Results of MIC ranges and interpretive category obtained by using the reference method for 134 BHS (75) and VGS (59) isolates

Antimicrobial agents	Organisms	MIC (mg/L) results			% of interpretive category		
		MIC range	MIC50	MIC90	S	I	R
Ampicillin	BHS	0.03-0.25	0.06	0.06	100		
	VGS	0.03-128	0.25	16	42.4	42.4	15.2
Penicillin	BHS	0.03-0.12	0.03	0.06	100		
	VGS	0.03-128	0.12	16	40.7	39.0	20.3
Cefotaxime	BHS	0.03-0.25	0.03	0.03	100		
	VGS	0.03-512	0.12	8	78.0	1.7	20.3
Meropenem	BHS	0.03-0.25	0.03	0.03	100		
	VGS	0.03-64	0.12	4	71.2	-*	-*
Erythromycin	BHS	0.03-512	0.03	256	72.0	4.0	24.0
	VGS	0.03-512	0.06	256	61.0		39.0
Clindamycin	BHS	0.03-512	0.06	256	63.7	2.6	34.7
	VGS	0.03-512	0.03	128	78.0		22.0
Levofloxacin	BHS	0.25-32	0.5	0.5	93.3		6.7
	VGS	0.25-32	0.5	2	86.4		13.6
Vancomycin	BHS	0.12-1	0.5	0.5	100		
	VGS	0.12-1	0.25	0.5	100		

*Any interpretive category other than susceptible was classified as a nonsusceptible category when meropenem was tested for VGS.

Abbreviations: MIC, minimal inhibitory concentration; S, susceptible; I, intermediate; R, resistant; BHS, β-hemolytic streptococci; VGS, viridans group streptococci.

Table 2. Comparison of MICs determined by using MICroSTREP with MICs determined using the CLSI reference method for 134 BHS (75) and VGS (59) isolates

Antimicrobial agents	Organisms	N of MICs by MICroSTREP within indicated log2 of reference MICs							% agreement (±1 dilution)
		-3	-2	-1	Same	+1	+2	+3	
Ampicillin	BHS			3	64	7	1		98.7
	VGS		2	10	39	8			96.6
	Total		2	13	103	15	1		97.8
Penicillin	BHS			25	43	7			100
	VGS		5	27	26	1			91.5
	Total		5	52	69	8			96.3
Cefotaxime	BHS				75				100
	VGS			8	48	3			100
	Total			8	123	3			100
Meropenem	BHS				75				100
	VGS		2	22	32	2		1	94.9
	Total		2	22	107	2		1	100
Erythromycin	BHS	3	16	55		1			94.7
	VGS			9	50				100
	Total	3	25	105		1			93.2
Clindamycin	BHS			15	59				98.7
	VGS			4	54	1			100
	Total			19	113	1			99.3
Levofloxacin	BHS			3	52	20			100
	VGS			11	42	6			100
	Total			14	94	26			100
Vancomycin	BHS			3	64	7	1		98.7
	VGS		2	10	39	8			96.6
	Total		2	13	103	15	1		97.8

Abbreviations: MIC, minimal inhibitory concentration; MICroSTREP, MicroScan MICroSTREP plus antimicrobial panel; BHS, β-hemolytic streptococci; VGS, viridans group streptococci.

Table 3. Interpretive category errors determined by comparing MICroSTREP and reference MICs for 134 BHS (75) and VGS (59) isolates

Antimicrobial agents	Organisms	N (%) interpretive category discrepancies	
		Complete agreement	Minor error
Ampicillin	BHS	74 (98.7)	1 (1.3)
	VGS	52 (88.1)	7 (11.9)
	Total	126 (94.0)	8 (6.0)
Penicillin	BHS	74 (98.7)	1 (1.3)
	VGS	50 (84.7)	9 (15.3)
	Total	124 (92.5)	10 (7.5)
Cefotaxime	BHS	75 (100)	
	VGS	57 (96.6)	2 (3.4)
	Total	132 (98.5)	2 (1.5)
Meropenem	BHS	75 (100)	
	VGS	51 (86.5)	
	Total	126 (94.0)	-*
Erythromycin	BHS	72 (96.0)	3 (4.0)
	VGS	59 (100)	
	Total	131 (92.4)	3 (2.2)
Clindamycin	BHS	74 (98.7)	1 (1.3)
	VGS	59 (100)	
	Total	133 (99.3)	1 (0.7)
Levofloxacin	BHS	75 (100)	
	VGS	57 (96.6)	2 (3.4)
	Total	133 (99.3)	1 (0.7)
Vancomycin	BHS	75 (100)	
	VGS	57 (100)	
	Total	134 (100)	

*Minor errors were not evaluated because any interpretive characteristic other than susceptibility was classified nonsusceptibility when meropenem was tested for VGS.
Abbreviations: MICroSTREP, MicroScan MICroSTREP plus antimicrobial panel; MIC, minimal inhibitory concentration; BHS, β-hemolytic streptococci; VGS, viridans group streptococci.

isolate, and 1 *S. agalactiae* isolate); and MIE only for ampicillin was obtained with 4 isolates (2 *S. mitis* isolates, 1 *S. anginosus* isolate, 1 *S. agalactiae* isolate). The frequency of total MIEs obtained with BHS isolates was lower than that obtained with VGS isolates (Table 4).

DISCUSSION

Healthcare professionals are faced with a variety of significant, fastidious organisms, including *S. pneumoniae*, BHS, and VGS, that are increasingly showing resistance to commonly used antimicrobial agents. Although, in the past, many laboratories may have chosen to screen VGS isolated from normally sterile body sites, e.g. cerebrospinal fluid, blood, and bone, to determine penicillin resistance, the rapid spread of multidrug-resistant strains requires a more aggressive approach. In addition, certain antimicrobials (penicillin, ampicillin, ertapenem, meropenem, and daptomycin) that may be used to treat VGS infections cannot be reliably tested using the disk diffusion method [9]. The CLSI also recommends the inclusion of penicillin (or ampicillin), cefepime (cefotaxime or ceftriaxone), erythromycin, clindamycin, and vancomycin in a routine, primary testing panel [9].

The accuracy and efficiency of AST dictates timely and appropriate decisions in choosing the antibiotic therapy. Automated systems with built-in expert systems can potentially increase the reproducibility and reliability of test results, and thus can be expected to improve the quality of patient care. In this study, penicillin-intermediate, and/or ampicillin-intermediate, and VGS isolates that were not susceptible to meropenem accounted for the bulk of the total MIEs. Despite the elevated frequency of MIEs obtained with VGS, the high EA values for penicillin, ampicillin, and meropenem suggest that these errors can be largely attributed to the MICs being close to the interpretive breakpoints (Table 4). In particular, all categorical errors for meropenem occurred when the MICroSTREP MIC was 2-fold dilution lower than the reference. The overall frequency of MIEs obtained with BHS was lower than that obtained with VGS, but MIEs for erythromycin and clindamycin were only detected with BHS. The highest MIE rates obtained for penicillin were similar to those reported by Guthrie et al. [12], who reported that penicillin was responsible for the highest MIE rate obtained with *S. pneumoniae*. When the reference MIC was close to a breakpoint value, 2 MIEs were observed for several isolates [13]. For most antibiotics, the MICroSTREP MICs tended to be lower than the reference MICs, which greatly contributed to the number of MIEs. The exceptions were the MIC results for ampicillin, levofloxacin, and vancomycin obtained with BHS: MICroSTREP reported higher MICs in these cases (Table 2). No VMEs or MEs were detected, and relatively few MIEs were observed. The only observed exceptions to the performance standards were categorical disagreement in cases of ampicillin, penicillin, and meropenem for VGS. The more the isolate population was concentrated near the MIC breakpoint level, the greater the possibility of categorical discrepancies between the results obtained using the testing instrument and the reference method. In contrast, the more the isolate population was distributed far away from MIC breakpoint level, the lesser the possibility of categorical discrepancies. Although diagnostic performance of the MIC testing system in BHS and VGS has not been reported, Jorgensen et al. [14] reported that MICroSTREP did not result in any VMEs or MEs and that high EA and CA

Table 4. Difference (log2 dilution) in MICs determined using MICroSTREP and reference method for 27 isolates showing categorical error by interpretive criteria

Organisms	MIC difference (log2 dilution) according to antimicrobial agents						
	Penicillin	Ampicillin	Cefotaxime	Meropenem	Erythromycin	Clindamycin	Levofloxacin
S. pyogenes					-2 (SI*)	-3 (SI)	
S. agalactiae	+1 (IS†)						
S. agalactiae		+2 (IS)					
S. dysgalactiae					-2 (SI)		
S. dysgalactiae					-2 (SI)		
S. mitis	-1 (SI)	-1 (SI)					
S. mitis	-1 (SI)	-1 (SI)					
S. mitis	-1 (SI)	+1 (IS)					
S. mitis	-2 (SI)		-1 (IR‡)				
S. mitis	-2 (IR)						
S. mitis	-1 (SI)						
S. mitis	+1 (IS)						
S. mitis		-1 (SI)					
S. mitis		-1 (SI)		-1 (SNS§)			
S. mitis				-1 (SNS)			
S. mitis				-1 (SNS)			
S. mitis				-1 (SNS)			
S. mitis				-1 (SNS)			
S. mitis				-1 (SNS)			
S. mitis							
S. mitis							-1 (SI)
S. mitis							-1 (SI)
S. anginosus	-1 (SI)	+1 (IS)					
S. anginosus		-2 (SI)		-1 (SNS)			
S. anginosus							
S. sanguinis	-1 (SI)			-1 (SNS)			
S. sanguinis		-1 (SI)					

*judged susceptible using MICroSTREP and intermediate using the reference method; †judged intermediate using MICroSTREP and susceptible using the reference method; ‡judged intermediate using MICroSTREP and resistant using the reference method; §judged susceptible using MICroSTREP and non-susceptible using the reference method.
Abbreviations: MIC, minimal inhibitory concentration; MICroSTREP, MicroScan MICroSTREP plus antimicrobial panel; BHS, β-hemolytic streptococci; VGS, viridans group streptococci.

values were achieved in testing with *S. pneumoniae*. The high EA and CA values obtained by using MICroSTREP satisfied the minimal performance criteria (CA, ≥90%; EA, ≥90%; VMEs, ≤1.5%; and MEs, ≤3%) of the Food and Drug Administration (FDA) [15]. The FDA suggested that 50% susceptible and 50% resistant distribution is desired to generate meaningful statistics regarding the performance characteristics of a method or system [15]. However, it was unlikely that sequentially collected isolates from clinical samples would be anything similar to the 50% susceptible and 50% resistant distribution suggested by the FDA.

The performance of MICroSTREP affirms the capability of the instrument as a reliable and efficient diagnostic tool for determining the appropriate antimicrobial agents for use against VGS and BHS infections. In conclusion, this instrument was able to decrease the turnaround time to results because of the reduced hands-on time required in comparison to that required in conventional laboratory methods.

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