Performance Evaluation of a Newly Developed and Fully Automated Bacteriological Analyzer "RAISUS ANY" for Antimicrobial Susceptibility Testing of Fastidious Bacteria *Haemophilus influenzae* and *Streptococcus pneumoniae*

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(Received October 24, 2016; Accepted February 1, 2017)

Objective: Antimicrobial susceptibility testing for fastidious bacteria, such as *Haemophilus influenzae* (*H. influenzae*) and *Streptococcus pneumoniae* (*S. pneumoniae*) has been performed manually. We evaluated the performance of a newly developed fully automated system for rapid bacterial identification and antimicrobial susceptibility testing "RAISUS ANY" (Nissui Pharmaceutical Co., Ltd.).
Methods: We evaluated the performance of "RAISUS ANY" for measurement of minimal inhibitory concentrations (MICs) of *H. influenzae* and *S. pneumoniae*, in comparison with the manual method (DP34, Eiken Chem. Co., Ltd.). The repeatability of MICs was studied using the reference strain of these bacteria, obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA).
Results: The comparison with the manual method for 35 and 36 clinical strains of *H. influenzae* and *S. pneumonia* showed 62.9–100% and 86.1–100% agreement, respectively. Five of 35 *H. influenzae* strains that showed a trailing effect were stably and accurately measured for MICs without a variation among the examiners.
Conclusion: In conclusion, the automated system "RAISUS ANY" provided a reliable MICs data for *H. influenzae* and *S. pneumonia*, suggesting its improvement in performance and reliability for routine antimicrobial susceptibility testing in clinical bacteriological laboratories.

Key words: fully automated system "RAISUS ANY", *Haemophilus influenzae*, *Streptococcus pneumoniae*

INTRODUCTION

Haemophilus influenzae (*H. influenzae*) and *Streptococcus pneumonia* (*S. pneumoniae*) are well-known fastidious pathogens that often cause pneumonia, and less frequently meningitis and bloodstream infections that could be life-threatening without the proper administration of the antimicrobial agents [1, 2]. Recently these pathogens showed a tendency of resistance against antimicrobial agents, such as penicillin [3]. Therefore, an accurate susceptibility testing has become increasingly important. Nowadays the measurement of antimicrobial susceptibility for the most of popular pathogens has been automated [4]. On the other hand, that has been manually performed for the fastidious bacteria, such as *H. influenzae* and *S. pneumonia* [5]. This manual method enforces a labor burden in the laboratory, and more importantly, the results have a variation among the examiners because of their visual judgment. These pathogens require NAD and iron for their growth [6]. Hematin and hemin are used as a source of hemoglobin, therefore a variety of susceptibility testing media have been developed and used with different prescription of these growth factors. This has raised an issue of inter-laboratory differences in the measurement results [7]. "RAISUS ANY" is a fully automated system for rapid bacterial identification and antimicrobial susceptibility testing, newly developed on the basis of precedent system "RAISUS". An oxidation-reduction color dye, Redox, was supplemented into *Haemophilus* Test Medium (HTM) broth for the automated measurement of MICs, which allowed us to measure bacterial growth with accuracy and a higher sensitivity. For the stable detection of *Streptococcus spp.*, horse serum was used instead of lysed horse blood (LHB). In this study, we have evaluated the performance of the "RAISUS ANY" in comparison with the manual methods in measurement of antimicrobial susceptibility of *H. influenzae* and *S. pneumoniae.*

MATERIAL AND METHODS

Bacterial samples

Bacterial samples used in this study were 35 clinical isolates of *H. influenzae* derived from specimens submitted to the clinical laboratory of Tokai University Hospital, including sputum (17), nasal mucus (13), pharyngeal mucus (4) and otorrhea (1), and 36 clinical isolates of *S. pneumoniae* derived from sputum (18), nasal mucus (12), blood (3), pharyngeal mucus (1), bronchoalveolar lavage (1) and vaginal discharge (1).

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Table 1 Within-run reproducibility of minimal inhibitory concentrations of *H. influenzae* and *S. pneumonia* by "RAISUS ANY"

1-1) *H. influenzae* (ATCC 49247)

Antimicrobial agent	Repeatability (n = 10)			
	Management range* (μg/mL)	Value (μg/mL)	CLSI category	Agreement
ABPC	2-8	2	I	100% (10/10)
CTRX	≤1	≤1	S	100% (10/10)
CCL		8	S	60.0% (6/10)
IPM		≤0.5	S	100% (10/10)
MEPM		0.25	S	100% (10/10)
LVFX	≤1	≤1	S	100% (10/10)
CAM	≤4-16	8	S	100% (10/10)

1-2) *S. pneumoniae* (ATCC 49619)

Antimicrobial agent	Repeatability (n = 10)			
	Management range* (μg/mL)	Value (μg/mL)	CLSI category	Agreement
PCG	0.25-1	0.25	S	100% (10/10)
CCL	1-4	1-2	S	80.0% (8/10)
MEPM	0.06-0.25	≤0.125	S	100% (10/10)
IPM	0.03-0.12	≤0.063	S	100% (10/10)
VCM	0.12-0.5	≤0.125	S	100% (10/10)
CLDM	0.03-0.12	≤0.25	S	100% (10/10)
LVFX	0.5-2	1	S	100% (10/10)

Management ranges*: the acceptable limits for QC strains when testing *Haemophilus* sp. on HTM (*Haemophilus* Test Medium). The repeatability of minimal inhibitory concentrations of *H. influenzae* and *S. pneumonia* by "RAISUS ANY" was studied using 10 samples of these bacteria (ATCC 49247 and ATCC 49619, respectively).

For the study of within-run reproducibility, reference strains of *H. influenzae* (ATCC 49247) and *S. pneumoniae* (ATCC 49619) were used.

The fully automated bacteriological instrument "RAISUS ANY"

The "RAISUS ANY" system is a fully automated instrument starting from the sample inoculation onto a microtiter plate, culture, identification, and MICs determination, until the acquisition of the test result once the prepared sample solution is set to a McFarland standard of 0.5 [1]. It identifies the bacterial strains using a fluorescent reagent (enzyme substrate or fluorescence indicator), and detects antimicrobial susceptibility using an oxidation-reduction indicator, Redox. Antimicrobial reagents used for MICs testing were as follows: imipenem (IPM), meropenem (MPEM), penicillin G (PCG), ampicillin (ABPC), vancomycin (VCM), levofloxacin (LVFX), clarithromycin (CAM), cefaclor (CCL) and ceftriaxone (CTRX).

The manual method of antimicrobial susceptibility testing

For comparison, a manual dry plate method, based on DP34 was used [8]. Dry microtiter plates using Mueller-Hinton broth for MICs measurement were prepared according to CLSI (Clinical Laboratory Standards Institute) broth microdilution susceptibility test standards [6]. The MICs were determined by broth microdilution methods according to CLSI criteria [6] (CLSI M45-A). Medical technologists with an experience of more than 5 years visually evaluated the results.

RESULTS

The within-run reproducibility

The within-run reproducibility of MICs for 10 samples from the reference strains of these bacteria revealed to be satisfactory (100% agreement), except for CCL (60% 6/10) (Table 1).

The comparison with the manual method

The comparison with the manual method of the MICs for 35 and 36 clinical isolates of *H. influenzae* and *S. pneumonia* strains, respectively showed 62.9-100% and 86.1-100% agreements as defined by CLSI M45-A document categories (Table 2). The visual evaluation of the MICs for 5 *H. influenzae* strains was problematic because of a trailing effect. In such cases, the endpoint was taken for the MICs at the well with the highest concentration of reagent where the trailing begins. There was also a similar tendency in the CCL measurement of *S. pneumonia* strains (86.1%, 31/36). The automated system did not take the minuscule spots of bacterial growth consistently (Figure A). As shown in Figure B, a magnified image revealed a minuscule spot of bacterial growth in a series of CCL dilution. This resulted in the visual misjudgment of MICs value higher by 3 of 5 examiners (4-16 μg/mL).

Table 2 The distribution of MICs value of the clinical isolates strains of *H. influenzae* and *S. pneumonia*

2-1) *H. influenzae* (n = 35)

		MICs (μg/mL)								Agreement rate by CLSI category*
		≤ 0.12	0.25	0.5	1	2	4	8	≤ 16	
MEPM	RAISUS	14	14	7						97.1% (34/35)
	DP34	19	14	1	1					
CTRX	RAISUS				35					100% (35/35)
	DP34	16	15	4						
LVFX	RAISUS				35					100% (35/35)
	DP34				35					
IPM	RAISUS			14	12	7	2			91.4% (32/35)
	DP34	3		11	12	4	5			
ABPC	RAISUS			6	6	11	7	2	3	88.6% (31/35)
	DP34	2	2	2	6	12	11			
CAM	RAISUS						15	13	7	85.7% (30/35)
	DP34						1	16	18	
CCL	RAISUS					5	4	2	24	62.9% (22/35)
	DP34			3		4	5	5	18	

2-2) *S. pneumoniae* (n = 36)

		MICs (μg/mL)							Agreement by CLSI category*
		≤ 0.06	0.12	0.25	0.5	1	2	4	≥ 8	
IPM	RAISUS	32	2	2						100% (36/36)
	DP34	33	3	0						
PCG	RAISUS	28	0	5	0	1	2			88.9% (32/36)
	DP34	28	2	0	6	0				
VCM	RAISUS			27	8	1				100% (36/36)
	DP34			28	8	0				
MEPM	RAISUS	30	2	1	2	1				94.4% (34/36)
	DP34		34	2	0	0				
LVFX	RAISUS				6	29	0	1		91.7% (33/36)
	DP34					28	4	4		
CLDM	RAISUS			13	23					88.9% (32/36)
	DP34			12	24					
CCL	RAISUS				7	13	6	6	4	86.1% (31/36)
	DP34				19	4	7	4	3	

Agreement by CLSI category*: the agreement between the automated and the manual methods are shown according to CLSI category.

DISCUSSION

We evaluated the performance of a newly developed fully automated bacteriological analyzer "RAISUS ANY" for antimicrobial susceptibility testing of fastidious bacteria *H. influenzae* and *S. pneumoniae*. The within-run reproducibility of measurement for *H. influenzae* and *S. pneumoniae* using ATCC reference strains revealed 100% agreement, except for CCL (Table 1). The excellent repeatability was considered as a result of a fully automated process, starting from the inoculation onto a microtiter plate, culture, identification, and a MICs acquisition. Furthermore, an integration of the turbidity meter into the instrument and automated measurement of the absorbance values provides an accurate and consistent number of bacteria. The "RAISUS ANY" measurement results of the clinical isolate strains of *H. influenzae* and *S. pneumoniae* showed a high coincidence with the manual method (DP34). However, *S. pneumoniae* showed a tendency of slightly higher MICs value as a whole [9]. This can be ascribed to that the chemical emission of coloring starts before visual detection of bacteria growth. The drug susceptibility tests of *H. influenzae* for CCL also brought in a lower coincidence than others. We suggest that this is an influence of a trailing effect phenomenon in the combination of *H. influenzae* and a CCL antimicrobial agent. The trailing effect is frequently observed in measurement of antimicrobial susceptibility for any combination of bacterial species and antimicrobial reagents. This phenomenon is a tiny or thin film of bacterial growth at the bottom of the microplate wells, resulting in an unclear endpoint and thus making it difficult to judge visually. The MICs value measured by "RAISUS ANY" was repeatable and stable without a variation among the examiners [10] (Figure A). The repeatability of an accurate and reliable MICs measurements together with a fully automation of the process would have a positive impact on the efficient operation of clinical bacteriological

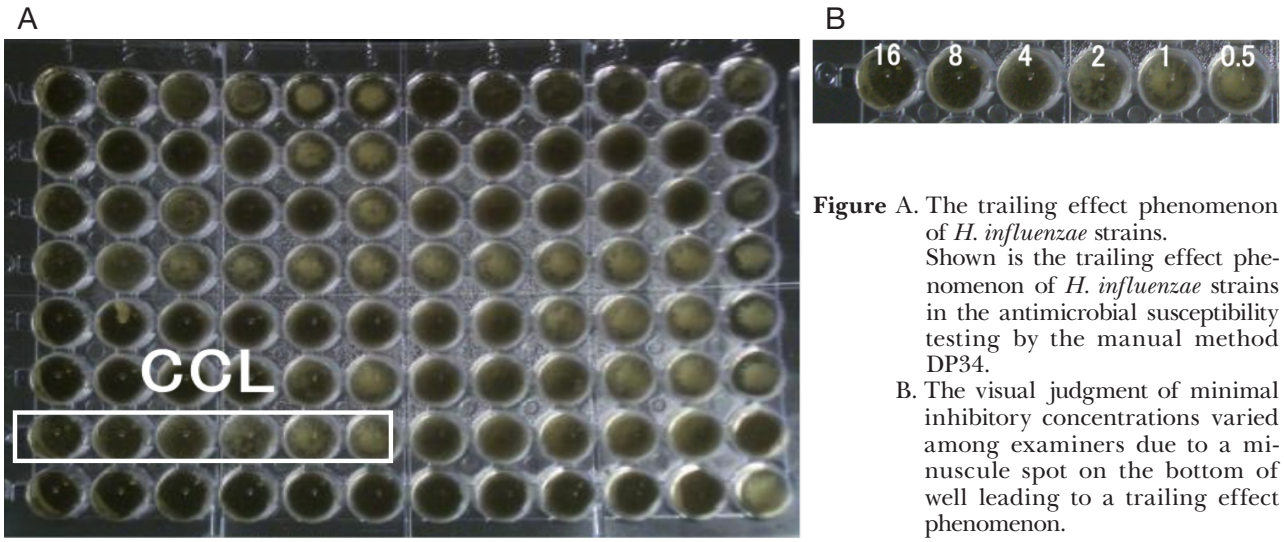

Figure A. The trailing effect phenomenon of *H. influenzae* strains.
Shown is the trailing effect phenomenon of *H. influenzae* strains in the antimicrobial susceptibility testing by the manual method DP34.
B. The visual judgment of minimal inhibitory concentrations varied among examiners due to a minuscule spot on the bottom of well leading to a trailing effect phenomenon.

laboratories. Furthermore, with the routine use of the "RAISUS ANY", it would be possible to standardize the MICs data among the laboratories and perform reliable surveillance monitoring for the MICs trends in these bacteria. In conclusion, in comparison with the manual method the new fully automated "RAISUS ANY" system showed improved performance and reliability of routine antimicrobial susceptibility testing of fastidious bacilli *H. influenzae* and *S. pneumoniae*.

ACKNOWLEDGEMENT

The authors thank Dr. Anar Damdinsuren for providing help with English editing of the manuscript.

All authors declare no conflict of interests.

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