31st ECCMID Online 9 – 12 July 2021
ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Evaluation of multiple methods for determining the linezolid minimum inhibitory concentration in clinical isolates of methicillin-resistant *Staphylococcus aureus*

J. Sasaki[1], S. Ishigaki [1], M. Matsumura [2], M. Asahara [1], Y. Atsukawa [1], S. Yonetani [3], Y. Ono [4], T. Furukawa [5]
[1] Teikyo University Hospital, Department of Clinical Laboratory, Tokyo, Japan, [2] Teikyo University, Department of Clinical Laboratory Science, Faculty of Medical Technology, Tokyo, Japan , [3] Kyorin University, Department of Medical Technology faculty of Health Scienecs, Tokyo, Japan , [4] Teikyo University School of Medicine, Department of Microbiology & Immunology, Tokyo, Japan, [5] Teikyo University School of Medicine, Department of Laboratory Medicine, Tokyo, Japan

TEIKYO

ABSTRACT

Background: At Teikyo University Hospital, the antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates is tested using MicroScan WalkAway 96 Plus system (Beckman Coulter) with the Prompt inoculation method, based on the Clinical and Laboratory Standards Institute (CLSI) breakpoints. Recently, increasing frequencies of MRSA isolates with a linezolid minimum inhibitory concentration (MIC) of 4 μg/mL, indicated increasing upper limits of linezolid sensitivity. We thus investigated the actual frequency of MRSA isolates with linezolid MIC of 4 μg/mL and evaluated their accuracy.

INTRODUCTION

Linezolid is an important drug in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Linezolid does not display cross-resistance with other antibacterial agents; consequently, the acquisition of resistance is rare. However, in our hospital, since 2010, MRSA isolates with linezolid minimum inhibitory concentration (MIC) of 4 μg/mL have been obtained with an increasing frequency, indicating that the upper limits of linezolid sensitivity are increasing. Therefore, we investigated the actual frequency of MRSA isolates with linezolid MIC of 4 μg/mL and evaluated their accuracy using multiple methods.

MATERIALS AND METHODS

1. Frequency of MRSA isolates with linezolid MICs of 4 μg/mL

- In total, 3,377 MRSA strains were isolated from 2010 to 2018 (one strain per patient each year).
- Strains were analysed using the MicroScan WalkAway 96 Plus system with the Prompt inoculation method (Table 1 summarizes the types of panels used).
- Frequency of strains with linezolid MIC of 4 μg/mL was determined.

2. Linezolid MIC was re-evaluated using multiple antimicrobial susceptibility testing methods and measurement instruments

- In total, 40 cryopreserved strains (in skim milk at -80 ℃) with linezolid MIC of 4 μg/mL were selected. Strains were subcultured three times using trypticase soy agar (TSA) containing 5% sheep blood for 24 hours at 35 ℃.
- Linezolid MIC was re-evaluated using turbidity inoculation and agar dilution methods, and VITEK 2, BD Phoenix M50, and DPS192iX systems (Table 1 summarizes the types of panels used).
- *Staphylococcus aureus* ATCC29213 was used for quality control tests, with an expected linezolid MIC of 1-4 μg/mL by CLSI M100-ED30.

Table 1. Instruments used for measuring antimicrobial susceptibility in this study

Instruments	Company	MIC Panel （Period of use）	Range of MIC for LZD
MicroScan WalkAway 96 Plus	Beckman Coulter	Pos Combo 6.1J（2010.1-12）	≤ 2～ > 4
		Pos Combo 3.1J（2011.1-2015.3）	≤ 2～ > 4
		Pos MIC 3.3J（2015.4-2018.12）	≤ 0.5～ > 4
DPS192iX	Eiken chemical	Dry Plate'Eiken'EP22	≤ 0.12～ > 4
BD Phoenix M50	BD Japan	PMIC-85	≤ 0.5～ > 8
VITEK2	Biomerieux Japan	AST-P625	≤ 0.5～ > 8

RESULTS

1. Frequency of MRSA isolates with linezolid MIC of 4 μg/mL (Figure 1)

- The number of MRSA isolates at our hospital decreased by approximately 20% from 2010 to 2011, then by approximately 10% annually until 2015.
- The number of MRSA isolates with linezolid MIC of 4 μg / mL increased from 8 in 2010 to 75 in 2014.
- The isolation rate of MRSA strains with a linezolid MIC of 4 μg/mL increased from 1.4% (8/568) in 2010 to 24.4% (75/307) in 2014 and was maintained thereafter at approximately 10%-15%.

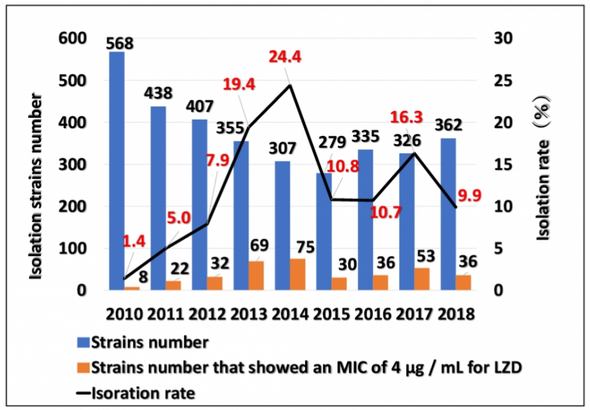

Figure 1. The transition in the isolation rate and strains Number that showed an MIC of 4 μg / mL for LZD in MRSA

RESULTS (CONTINUED)

2. Linezolid MIC re-evaluated using multiple antimicrobial susceptibility testing methods and measurement instruments (Tables 2 and 3)

- The MIC of all 40 strains was ≤2 μg/mL when re-measured using turbidity inoculation and agar dilution methods.
- The MIC of all 40 strains was also ≤2 μg/mL when re-measured using the DPS192iX and BD Phoenix M50 systems.
- The MIC of one strain was 4 μg/mL and that of the remaining 39 strains was ≤2 μg/mL when re-measured using the VITEK2 system.
- The MIC of *S. aureus* ATCC29213 quality control strain was 4 μg / mL for the Prompt inoculation method, 2 μg / mL for the turbidity inoculation and agar dilution methods and VITEK2 system, and 1 μg / mL for the DPS192iX and BD Phoenix M50 systems.
- Compared with the MIC obtained using agar dilution, the MIC of all strains measured using the Prompt method was more than +1 doubling dilution. For other methods, the MIC was within ± 1 doubling dilution in all the strains. However, BD Phoenix M50 showed -1 doubling dilution in 27 strains.

Table 2. Comparison of MIC for LZD in multiple antimicrobial susceptibility testing methods and measuring instruments（n=40）

Instruments / Methods		MIC (μg/mL) 1	2	4
MicroScan WalkAway 96 Plus	Prompt inoculation	0	0	40
	Turbidity inoculation	7	33	0
DPS192iX		0	40	0
BD Phoenix M50		29	11	0
VITEK2		8	31	1
Agar dilution		2	38	0

Table 3. Comparison of MIC for LZD based on agar plate dilution with multiple antimicrobial susceptibility testing methods and measuring instruments（n= 40）

Instruments / Methods		Number of strains for with different MIC doubling dilution -2	-1	same	+1	+2
MicroScan WalkAway 96 Plus	Prompt inoculation	0	0	0	38	2
	Turbidity inoculation	0	7	31	2	0
DPS192iX		0	0	38	2	0
BD Phoenix M50		0	27	13	0	0
VITEK2		0	7	31	2	0

CONCLUSIONS

- The frequency of MRSA isolates with a linezolid MIC of 4 μg/mL was increased.
- The MIC of linezolid for MRSA isolates measured using the Prompt inoculation method with the WalkAway 96 Plus system is approximately +1 doubling dilution higher than that obtained using other methods and instruments.
- In most cases, a difference of ±1 doubling dilution is considered acceptable. However, the difference would increase to >2 doubling dilutions when other confounding factors are involved.
- Thus, it is important to perform various evaluations to understand the characteristics of different antimicrobial susceptibility testing methods and instruments.