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Note

Effect of sodium mercaptoacetic acid on different antimicrobial disks in the sodium mercaptoacetic acid double disk synergy test for detection of IMP-1 metallo-β-lactamase-producing *Pseudomonas aeruginosa* isolates in Japan☆

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ABSTRACT

We determined the optimal antimicrobial in the sodium mercaptoacetic acid double disk synergy test (SMA-DDST) for the detection of IMP-1-producing *Pseudomonas aeruginosa* isolates in Japan and evaluated the performance of the test.

Fifty-four *P. aeruginosa* clinical isolates were tested, including 39 IMP-1 producers and 15 non-metallo-β-lactamase (MBL)-producing carbapenem- and ceftazidime (CAZ)-resistant isolates. The SMA-DDST was performed with CAZ, cefepime (CFPM), imipenem (IPM), meropenem (MEPM), doripenem (DRPM), or biapenem (BIPM)-containing disks. The sensitivity of the SMA-DDST with CAZ, CFPM, IPM, MEPM, DRPM, and BIPM was 39/39 (100%), 36/39 (92%), 18/39 (46%), 8/39 (21%), 19/39 (49%), and 36/39 (92%), respectively. The specificity was 15/15 (100%) for all SMA-DDSTs. This suggests that the isolates may have a resistance mechanism other than MBL production for IPM, MEPM, or DRPM. Since the CAZ resistance mechanism in *P. aeruginosa* is the same as that of CFPM, but differs from that of carbapenems, we conclude that combining CAZ with BIPM SMA-DDSTs can prevent any failure in the detection of IMP-1-producing *P. aeruginosa*.

The prevalence of clinical isolates of *Pseudomonas aeruginosa* with high-level carbapenem resistance has been on the rise, posing an increasingly urgent public health concern. Carbepenem resistance is often attributed to the production of carbapenemases, primarily metallo-β-lactamases (MBLs). The most commonly used tests for the detection of MBLs include genetic methods, such as the polymerase chain reaction (PCR), and phenotypic methods, based on the inhibition of MBL activity by ethylenediaminetetraacetic acid and a thiol compound or dipicolinic acid in a double-disk synergy test (DDST) [1–3]. In Japan, sodium mercaptoacetic acid (SMA)-containing disks are commercially available for DDSTs that require no special device or operation, and are widely applicable in clinical laboratory testing due to their high economic efficiency and convenience. Moreover, mechanisms other than MBL production, such as the efflux system with *ampC* and *oprD* expression, have been shown to elevate the minimum inhibitory concentration (MIC) of carbapenem in *P. aeruginosa* [4]. Since the antimicrobials used in MBL detection should not be affected by mechanisms unrelated to MBL production, selecting the optimal antimicrobial disk for SMA-DDSTs is crucial. Therefore, the aim of the present study was to determine which antimicrobial disks to use in SMA-DDSTs for the detection of MBL-producing *P. aeruginosa* isolates in Japan.

A total of 54 non-repetitive (one per patient) clinical isolates, including 39 IMP-1-producing *P. aeruginosa* and 15 non-MBL-producing *P. aeruginosa*, were collected from 11 hospitals (A–K)

Abbreviations: BIPM, biapenem; CAZ, ceftazidime; CFPM, cefepime; DRPM, doripenem; IPM, imipenem; MEPM, meropenem; MIC, minimum inhibitory concentration; SMA-DDST, sodium mercaptoacetic acid double disk synergy test.

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in six prefectures in Japan during 2011–2016. All of them showed reduced susceptibility to ceftazidime (CAZ), cefepime (CFPM), carbapenems, imipenem (IPM), meropenem (MEPM), doripenem (DRPM), and/or biapenem (BIPM). The background MBL genotypes were characterized by PCR using a previously reported methodology [5,6]. Table S1 summarizes the characteristics of the isolates tested in this study. SMA-DDSTs were performed according to a previously published method using the following antimicrobial disks: CAZ 30 μg/disk, CFPM 30 μg/disk, IPM 10 μg/disk, MEPM 10 μg/disk, DRPM 10 μg/disk, or BIPM 10 μg/disk (Eiken Chemical Co., Tokyo, Japan) [7]. Performance of each antimicrobial disk in the SMA-DDST for the detection of IMP-1-producing *P. aeruginosa* was evaluated against PCR as the gold-standard method [5,6]. Sensitivity was calculated from the number of correctly determined IMP-1-producing *P. aeruginosa* isolates, whereas specificity was calculated from the number of correctly determined non-MBL-producing *P. aeruginosa* isolates.

Table 1 shows the performance of each antimicrobial disk in SMA-DDST. While the sensitivity of six SMA-DDSTs ranged from 21% to 100%, specificity was 100% in all tests. Fig. 1 shows the extent of contribution of SMA to the expansion of inhibition zone produced by six antimicrobials around the IMP-1-producing isolates.

Although the package insert of the Metallo-β-lactamase SMA Eiken kit recommends the use of CAZ or IPM disks in the DDST, a previous study demonstrated compromised detection of MBL-positive isolates of *Enterobacteriaceae* by CAZ and IPM SMA-DDSTs, and increased detection by MEPM SMA-DDSTs [8]. However, only the performance of CAZ or IPM SMA-DDST in the detection of MBL-positive *P. aeruginosa* has been evaluated till date [9,10]. Thus, in this study, we re-evaluated the detection of IMP-1-producing *P. aeruginosa* in Japan using selective antimicrobial disks for SMA-DDST.

The CAZ SMA-DDST showed 100% sensitivity with stable expansion of inhibition zone by SMA. In *P. aeruginosa*, CAZ resistance is usually affected by de-repression of AmpC β-lactamase production; thus, the IMP-1-producing isolates, tested in this study, might lack this CAZ-resistance mechanism [4]. However, we believe that isolates that de-repress AmpC β-lactamase production may emerge in the future. In addition, the CFPM and BIPM SMA-DDSTs also showed high sensitivities (92%). Since the mechanism of CAZ resistance in *P. aeruginosa* is the same as that for CFPM resistance, but is distinct from that of carbapenem resistance, our results suggest that the combination of CAZ and BIPM SMA-DDSTs may effectively avoid cross-resistance and reduce failure to detect isolates having CAZ resistance mechanisms other than carbapenemase production [4].

In contrast, the IPM, MEPM, and DRPM SMA-DDSTs showed low sensitivity (<50%), the MEPM SMA-DDST having the lowest. This finding contradicts the previous study that evaluated the performance of SMA-DDSTs for *Enterobacteriaceae* [8]. Hence, our result indicated that the optimal choice of an antimicrobial disk for SMA-DDSTs differs between the evaluation of *Enterobacteriaceae* and that of *P. aeruginosa*. The *P. aeruginosa* isolates may utilize a different resistance mechanism for IPM, MEPM, and DRPM, such as the loss of porin and up-regulation of efflux systems [4,11–13]. In this context, we believe that the IPM, MEPM, and DRPM SMA-DDSTs would have reduced utility.

Previous studies demonstrated that BIPM and IPM have similar effects against *P. aeruginosa* [11–14]. However, in the present study, the BIPM SMA-DDST showed higher sensitivity than the IPM SMA-DDST. Further study would be required to determine the factors contributing to this difference.

Unfortunately, we were unable to test more diverse MBL types in this study owing to the epidemiology related to our region. Hence,

Table 1
Summary of sodium mercaptoacetic acid double disk synergy test (SMA-DDST) results.

Isolate types (*n*)	Number (%) of positive isolates by the SMA-DDST with:					
	Ceftazidime	Cefepime	Imipenem	Meropenem	Doripenem	Biapenem
IMP-1 producers (39)	39 (100)	36 (92)	18 (46)	8 (21)	19 (49)	36 (92)
Non-MBL producers (15)	0	0	0	0	0	0

Abbreviation: MBL, metallo-β-lactamase.

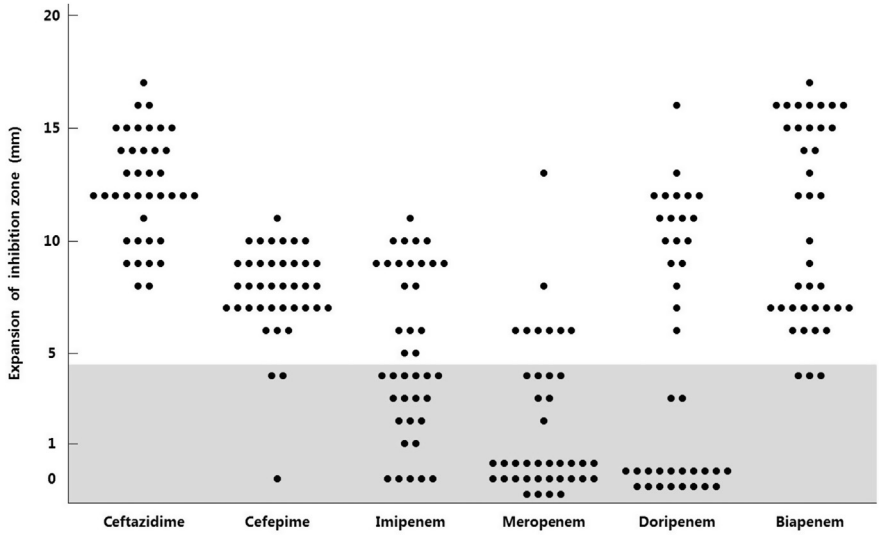

Fig. 1. Expansion of the inhibition zone (mm) by sodium mercaptoacetic acid (SMA) in SMA double disk synergy tests (SMA-DDSTs) using antimicrobials. The zones with grey background represent negative result of the SMA-DDST.

the main limitation of this study was that only MBL-producing isolates, harboring IMP-1, were tested. Thus, our finding might not be applicable to other MBL-producing *P. aeruginosa* strains, such as those producing VIM or NDM.

In conclusion, our results strongly suggest that IPM, MEPM, or DRPM SMA-DDSTs are inappropriate for the detection of IMP-1-producing *P. aeruginosa* strains. Further, our results indicate that CAZ, combined with BIPM SMA-DDSTs, can prevent failure to detect IMP-1-producing *P. aeruginosa*.

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Conflicts of interest

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jiac.2018.07.005.

References

[1] Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. Clin Microbiol Infect 2001;7:88–91.
[2] Arakawa Y, Shibata N, Shibayama K, Kurokawa H, Yagi T, Fujiwara H, et al. Convenient test for screening metallo-β-lactamase-producing gram-negative bacteria by using thiol compounds. J Clin microbial 2000;38:40–3.
[3] Kimura S, Ishii Y, Yamaguchi K. Evaluation of dipicolinic acid for detection of IMP- or VIM-type metallo-β-lactamase–producing *Pseudomonas aeruginosa* clinical isolates. Diagn Microbiol Infect Dis 2005;53:241–4.
[4] Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin Infect Dis 2002;34:634–40.
[5] Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119–23.
[6] Notake S, Matsuda M, Tamai K, Yanagisawa H, Hiramatsu K, Kikuchi K. Detection of IMP metallo-β-lactamase in carbapenem-nonsusceptible *Enterobacteriaceae* and non-glucose-fermenting Gram-negative rods by immunochromatography assay. J Clin Microbiol 2013;51:1762–8.
[7] Sakanashi D, Kawachi M, Uozumi Y, Nishio M, Hara Y, Suematsu H, et al. Evaluation of commercial phenotypic assays for the detection of IMP- or New Delhi metallo-β-lactamase-producing *Enterobacteriaceae* isolates in Japan. J Infect Chemother 2017;23:474–80.
[8] Sakanashi D, Kawachi M, Uozumi Y, Nishio M, Hara Y, Suematsu H, et al. Evaluation of the modified sodium mercaptoacetic acid double disk synergy test for detecting the metallo-β-lactamase producing *Enterobacteriaceae*. Jpn J Antibiot 2017;70:155–65.
[9] Picao RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, et al. Metallo-β-lactamase detection: comparative evaluation of double-disk synergy versus combined disk tests for IMP-, GIM-, SIM-, SPM-, or VIM-producing isolates. J Clin Microbiol 2008;46:2028–37.
[10] Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J Clin Microbiol 2003;41:4623–9.
[11] Chen HY, Livermore DM. In-vitro activity of biapenem, compared with imipenem and meropenem, against *Pseudomonas aeruginosa* strains and mutants with known resistance mechanisms. J Antimicrob Chemother 1994;33:949–58.
[12] Iyobe S, Watanabe M, Mitsuhashi S, Inoue M. Estimation of outer membrane permeability of carbapenem antimicrobials to *Pseudomonas aeruginosa*. J Infect Chemother 1999;5:168–70.
[13] Okamoto K, Gotoh N, Nishino T. Alterations of susceptibility of *Pseudomonas aeruginosa* by overproduction of multidrug efflux systems, MexAB-OprM, MexCD-OprJ, and MexXY/OprM to carbapenems: substrate specificities of the efflux systems. J Infect Chemother 2002;8:371–3.
[14] Yang Y, Bhachech N, Bush K. Biochemical comparison of imipenem, meropenem and biapenem: permeability, binding to penicillin-binding proteins, and stability to hydrolysis by β-lactamases. J Antimicrob Chemother 1995;35:75–84.