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A highly sensitive carbapenemase assay using laser desorption/ionization mass spectrometry based on a parylene-matrix chip

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ABSTRACT

A quantitative carbapenemase assay was developed using laser desorption/ionization mass spectrometry (LDI-MS) based on a parylene-matrix chip. As a first step, the reproducibility (spot-to-spot, shot-to-shot, and day-to-day) of LDI-MS based on a parylene-matrix chip and the quantification ranges for four carbapenem antibiotics (doripenem, ertapenem, imipenem, and meropenem) were determined. A carbapenem-susceptibility test was performed using the four carbapenems and 51 bacterial strains that displayed (1) carbapenem resistance with carbapenemase, (2) carbapenem resistance without carbapenemase, or (3) carbapenem susceptibility. The susceptibility test results showed that LDI-MS based on a parylene-matrix chip was more sensitive and selective for detecting the carbapenemase reaction than conventional MALDI-TOF MS based on a 2,5-dihydroxybenzoic acid matrix.

1. Introduction

Carbapenems are a class of β-lactam antibiotics with broad-spectrum antibacterial activity [1]. One of the major mechanisms of carbapenem resistance in gram-negative bacteria is the production of carbapenemases, of which there are four families that comprise more than 100 enzymes [2,3]. To directly detect and assess the activity of carbapenemase, the modified Hodge test (MHT) [4] and the double-disk synergy test using carbapenems and ion chelators [5] have been used widely. However, these tests are difficult to standardize, and false-positive results are sometimes observed [1,6]. Additionally, it takes at least 18 h to obtain results using these susceptibility-testing methods [2]. Polymerase chain reaction (PCR)-based methods simplify the detection of carbapenemase-producing isolates [7–9] and yield results on the same day; however, they have a high cost per reaction. The specificity of PCR implies that it can only detect isolates expressing known enzymes [10,11], and it becomes increasingly difficult to comprehensively assay for carbapenemase-producing isolates by PCR as more types of carbapenemases are being discovered [1,2].

Recently, a MALDI-TOF MS-based assay to analyze carbapenemases was described [2,12–14]. The carbapenemase assay using MALDI-TOF MS can be completed within 1–2.5 h, at low cost [2]. However, differences in the ionized products of the carbapenemase reactions produced by MALDI-TOF MS are frequently difficult to resolve depending on, for example, the use of different instruments, matrices, and buffers [1,2]. MALDI-TOF MS uses organic matrix molecules to transfer energy from laser radiation to ionize analyte molecules, such as proteins and DNA, with minimum fragmentation [15–19]. During the ionization of the analytes, the organic matrix molecules are fragmented to produce their own mass peaks in the mass spectrum in the low mass-to-charge (m/z) ratio range ($<$ 500). The mass peaks of organic matrix molecules are usually not reproducible and can therefore not be completely distinguished from those of the analytes [17,20]. For this reason, the application of MALDI-TOF MS has been restricted to analytes with small molecular weights in the m/z ratio range of matrix molecules ($<$ 500). As shown in Fig. 1(a), typical carbapenem antibiotics have molecular weights in the range of 300–600 Da and their mass peaks are located within the m/z ratio range of the fragmented matrix molecules. To solve this problem, the parylene-matrix chip was developed for the quantitative analysis of small molecules without interference from the fragmented matrix molecules [15,21–23]. As shown in Fig. 1(b), this chip is produced by coating a thin parylene film onto a dried matrix. For analysis, the sample is simply dropped onto the parylene-matrix chip and then laser radiation is applied, without any additional organic matrix. This method was termed laser desorption/ionization mass spectrometry (LDI-MS) based on a parylene-matrix chip.

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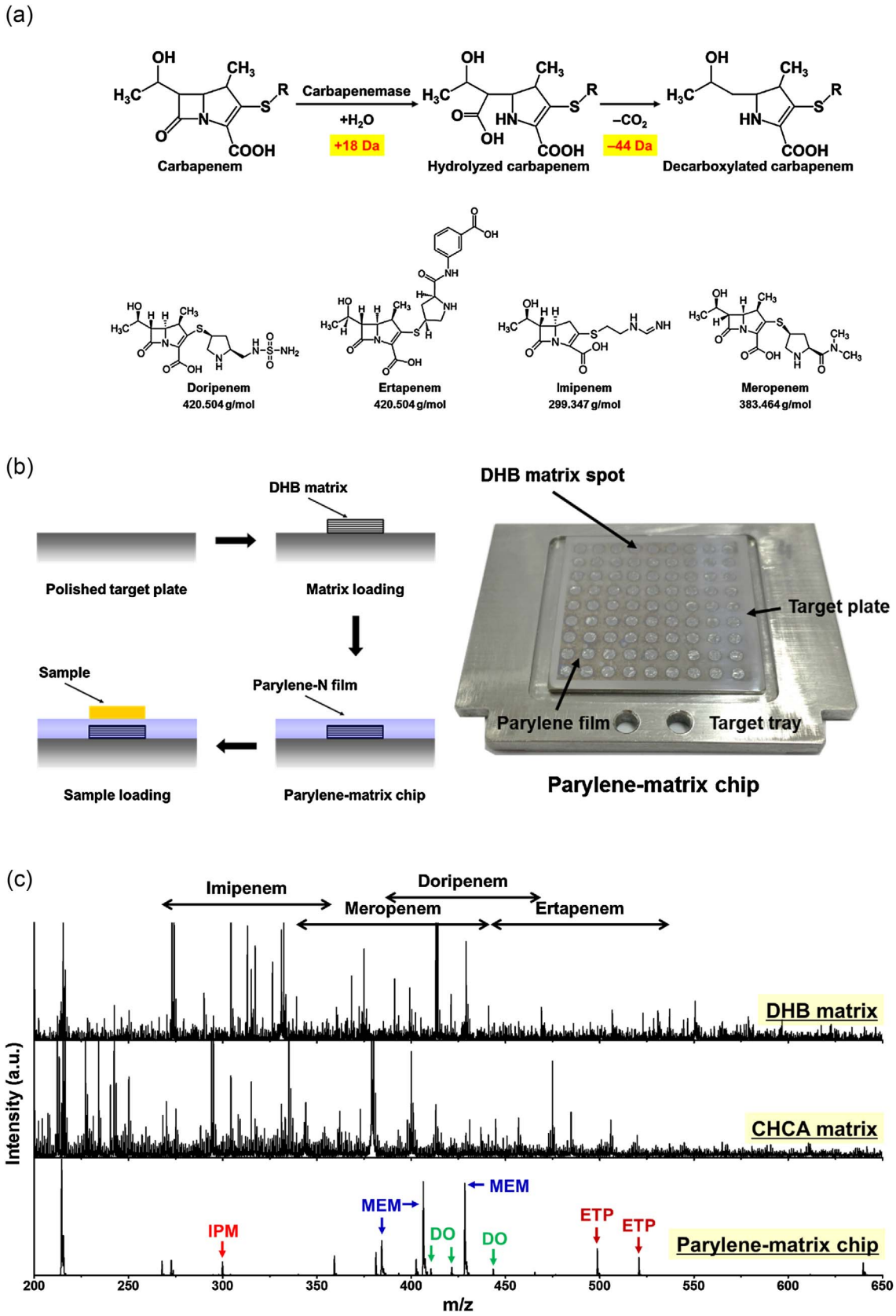

Fig. 1. Laser desorption/ionization mass spectrometry (LDI-MS) based on a parylene-matrix chip for carbapenem-susceptibility testing. (a) Carbapenem antibiotics and the hydrolysis reaction of carbapenemase. (b) Preparation of the parylene matrix chip. (c) Mass spectra of four carbapenem antibiotics obtained by conventional MALDI-TOF MS based on 2,5-dihydroxybenzoic acid (DHB) (top) and α-4-hydroxycinnamic acid (middle) matrices and by LDI-MS based on a parylene-matrix chip (bottom).