Short Communication (Note)

Title

Detection of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* using the MALDI Biotyper Selective Testing of Antibiotic Resistance–β-Lactamase (MBT STAR-BL) assay

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Running title

Accurate detection of ESBL by MBT STAR-BL

Abstract

The MALDI Biotyper Selective Testing of Antibiotic Resistance–β-Lactamase (MBT STAR-BL) assay, which analyzes bacterial induced hydrolysis of cefotaxime using MALDI-TOF MS, correctly identified 100.0% of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* as positive and 94.7% of non-ESBL producers as negative in 80 strains tested.

Key words: MALDI-TOF MS, ESBL, Antibiotic resistance

Text

Extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* represent a significant public health concern. A previous study from Japan reported that ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* are spreading, accounting for 23.0% of *E. coli* and 10.7% of *K. pneumoniae* infections in 2014-2015 (Takesue et al., 2017).

We recently reported the reliable performance of the MALDI Biotyper Selective Testing of Antibiotic Resistance–β-Lactamase (MBT STAR-BL) assay, which analyzes bacterial induced hydrolysis of β-lactam antibiotics using matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS), for detecting IMP metallo-β-lactamase (MBL) activity in *Enterobacteriaceae* (Kawamoto et al., 2018). In the present study, we also investigated the utility of the MBT STAR-BL assay for the detection of ESBL activity in *Enterobacteriaceae*.

We used *Enterobacteriaceae* (*E. coli* and *K. pneumoniae*) clinically isolated at the Nagasaki University Hospital between January 2011 and May 2016. MICs were determined using a BD Phoenix Automated Microbiology System (BD Diagnostics). ESBL production was also detected using the BD Phoenix system (Leverstein-van Hall et al., 2002; Sanguinetti et al., 2003). The presence of ESBL genes was evaluated by

PCR. PCR amplification of ESBL genes (variants of CTX-M–group 1, including CTX-M-1, CTX-M-3, and CTX-M-15; variants of CTX-M–group 2, including CTX-M-2; variants of CTX-M–group 9, including CTX-M-9 and CTX-M-14; TEM variants, including TEM-1 and TEM-2; and SHV variants, including SHV-1) was performed using previously described primers (Dallenne et al., 2010) under the following conditions: 10 min at 95°C, 30 cycles consisting of 40 s at 95°C, 40 s at 60°C, 1 min at 72°C, and 7 min at 72°C for the final extension for ESBL genes, as described previously (Higashino et al., 2017). Analysis of cefotaxime hydrolysis using the MBT STAR-BL assay (including calibration) was performed according to the manufacturer's instructions, as described previously (Kawamoto et al., 2018). Three to five individual bacterial colonies were randomly collected using a 1-μL inoculation loop, suspended in 50 μL of solution (10 mM NH_4CO_3, 10 μg/mL $ZnCl_2$ [pH 8]) containing 0.5 mg/mL of cefotaxime, and incubated at 37°C for 2 h according to the manufacturer's instructions.

Signal peak intensity was used to calculate the logRQ value (a measure of hydrolysis efficiency), which was the logarithm of the ratio of the summed intensity of the hydrolyzed form (molecular peaks of $[M_{hydrolyzed/deacetyl} + H]^+$ at 414 *m/z* and $[M_{hydrolyzed/decarboxylated/deacetyl} + H]^+$ at 370 *m/z*) to the summed intensity of the non-hydrolyzed form (peaks of $[M + H]^+$ at 456 *m/z*, the sodium adducts $[M + Na]^+$ at 478

76 *m/z* and $[M + 2Na]^+$ at 500 *m/z*, and $[M_{deacetyl} + H]^+$ at 396 *m/z*). Higher logRQ values
77 indicated a higher degree of antibiotic hydrolysis (Kawamoto et al., 2018; Oviano and
78 Bou, 2017). The 95% confidence intervals (CIs) for sensitivity and specificity were
79 calculated using R statistical software (https://cran.ism.ac.jp/) (Kosai et al., 2017;
80 Yamakawa et al., 2018). Dot plots were generated using EZR
81 (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html).
82 A total of 80 strains (42 *E. coli* and 38 *K. pneumoniae*) were used for the
83 cefotaxime hydrolysis assay, and the characteristics of these strains are presented in
84 Table 1. Of the 80 strains, 42 (22 *E. coli* and 20 *K. pneumoniae*) harbored ESBL genes
85 that were not detected in the remaining 38 strains. The most prevalent ESBL genotype
86 was the CTX-M type, and 23 strains harbored multiple ESBL genes. The results of
87 ESBL production assays using the BD Phoenix system were consistent with the
88 presence or absence of ESBL genes in all 80 strains. ESBL producers and non-
89 producers were defined as strains positive for both ESBL genes and ESBL production
90 and as strains negative for both ESBL genes and ESBL production, respectively.
91 Representative spectra of the cefotaxime hydrolysis assay analyzed using the MBT
92 STAR-BL assay are presented in Figure 1. The distribution of normalized logRQ values
93 of the strains examined is shown in Figure 2. All 42 ESBL producers were accurately

identified as positive. With respect to the 38 non-ESBL producers, 36 were correctly identified as negative, whereas 1 strain was indeterminate and 1 strain was identified as positive. The sensitivity and specificity of the cefotaxime hydrolysis assay for the detection of ESBL activity were 100.0% (95% CI, 91.6–100.0) and 94.7% (95% CI, 82.3–99.4), respectively.

Our results indicate that the MBT STAR-BL assay exhibits excellent performance, with high sensitivity and specificity. The results of previous studies using similar detection systems were consistent with our present results, suggesting that the assay is reliable for the detection of ESBL-producing strains (Oviano et al., 2014; Oviano et al., 2017). The resistance of the two non-ESBL producers identified as indeterminate or positive by the cefotaxime hydrolysis assay could be associated with plasmid-mediated inducible β-lactamases not evaluated in this study (Empel et al., 2010; Jacoby, 2009; Yong et al., 2005).

There are some limitations to the present study. First, we examined only cefotaxime under a single condition (concentration, 0.5 mg/mL; incubation time, 2 h). However, it should be recognized that differences in bacterial concentration, antibiotic, drug concentration, and incubation time could affect the results of this assay (Mirande et al., 2015; Monteferrante et al., 2016). In addition, we used strains isolated at a single

institution, and CTX-M was the major genotype of these strains. Because epidemiologic differences could have affected the results, further studies using strains isolated in other regions and including a variety of genotypes will be necessary in order to confirm the assay performance.

In conclusion, this study demonstrated that the MBT STAR-BL assay enables detection of ESBL-producing *Enterobacteriaceae* with high accuracy, thereby making it suitable for screening ESBL producers.

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Figure legends

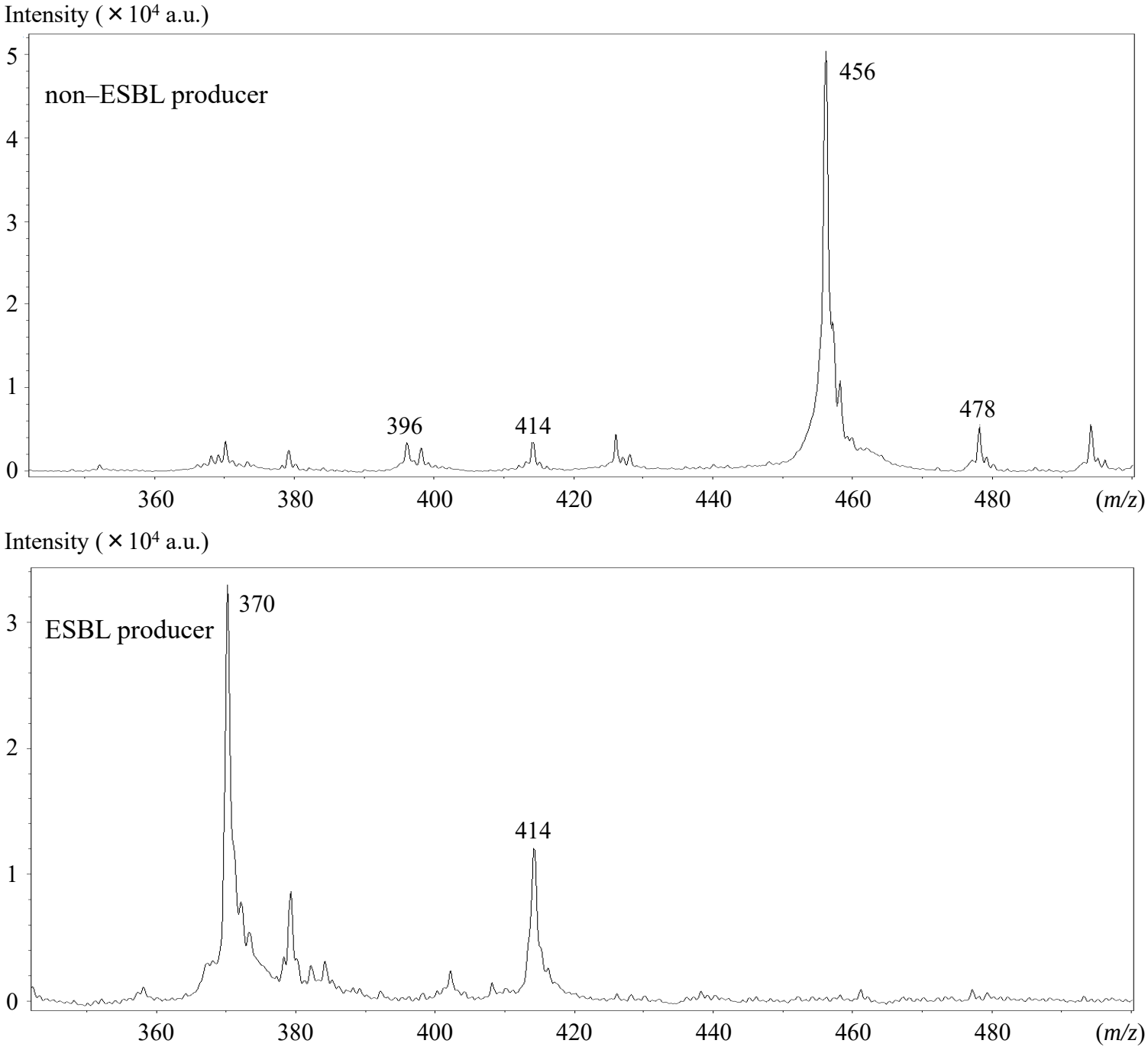
Figure 1. Representative spectra of the cefotaxime hydrolysis assay analyzed using the MBT STAR-BL assay. Non-ESBL producers exhibited peaks at 456, 478, and 396 *m/z*, corresponding to the non-hydrolyzed form of cefotaxime. Hydrolysis of cefotaxime by ESBL producers was characterized by disappearance of the non-hydrolyzed form of cefotaxime. ESBL, extended-spectrum β-lactamase.

Figure 2. Distributions of normalized logRQ values for strains tested using the cefotaxime hydrolysis assay. Normalized logRQ values >0.4 or <0.2 indicate positive or negative results, respectively. Values between 0.2 and 0.4 were considered indeterminate. ESBL, extended-spectrum β-lactamase; MIC, minimum inhibitory concentration.

Table 1. Characteristics of *Enterobacteriaceae* evaluated in this study.

Bacterial species	ESBL genotype	ESBL production	MIC for cefotaxime (μg/mL)	n
Escherichia coli	CTX-M group 1	Positive	>32	2
	CTX-M group 9	Positive	>32	12
	CTX-M group 1, TEM	Positive	>32	1
	CTX-M group 9, TEM	Positive	>32	7
	Negative	Negative	≤1	20
Klebsiella pneumoniae	CTX-M group 9	Positive	>32	1
	SHV	Positive	>32	1
	SHV	Positive	≤1	3
	CTX-M group 1, SHV	Positive	>32	6
	CTM-M group 2, SHV	Positive	>32	3
	CTM-M group 9, TEM	Positive	>32	2
	TEM, SHV	Positive	≤1	1
	CTX-M group 1, TEM	Positive	>32	1
	CTX-M group 1, TEM, SHV	Positive	>32	2
	Negative	Negative	≤1	18
Total				80

ESBL, extended-spectrum β-lactamase; MIC, minimum inhibitory concentration.



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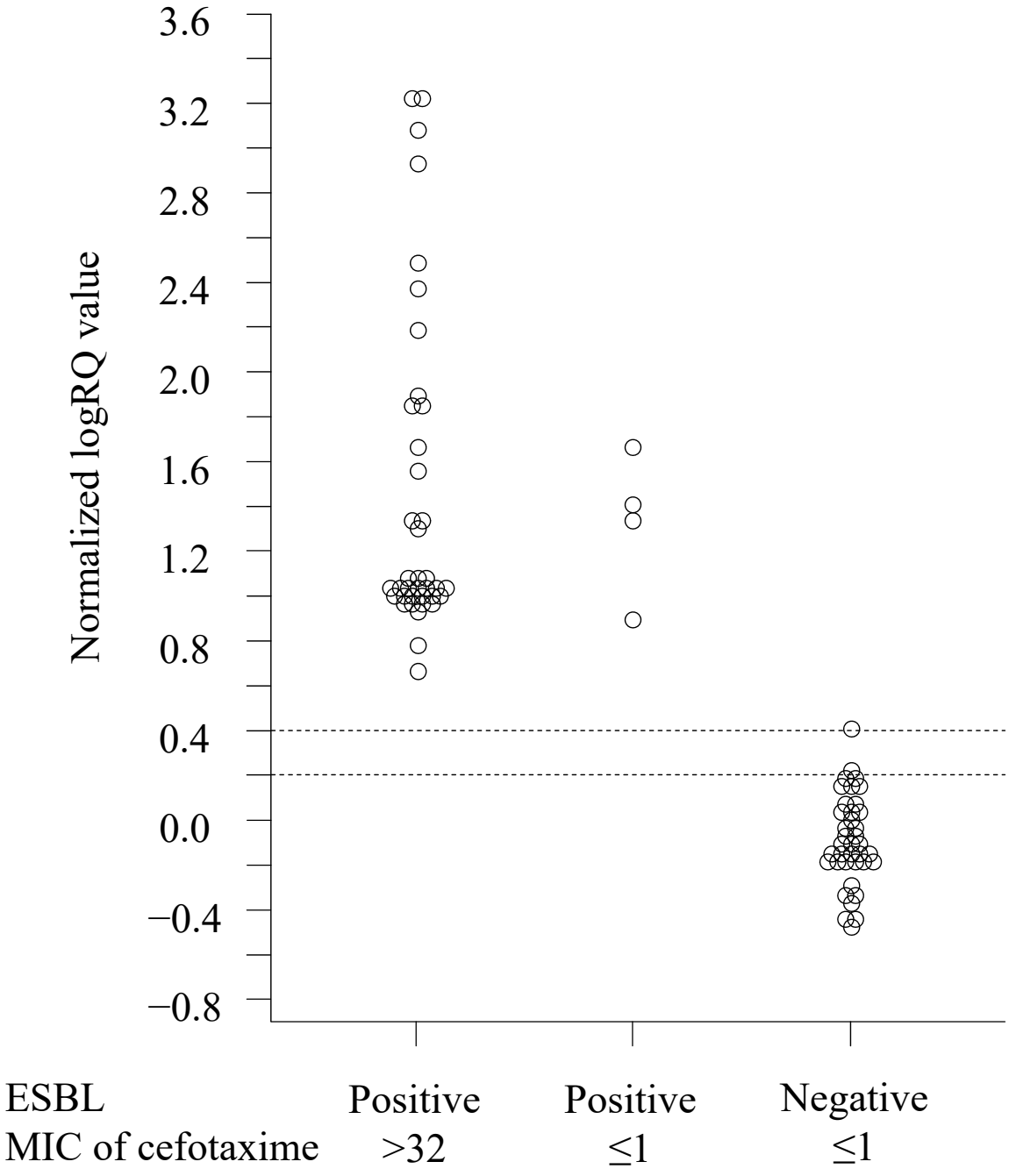

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