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Typing of *emm1* Group A Hemolytic Streptococci Using MALDI-TOF MS

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Introduction and Overview

Microbial identification techniques using matrix-assisted laser desorption/ionization mass spectrometers (MALDI-TOF MS) are becoming more common as the process of identification is faster and simpler than conventional methods. MALDI-TOF MS has already gained a position as an instrument for identification techniques. For further expansion, numerous efforts are being made to utilize MALDI instruments for microbial tests other than identification.
Recently, invasive infections caused by group A streptococcus (GAS) are on the increase. Among these, the *emm*1 group A streptococci has high pathogenicity, and its invasive infection cases indicate a significantly high possibility of developing into a fulminant form (Fig. 1). It is said that both its case fatality rate and rate of complications are high. This article introduces an example of differentiating between the highly pathogenic *emm*1 type and other types using MALDI-TOF MS and the statistical analysis software eMSTAT Solution[1].

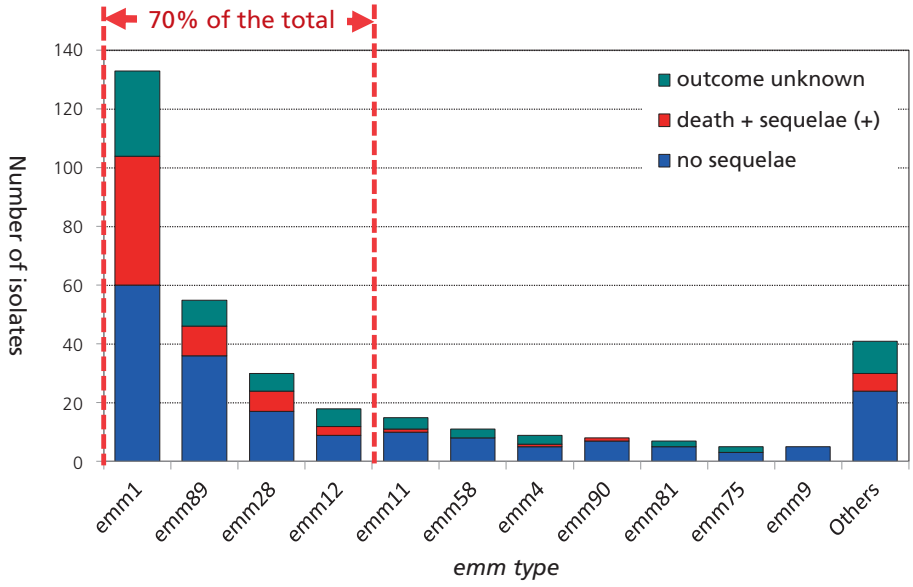

Fig. 1 *emm* types of GAS strains from invasive infections[2]

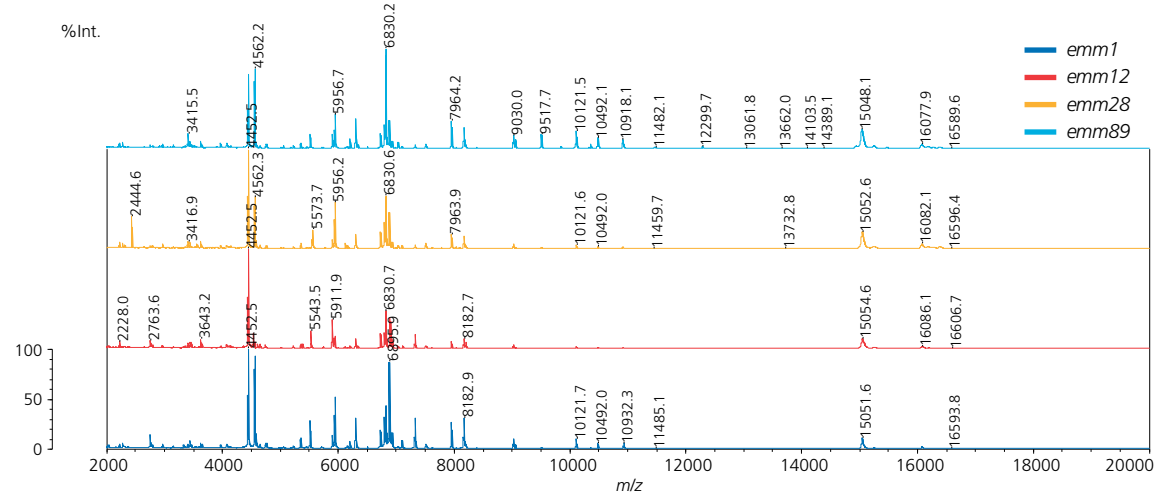

Fig. 2 Comparison of Mass Spectra of the Group A Streptococcus *emm*1 Type and the Other Types

Methods and Materials

Bacterial strains

Among the GAS strains derived from invasive infections, we used 10 strains each of *emm1*, *emm12*, *emm28* and *emm89*, which have a high isolation frequency, and conducted a search for markers that help to differentiate *emm* types. The above four *emm* types were cultured on a blood agar medium for 24 hours, Furthermore, we used 379 strains derived from pharyngitis and tonsillitis, including groups B, C, and G streptococci for blind tests.

MALDI-TOF MS

Instrument:	iD^{plus} MALDI microbial identification platform (Shimadzu)
Tuning mode:	Linear positive
Matrix solution:	Sinapinic acid 10 mg/mL (50 % acetonitrile 1 % TFA)
Sample preparation:.	Bacterial cells were collected and suspended in 0.5 mL of 70%(v/v) ethanol. Cells were then separated by centrifugation at 15,000 x rpm for 5 min and dried in air. Dried cells were extracted with 10 µL of 35%(v/v) formic acid. 1.5 µL of extracted cell suspensions were mixed with 10 µL of matrix solution. Then 1.5 µL of mixed solution was spotted onto the metal MALDI target and dried in air.

In order to ensure the repeatability of markers, samples were prepared from the re-cultured colonies and each type was measured nine times in total. The peak lists obtained from the mass spectra were subjected to multivariate analysis using the eMSTAT Solution software to differentiate *emm* types.

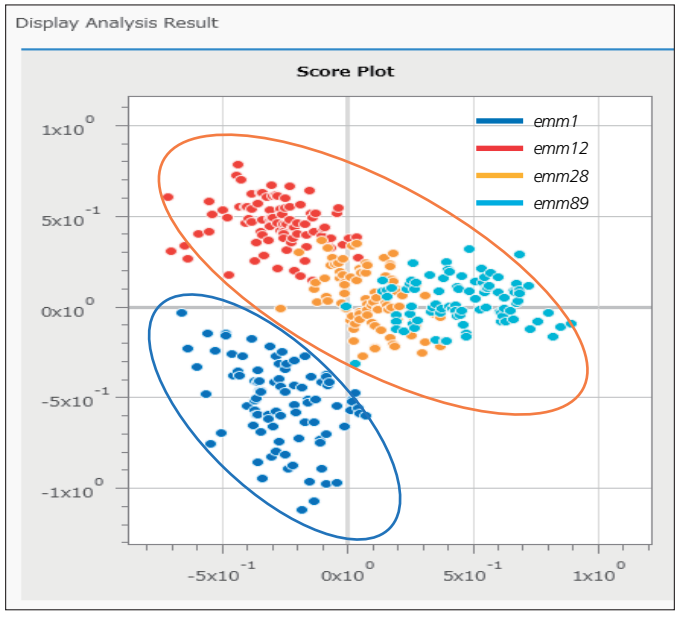

Fig. 3 Results of Multivariate Analysis of Group A Streptococcus (Score Plot)

Results and Discussion

Fig. 2 shows mass spectra of the group A streptococcus *emm1* type and other types. The mass spectrum patterns were similar and it was a challenge to differentiate them visually. However, by performing multivariate analysis (algorithm: PLS-DA), they were classified into two groups: the *emm1* type and the other types (Fig. 3).
Using the eMSTAT Solution software, it was possible to distinguish marker peaks detected in the MALDI spectra which help to differentiate the *emm1* type from the other types. As a result of the search, we confirmed from the Peak Matrix table that the peak at *m/z* 10932 was detected with all 90 samples of the *emm1* type and, in contrast to this, was not detected with all samples of the other types (Fig. 4). This was confirmed in the mass spectra where we can see that the peak at *m/z* 10932 was detected only with the *emm1* type (Fig. 5).

Peak Matrix
1519 peaks

m/z	ANOVA	emm1.0	emm12.0	emm28.0	emm89.0
10529.53	0.45215	0	1	0	0
10580.12	0.022573	0	0	0	4
10689.77	0.13804	5	1	9	2
10697.97	1.1797E-16	64	80	55	84
10706.51	0.32824	3	2	0	0
10717.11	0.76438	2	1	2	3
10725.56	0.27327	0	0	1	2
10734.97	0.26564	0	2	0	4
10918.12	4.173E-74	0	88	90	89
10931.66	8.8298E-56	90	0	0	0
10951.27	0.45215	0	1	0	0
10963.42	0.0097304	0	0	0	7
10970.66	0.45215	1	0	0	0
11000.27	0.0046591	6	0	0	0
11007.57	0.45215	1	0	0	0
11025.18	0.13935	0	3	0	0

Fig. 4 Identification of Marker Peaks using the Peak Matrix Function

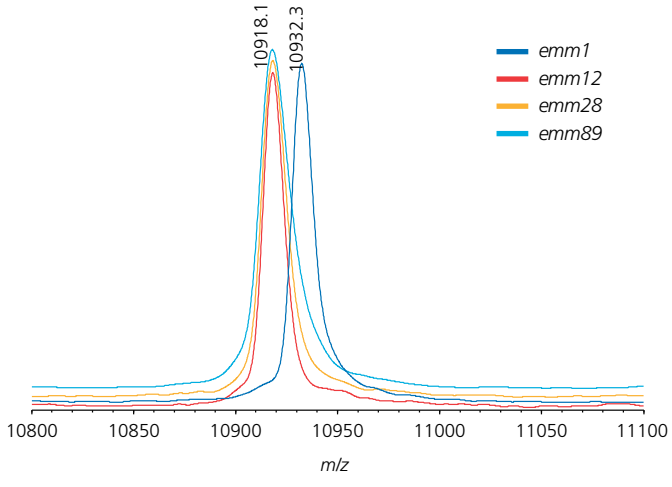

Fig. 5 Enlarged Mass Spectra of the Group A Streptococcus *emm1* Type and the Other Types

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Next, we conducted a blind test of 379 clinically isolated strains using a marker peak of *m/z* 10932 as an indicator (Table 1). Among 379 strains, 97 strains were typed as the *emm1* type using a conventional genetic analysis, while 92 strains among these 97 strains (94.8 %) were typed as the *emm1* type using the MALDI-TOF MS technique, which indicates a high positive agreement rate. In addition, 3 strains typed as non *emm1* type using the MALDI-TOF MS method were typed as *emm11* (n=1) and *emm28* (n=2) by the conventional method, which indicates a negative agreement rate of 98.9 % (Table 2).

Table 1 Clinically Isolated Strains Used for Blind Test

emm1	97
emm2	2
emm3	4
emm4	31
emm6	2
emm9	7
emm11	11
emm12	92
emm28	59
emm75	6
emm77	1
emm89	58
emm112	3
Other	6
Total	379

Table 2 Results of Blind Test

emm1 Type		Conventional Method (Genetic Analysis)		Total
		(+)	(-)	
Mass Spectrometry	(+)	92 (94.8 %)	3 (1.1 %)	95
	(-)	5 (5.2 %)	279 (98.9 %)	284
Total		97	282	379

Conclusions

Conventionally, the *emm* typing techniques need culturing, GAS isolation, *emm* gene amplification by PCR, and sequence analysis, and it takes three days before the result is obtained. On the other hand, using MALDI-TOF MS, the result can be obtained within several tens of minutes after culturing. This is a new technique for typing the highly pathogenic *emm1* type in a short time, and its future development can be expected.

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Reference

1) Sakuma M, Morozumi M, Ubukata K, Iwata T, 2018. Discrimination of group A streptococcus *emm1* type using MALDI-TOF MS.
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