Performance of MALDI-TOF Mass Spectrometry (VITEK MS) in the Identification of *Salmonella* Species

Gyu Ri Kim 1,†, Si Hyun Kim 2,†, Eun-Young Kim 3, Eun Hee Park 3, In Yeong Hwang 3, Seok Hoon Jeong 4, Hyun Soo Kim 5, Young Ah Kim 6, Young Uh 7, Kyeong Seob Shin 8, Young Ree Kim 9, Namhee Ryoo 10, Jong Hee Shin 11,‡ and Jeong Hwan Shin 1,‡

Abstract: *Salmonella* is a major pathogen causing foodborne infections in humans. *Salmonella* isolates are identified using biochemical and serological tests, including automated systems such as the VITEK2 system. However, there are few reports on *Salmonella* identification using VITEK MS. Therefore, we aimed to evaluate the usefulness of MALDI-TOF VITEK MS for *Salmonella* identification. A total of 1389 *Salmonella* isolates were identified using VITEK MS ver3.0 or ver3.2. All *Salmonella* isolates were confirmed by serotyping using the Kauffmann-White scheme, and the results were compared with the VITEK MS results. A total of 1389 *Salmonella* isolates, including 66 serotypes, were correctly identified at the genus level by VITEK MS. However, these systems failed to correctly identify typhoidal *Salmonella*. Among the five *Salmonella enterica* ssp. *diarizonae* isolates, only one was correctly identified, whereas one and three isolates were partially identified and misidentified, respectively. On the other hand, the VITEK2 system successfully identified all typhoidal *Salmonella* (Typhi and Paratyphi A) and *Salmonella enterica* ssp. *diarizonae* isolates. VITEK MS was useful for identifying *Salmonella* species isolated from clinical specimens; however, additional biochemical tests, such as the VITEK2 System, should be considered to accurately identify *Salmonella* ser. Typhi, and *Salmonella* ser. Paratyphi A.

Keywords: MALDI-TOF MS; VITEK MS; mass spectrometry; *Salmonella* species

1. Introduction

*Salmonella* is a major pathogen causing foodborne infections, including gastroenteritis and enteric fever, in humans [1]. The genus *Salmonella* includes two species, *S. enterica* and *S. bongori*, which have similar phenotypes and genotypes [2]. *Salmonella enterica* is the most frequently isolated species of *Salmonella* and is closely associated with human infections [3]. *Salmonella* identification is routinely performed using biochemical and serological tests. Biochemical identification using automated systems, such as the VITEK2 system...
(bioMérieux, Lyon, France), is commonly performed in clinical microbiology laboratories [4]. Owing to advantages of speed and accuracy, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has recently become a routine identification system [5]. Two common MALDI-TOF MS systems, MALDI Biotyper® (Bruker Daltonik GmbH, Bremen, Germany) and VITEK MS system (bioMérieux, Lyon, France), are used in clinical laboratories.

The performance of VITEK MS in identifying bacteria, fungi, and mycobacteria has been steadily evaluated [6–10]. However, only a few studies have evaluated the performance of VITEK MS in identifying Salmonella strains and serotypes [11–13]. This study aimed to evaluate the usefulness of MALDI-TOF VITEK MS for Salmonella identification using more than 1000 Salmonella strains, including 60 serotypes, isolated from human specimens.

2. Materials and Methods

2.1. Clinical Isolates

In total, 1389 Salmonella strains isolated from various human specimens such as stool, blood, urine, body fluids, and tissues were included in this study.

2.2. Final Identification by Serotyping

Salmonella strains were identified by serotyping using the White-Kauffmann-Scheme with the slide agglutination test for somatic antigens and the tube agglutination test for flagella antigens, as in our previous report [14,15].

2.3. Identification by VITEK MS and VITEK2 Systems

All Salmonella strains were identified using the VITEK MS system (bioMérieux, Lyon, France), according to the manufacturer’s instructions. The results were interpreted using VITEK MS v3.0, (n = 1167) until October 2019, and VITEK MS v3.2 (n = 222) was used thereafter. A fresh colony was smeared onto a 48-wall target plate and covered with 1 µL of α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution. After drying, the target plate was loaded into the MALDI-TOF VITEK MS system [4]. The developed MS fingerprint was automatically compared to the VITEK MS database v3.0 and v3.2. Escherichia coli ATCC 8739 was used as the quality control strain. Additionally, we tested the VITEK2 system for 93 typhoidal Salmonella, including 20 Salmonella ser. Paratyphi A, 9 Salmonella ser. Paratyphi B, 64 Salmonella ser. Typhi, and 5 Salmonella enterica subsp. diarizonae.

2.4. Database and Analysis

Table 1 shows the database of the VITEK MS ver3.0, ver3.2, and VITEK2 systems for Salmonella spp. VITEK MS v3.0 reports the results for Salmonella ser. Typhi, Salmonella ser. Paratyphi A, Salmonella ser. Gallinarum, Salmonella enterica subsp. arizonae/Salmonella enterica subsp. diarizonae, and Salmonella groups. The Salmonella group refers to all S. enterica strains other than those mentioned above. The reports of VITEK MS v3.2 were simplified as two results: Salmonella enterica subsp. arizonae/Salmonella enterica subsp. diarizonae and Salmonella enterica subsp. enterica. For the VITEK2 system, six results were included in the database, including the Salmonella ser. Typhi, Salmonella ser. Paratyphi A, Salmonella ser. Gallinarum, Salmonella enterica subsp. arizonae, Salmonella enterica subsp. diarizonae, and Salmonella groups.

The results of the VITEK MS were compared with those of the final identification by serotyping. The difference in the results between the VITEK2 system and VITEK MS for 93 typhoidal Salmonella and 5 Salmonella enterica subsp. diarizonae were analyzed.
Table 1. Database of VITEK MS v3.0, v3.2, and VITEK2 system for Salmonella.

| System Type         | Database List                                      |
|---------------------|--------------------------------------------------|
| VITEK2 system       | Salmonella group                                  |
|                     | Salmonella ser. Gallinarum                       |
|                     | Salmonella ser. Paratyphi A                      |
|                     | Salmonella ser. Typhi                            |
|                     | Salmonella enterica ssp. arizonae                |
|                     | Salmonella enterica ssp. diarizonae              |
| VITEK MS v3.0       | Salmonella group                                  |
|                     | Salmonella ser. Gallinarum                       |
|                     | Salmonella ser. Paratyphi A                      |
|                     | Salmonella ser. Typhi                            |
|                     | Salmonella enterica ssp. arizonae/diarizonae     |
| VITEK MS v3.2       | Salmonella enterica ssp. enterica                |
|                     | Salmonella enterica ssp. arizonae/diarizonae     |

3. Results

A total of 1389 Salmonella strains were included in this study (Table 2). These comprised 66 serotypes of 1167 Salmonella isolates for VITEK MS v3.0 analysis, and 27 serotypes of 222 Salmonella isolates for VITEK MS v3.2. In the analysis using VITEK MS v3.0, 72 typhoidal Salmonella spp. (14 Salmonella serovar. Paratyphi A, 7 Salmonella ser. Paratyphi B, 51 Salmonella ser. Typhi), 2 Salmonella enterica subsp. diarizonae (1 Salmonella ser. IIIb 47:r:z and 1 Salmonella ser. IIIb 48:k:z), and 1093 other Salmonella enterica subsp. enterica were included. In the analysis using VITEK MS v3.2, 21 typhoidal Salmonella isolates (6 Salmonella ser. Paratyphi A, 2 Salmonella serovars. Paratyphi B, and 13 Salmonella ser. Typhi), 3 Salmonella enterica subsp. diarizonae (1 Salmonella ser. IIIb 47:r:z and 2 Salmonella ser. IIIb 48:k:z), and 198 other Salmonella enterica subsp. enterica were included.

All 1389 Salmonella strains were correctly identified as Salmonella by VITEK MS at the genus level (Table 3). The results of VITEK MS v3.0 (n = 1167) were as follows: Salmonella group (n = 1157), Salmonella ser. Paratyphi A (n = 3), Salmonella ser. Paratyphi A/Salmonella group (n = 3), Salmonella ser. Typhi/Salmonella group (n = 3), and Salmonella enterica ssp. arizonae/Salmonella enterica ssp. diarizonae (n = 1). The results of VITEK MS v3.2 (n = 222) were as follows: Salmonella enterica subsp. enterica (n = 221), and Salmonella enterica ssp. enterica/S. enterica ssp. arizonae/S. enterica ssp. diarizonae (n = 1).

We compared the results of VITEK MS v3.0 and 3.2 with those of serotyping and the VITEK2 system for 98 Salmonella isolates (Table 4). VITEK MS v3.0 did not correctly identify typhoidal Salmonella and Salmonella enterica ssp. diarizonae, although these were included in the ver3.0. Only 3 of the 14 Salmonella ser. Paratyphi A isolates were correctly identified, and the other three isolates were partially identified as Salmonella ser. Paratyphi A/Salmonella group. The remaining eight isolates were assigned to the Salmonella group. Most of the Salmonella ser. Typhi was reported to belong to the Salmonella group, although three isolates were partially identified as Salmonella ser. Typhi/Salmonella group. For two Salmonella enterica ssp. diarizonae isolates, Salmonella ser. IIIb 47:r:z was reported as Salmonella group and Salmonella ser. IIIb 48:k:z was reported as Salmonella enterica ssp. arizonae/Salmonella enterica subsp. diarizonae. However, all Salmonella ser. Paratyphi A, Salmonella ser. Typhi, and Salmonella enterica ssp. diarizonae were correctly identified using the VITEK2 system.
Table 2. Serotype distribution of *Salmonella* in this study.

| Serotype               | VITEK MS v3.0 (n = 1167) | VITEK MS v3.2 (n = 222) |
|------------------------|---------------------------|-------------------------|
|                        | N                         | N                       |
| I4,[5],12:i:-          | 220                       | 38                      |
| Enteritidis            | 171                       | 65                      |
| Bareilly               | 121                       | 21                      |
| Typhimurium            | 87                        | 10                      |
| Infantis               | 83                        | 22                      |
| Thompson               | 56                        | 1                       |
| Agona                  | 53                        | 6                       |
| Typhi                  | 51                        | 13                      |
| Montevideo             | 34                        | 4                       |
| Livingstone            | 34                        | 0                       |
| Stanley                | 26                        | 1                       |
| Virchow                | 20                        | 2                       |
| Panama                 | 20                        | 2                       |
| Newport                | 16                        | 6                       |
| Saintpaul              | 15                        | 2                       |
| Paratyphi A            | 14                        | 6                       |
| Mbendaka               | 14                        | 1                       |
| Braenderup             | 11                        | 4                       |
| Othmarschen            | 10                        | 3                       |
| Rissen                 | 9                         | 3                       |
| Paratyphi B            | 7                         | 2                       |
| Others †               | 95                        | 10                      |

† Others: VITEK MS v3.0: *Salmonella* ser. Agama, Aqbeni, Albany, Bovisporificans, Brunei, Cerro, Choleraesuis, Derby, Dessau, Ebrie, Essen, Give, Hadar, Hato, Heidelberg, Hindmarsh, I4,[5],12:-:-, Inganda, Kentucky, Kingston, Kottbus, Litchfield, London, Muenchen, Muenster, Ohio, Oslo, Ponoma, Poona, Reading, Sandiego, Schleissheim, Schwarzegrund, Serftenberg, Simi, Singapore, Sinstorf, Uganda, Urbana, Wa, Weltevreden, Weltevreden var. 15+, IIb 47:r:z, and IIb 48:k:z. VITEK MS v3.2: *Salmonella* ser. Derby, Give, London, Ohio, Simi, IIb 47:r:z, IIb 48:k: z.

Table 3. *Salmonella* identification results by VITEK MS v3.0 and v3.2.

| VITEK MS (N) | MALDI-TOF VITEK MS Results | n (%) |
|--------------|----------------------------|-------|
| v3.0 (1167)  | *Salmonella* group         | 1157 (99.1) |
|              | *Salmonella* ser. Paratyphi A | 3 (0.3)    |
|              | *Salmonella* ser. Paratyphi A/*Salmonella* group | 3 (0.3)    |
|              | *Salmonella* ser. Typhi/*Salmonella* group | 3 (0.3)    |
|              | *Salmonella enterica* ssp. arizonae/*Salmonella enterica* ssp. diarizonae | 1 (0.1)    |
| v3.2 (222)   | *Salmonella enterica* ssp. enterica | 221 (99.5) |
|              | *Salmonella enterica* ssp. enterica/*Salmonella enterica* ssp. arizonae/*Salmonella enterica* ssp. diarizonae | 1 (0.5)    |

There were a few changes in the VITEK MS v3.2 as described in the Methods. VITEK MS v3.2 system identified all six *Salmonella* ser. Paratyphi A and 13 *Salmonella* ser. Typhi as *Salmonella enterica* subsp. *enterica*. Two *Salmonella enterica* ssp. *diarizonae*, including one *Salmonella* ser. IIb 47:r:z and one *Salmonella* ser. IIb 48:k:z were misidentified as *Salmonella enterica* subsp. *enterica*. The remaining one *Salmonella enterica* ssp. *diarizonae* was partially identified as *Salmonella enterica* ssp. *enterica*. Two *Salmonella enterica* ssp. *arizonae*/*Salmonella enterica* ssp. *diarizonae*. All *Salmonella* isolates were correctly identified using the VITEK2 system.
Table 4. Comparison between the performance of VITEK MS and VITEK2 systems in identifying serotype or subspecies.

| VITEK MS | Serotype (n) | VITEK MSs | VITEK2 System |
|----------|--------------|-----------|---------------|
| v3.0     | Paratyphi A (14) | 8         | Salmonella group |
|          |               | 3         | Salmonella ser. Paratyphi A |
|          |               | 3         | Salmonella ser. Paratyphi A/Salmonella group |
|          | Paratyphi B (7) | 7         | Salmonella group |
|          | Typhi (51)    | 48        | Salmonella group |
|          |               | 3         | Salmonella ser. Typhi Salmonella group |
| IIIb 47:rz (1) | 1            | Salmonella group |
| IIIb 48:k:z (1) | 1            | Salmonella enterica ssp. arizonae/Salmonella enterica ssp. diarizonae |
| v3.2     | Paratyphi A (6) | 6         | Salmonella enterica ssp. enterica |
|          | Paratyphi B (2) | 2         | Salmonella enterica ssp. enterica |
|          | Typhi (13)    | 13        | Salmonella enterica ssp. enterica |
| IIIb 47:rz (1) | 1            | Salmonella enterica ssp. enterica |
| IIIb 48:k:z (2) | 1            | Salmonella enterica ssp. enterica/Salmonella enterica ssp. arizonae/Salmonella enterica ssp. diarizonae |

4. Discussion

More than 2600 serotypes of *Salmonella*, including 46 type O serogroups and 114 type H serogroups, have been reported [2]. The two major antigens that determine the serotype are bacterial somatic antigens corresponding to O-polysaccharide and flagella antigens corresponding to flagellin proteins. Accurate serotype identification is crucial because the virulence of *Salmonella*, especially typhoidal *Salmonella*, varies depending on the serotype [15].

*Salmonella* identification has been performed using biochemical and serological tests. Automated identification systems such as the VITEK2 system provide rapid, reliable, and highly reproducible results [16]. Recently, MALDI-TOF MS has been commonly used as a routine identification method [5,6]. However, there are a few previous reports on the performance of VITEK MS in identifying *Salmonella* [11–13]. Guo et al. [17] using 1025 bacteria isolated from clinical specimens, reported that VITEK MS exhibited good performance; however, only two strains of *Salmonella enterica* were included in the report. Richter et al. [6] reported that VITEK MS exhibited good performance in identifying *Enterobacteriaceae* at the genus and species levels. They reported that 35 isolates of *Salmonella enterica* ssp. *enterica* were included, and 33 and 2 strains were correctly identified at the species and genus levels, respectively, using VITEK MS v2.0. Therefore, they concluded that VITEK MS would be appropriate for the identification of *Salmonella* at the genus and species levels with high accuracy. Wattal et al. [18] evaluated VITEK MS using 12,003 microbial isolates, including *Enterobacteriales*, other gram-negative bacteria, gram-positive bacteria, yeast, fungi, and mycobacteria. They reported that VITEK MS correctly identified 95.8% of the isolates at the species level. Among the 145 *Salmonella* isolates, 138 isolates were identified as *Salmonella*; however, seven *Salmonella* ser. Typhi isolates showed no identification results. Interestingly, they reported that all 18 *Salmonella* spp. Paratyphi A and 44/51 *Salmonella* ser. Typhi were correctly identified at the serotype level. In our study, we evaluated the performance of VITEK MS with numerous *Salmonella* isolates consisting of 66 confirmed serotypes, and all 1389 *Salmonella* isolates were correctly identified as *Salmonella*, consistent with the above studies. Collectively, these findings demonstrate that VITEK MS is suitable for the identification of *Salmonella* at the genus level.
In our study, only three *Salmonella* ser. Paratyphi A isolates were correctly identified using the VITEK MS v3.0 among 14 *Salmonella* ser. Paratyphi A and 51 *Salmonella* ser. Typhi isolates, which is in contrast with the above report by Wattal et al. [18]. Moreover, three *Salmonella* ser. Paratyphi A and three *Salmonella* ser. Typhi isolates were partially identified, whereas the others were identified as *Salmonella* group using VITEK MS v3.0. In the updated VITEK MS v3.2, the serotypes of *Salmonella* ser. Paratyphi A, *Salmonella* ser. Typhi, and *Salmonella* ser. Gallinarum were removed from the database. Another MALDI-TOF MS device, MALDI Biotyper® (Bruker Daltonik GmbH, Germany) is also widely used in clinical laboratories. Bastin et al. [19] reported that the MALDI Biotyper could identify 100% of *Salmonella* isolates at the genus level; however, it failed to correctly identify the serotype for typhoidal *Salmonella*.

Using the VITEK2 system, *Salmonella* ser. Typhi isolates were correctly identified at the serotype level. Therefore, additional biochemical tests, such as the VITEK2 system should be performed for the accurate identification of *Salmonella* ser. Paratyphi A and *Salmonella* ser. Typhi. Nevertheless, *Salmonella* ser. Paratyphi B cannot be correctly identified at the serotype level by either the VITEK2 system or VITEK MS, and additional tests are needed.

There are few reports on the identification of *Salmonella enterica* ssp. *arizonae/Salmonella enterica* ssp. *diarizonae* using VITEK MS. In this study, we found that the results for *Salmonella enterica* ssp. *arizonae/S. enterica* ssp. *diarizonae* using VITEK MS v3.0 and v3.2 were not suitable for the final identification of the pathogens (Table 4). Only one *Salmonella enterica* ssp. *diarizonae* isolate was correctly identified by VITEK MS. In contrast, one and three *Salmonella enterica* ssp. *diarizonae* isolates were partially identified and misidentified, respectively. Nevertheless, these five isolates were correctly identified as *Salmonella enterica* ssp. *diarizonae* using the VITEK2 system. Therefore, we believe that biochemical identification systems, such as the VITEK2 system, are useful for the accurate identification of *Salmonella enterica* subsp. *diarizonae*.

5. Conclusions

In this study, we demonstrated that VITEK MS can identify most of the common serotypes of *Salmonella* in the *Salmonella* group or *Salmonella enterica* subsp. *enterica* with 100% sensitivity. However, additional tests, such as the VITEK2 system, are required to confirm the presence of typhoidal *Salmonella* spp. (*Salmonella* ser. Typhi, and *Salmonella* ser. Paratyphi A).

**Author Contributions:** Conceptualization, G.R.K., S.H.K. and J.H.S. (Jeong Hwan Shin); Methodology, G.R.K., E.H.P. and I.Y.H.; Validation, S.H.K. and E.-Y.K.; Writing—Original Draft Preparation, G.R.K. and S.H.K.; Writing—Review and Editing, S.H.K., E.-Y.K. and J.H.S. (Jeong Hwan Shin); Resources, S.H.J., H.S.K., Y.A.K., Y.U., K.S.S., Y.R.K., N.R. and J.H.S. (Jong Hee Shin); Supervision, J.H.S. (Jeong Hwan Shin). All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the Research Program funded by the Korean Disease Control and Prevention Agency (2020E540600). This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2021R1C1C1007969).

**Institutional Review Board Statement:** This study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Inje University Busan Paik Hospital (2022-08-054) with consent exemption.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Patient consent was waived because personal information was not used.

**Conflicts of Interest:** The authors declare no conflict of interest.
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