Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Matrix-assisted laser desorption-ionization-time-of-flight mass spectrometry as an efficient proteomic approach for identification of *Salmonella* isolates recovered from food of animal origins.

Ahmed Hamad*, Faten S. Hassanin, Amani M. Salem, Reham A. Amin

Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Toukh, Qalyubia 13736, Egypt.

ARTICLE INFO

Keywords

Food safety
Foodborne pathogens
Mass spectrometry
Microbial identification
VITEK-MS

Received 03/05/2024
Accepted 23/05/2024
Available On-Line 01/07/2024

ABSTRACT

Golden standard isolation techniques based on the phenotypic and genotypic characteristics of the microorganisms are commonly applied for identifying foodborne pathogens. Novel proteomics analysis is a quick, precise, and economical method of foodborne pathogen isolates identification. This study aimed to assess the efficacy, sensitivity, and dependability of *Salmonella* species recovered from animal-based food using matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS). In total, 215 samples were gathered from supermarkets, and retail stores in Qalyubia Governorate, Egypt, including raw chicken breast, breaded chicken, shrimp, tilapia, seabass, mullet, and mackerel fish then identifying *Salmonella* species recovered from food of animal origins and finally confirm the results using MADLFI-TOF MS. The result revealed that *Salmonella* isolates were detected in 24 samples based on the morphological characteristics. MALDI-TOF MS was used to assess all *Salmonella* isolates. In contrast to traditional identification, MADI-TOF MS identified only 19 isolates as *Salmonella enterica* with a 99 % confidence value. This study proposed that MALDI-TOF MS is a quick and inexpensive technology for identifying *Salmonella* species as an alternate tool in the food analysis laboratory for routine identification of foodborne pathogen isolates. Further study is needed to validate the MALDI-TOF MS approach and expanding the MS instrument's database to increase the identification accuracy.

1. INTRODUCTION

Animal-derived foods are a significant source of nutrients for many people worldwide. Yet they also have certain adverse health consequences after consumption. This puts the safety of meals generated from animals at the top of the list of public concerns, which is one of the ongoing issues facing food producers today (Edris et al., 2023).

Salmonella is a Gram-negative, rod-shaped, facultative anaerobic *Enterobacteriaceae* member with over 2500 serovars. Salmonellosis, one of the most common foodborne infections, is a worldwide public health concern (Abd El-basit et al., 2019). In humans, it can cause severe illness, particularly in newborns and the elderly, resulting in diseases such as typhoid fever, gastroenteritis, and even death (Xiong et al., 2017). Overall, animal-derived foods had the greatest incidence rate of *Salmonella enterica*, and it was slightly higher prevalent in ready-to-eat foods than in raw foods (Sabeq et al., 2022).

Rapid detection of *Salmonella* species is critical for successful food industry regulation and monitoring. The conventional detection methods e.g., morphological, biochemical and serotyping methods may produce ambiguous results and require high expertise skills (Yang et al., 2021). Unfortunately, these methods are time-consuming, expensive, complicated, and arduous, requiring more than 150 unique antisera (Dieckmann and Malorny, 2011; Diep et al., 2019). Although whole-genome sequencing is becoming more accessible and effective for identification because it gives extensive genetic information, sequencing several isolates simultaneously is not feasible (Xiong et al., 2017).

MALDI-TOF MS can be used to identify numerous foodborne isolates such as *Brucella* (Mesureur et al., 2016), Enterobacteriaceae including *Salmonella* (Singhal et al., 2015), Mycobacteria (Panda et al., 2013), and *Salmonella* (shell *et al.*, 2017). Bacteria are identified using MALDI-TOF MS analysis based on their protein profiles. The identification is based on comparing bacterial protein mass spectra to known protein reference spectra in the database (Croxatto et al., 2012; Neuschlova et al., 2017).

Rapid detection of *Salmonella* species is critical for successful food industry regulation and monitoring. Therefore, a method for identifying foodborne pathogens in food samples that is quick, sensitive, specific, dependable, and cost-effective is required. This study aimed to assess the efficacy, sensitivity, and dependability of MALDI-TOF MS in identifying *Salmonella* species recovered from food of animal origins.

2. MATERIAL AND METHODS

2.1. Sample collection and study area:

A total of 215 foods originating from animals were collected between September 2020 and September 2022 from supermarkets, markets, and retail stores in Qalyubia Governorate, Egypt. Samples included raw chicken breast (n=45), breaded chicken products (n=45), shrimp (n=25),

* Correspondence to: ahmed.alhussaini@fvtm.bu.edu.eg

Mackerel fish (n=25), sea bass fillet (n=25), tilapia fillet (n=25) and mugil fillet (n=25). For microbiological (*Salmonella*) analysis, samples were gathered and delivered to the lab in sterile plastic bags in ice boxes in less than an hour.

2.2. Isolation and conventional identification

Standard cultivation procedures for isolation and identification of *Salmonella* were employed according to ISO 6579-1: 2017 (ISO, 2017). Shortly, each sample, weighed out 25 g, was homogenized using the Stomacher 400R (Seward, UK) for 2 minutes at 10xg in 225 mL of 10% buffered peptone solution. For *salmonella* pre-enrichment, 1 ml of the prepared sample was inoculated into 10 mL of Rappaport Vassiliadis (RV) broth (Lab M, UK), and incubated for 18-24 hours at 41°C. Then, every suspicious tube from the enrichment broth was streaked onto selective xylose lysine deoxycholate (XLD) agar (BioLife, USA) and incubated for 18-24 hours at 37 °C. Atypical colonies were additionally verified using Bismuth Sulfite Agar (BioLife, USA). The API 20E® system (BioMerieux, France) was used to biochemically confirm suspected colonies with typical *Salmonella* morphology.

2.3. Bacterial strains

A total of 24 suspected *Salmonella* strains were recovered from food samples. Until they were ready for use, the strains were kept at -72 °C and 50% glycerol in the Central Laboratory of the Faculty of Veterinary Medicine, Benha University. Prior to examination, the bacteria were cultivated for 24 hours at 37 °C on Tryptic Soy Agar (TSA) (BioLife, USA). As controls, *Salmonella enterica* ATCC 35664 strains were employed.

2.4. MALDI-TOF identification and result interpretation.

Using the VITEK®-MS system (BioMérieux, France), all *Salmonella* strains were identified in accordance with the instructions of the manufacturer. Whole *Salmonella* colonies incubated for the previous night were selected and placed on a 48-well single-use target slide. The deposits were then covered with 0.5 µL of formic acid (BioMérieux, France) and left to dry. Afterward, they were covered with 1 µL of α-cyano-4-hidroxycinnamic acid (CHCA) matrix solution (BioMérieux, France) and let to dry once more. The VITEK®-MS device was filled with the slide. Each plate was calibrated using the reference strain of *Escherichia coli*, ATCC 8739. The obtained mass spectra were compared using the VITEK®-MS IVD V3.2 database. The program generated a confidence-related identification. The results were defined using the following levels: (1) A single identification with a confidence grade ranging from 60.0 to 99.9% indicates a perfect match. (2) Two or more identification results with confidence ratings of more than 60% were interpreted as indicating a "low discrimination result." Therefore, biological reactions should be supplied. (3) A confidence level of less than 60% was regarded as inadequate, or "no identification".

2.5. Statistical analysis

Statistical analysis was performed using STATA 18 (StataCorp., LLC, USA) (StataCorp., 2021). Frequency, percentage, and proportion as a descriptive statistic that were used to calculate the data obtained from the various food samples and identification results.

3. RESULTS

3.1. Isolation and conventional identification

Microbiological examination of 215 animal source food samples revealed the identification of 24 *Salmonella* isolates.

3.2. MALDI-TOF identification and result interpretation

Using MALDI-TOF MS systems, out of 24 identified *Salmonella* isolates, only 19 *Salmonella* strains were correctly identified at the genus, species, and subspecies level. All 19 Salmonella strains were identified as *Salmonella entrica* ssp. entrica over a 99% confidence value, compared to ATCC *Salmonella entrica* ssp. *entrica* reference strain. Five isolates (n=5) were also correctly identified to the species level but with a confidence value under 60% (Table 1).

The isolates of Salmonella spp. detected by the VITEK-MS MALDI-TOF as *Salmonella entrica* ssp. *entrica* comprised all seven isolates of raw chicken breast, five isolates of breaded chicken product, two isolates of Tilapia fillet, two isolates of sea bass fillet, and the isolate found in shrimp, mugil fillet, and mackerel fish (Table 1).

Using MALDI-TOF MS to analyze *Salmonella* isolates that were found in animal-derived food, eight to ten significant ion peaks were found in the mass spectra. These notable ion peaks have a range of 2800–12,000 m/z, with 5700–10960 m/z being the highest peaks of intensity (Figure 1). Based on this, all *Salmonella* isolates were accurately identified at the species level by the score values obtained by MALDI-TOF MS.

Table 1 Species identification of *Salmonella* isolates by MALDI-TOF analysis

Sample No.	Colony morphology	Food Source	MADLI-TOF VITEK-MS			
			Genus	Species	Subspecies	Confidence value (%)
N1	Salmonella	Raw chicken breast	*Salmonella*	*Enterica*	*enterica*	≥ 99
N2	Salmonella	Raw chicken breast	*Salmonella*	*Enterica*	*enterica*	≥ 99
N3	Salmonella	Raw chicken breast	*Salmonella*	*Enterica*	*enterica*	≥ 99
N4	Salmonella	Raw chicken breast	*Salmonella*	*Enterica*	*enterica*	≥ 99
N5	Salmonella	Raw chicken breast	*Salmonella*	*Enterica*	*enterica*	< 10
N6	Salmonella	Raw chicken breast	*Salmonella*	*Enterica*	*enterica*	≥ 99
N7	Salmonella	Raw chicken breast	*Salmonella*	*Enterica*	*enterica*	≥ 99
N8	Salmonella	Raw chicken breast	*Salmonella*	*Enterica*	*enterica*	< 10
N9	Salmonella	Raw chicken breast	*Salmonella*	*Enterica*	*enterica*	≥ 99
N10	Salmonella	Breaded chicken product	*Salmonella*	*enterica*	*enterica*	< 10
N11	Salmonella	Breaded chicken product	*Salmonella*	*enterica*	*enterica*	≥ 99
N12	Salmonella	Breaded chicken product	*Salmonella*	*enterica*	*enterica*	≥ 99
N13	Salmonella	Breaded chicken product	*Salmonella*	*enterica*	*enterica*	≥ 99
N14	Salmonella	Breaded chicken product	*Salmonella*	*enterica*	*enterica*	≥ 99
N15	Salmonella	Breaded chicken product	*Salmonella*	*enterica*	*enterica*	≥ 99
N16	Salmonella	Tilapia fillet	*Salmonella*	*enterica*	*enterica*	≥ 99
N17	Salmonella	Tilapia fillet	*Salmonella*	*enterica*	*enterica*	≥ 99
N18	Salmonella	Tilapia fillet	*Salmonella*	*enterica*	*enterica*	< 10
N19	Salmonella	Sea bass fillet	*Salmonella*	*enterica*	*enterica*	≥ 99
N20	Salmonella	Sea bass fillet	*Salmonella*	*enterica*	*enterica*	≥ 99
N21	Salmonella	Shrimp	*Salmonella*	*enterica*	*enterica*	≥ 99
N22	Salmonella	Mugil fillet	*Salmonella*	*enterica*	*enterica*	< 10
N23	Salmonella	Mugil fillet	*Salmonella*	*enterica*	*enterica*	≥ 99
N24	Salmonella	Mackerel fish	*Salmonella*	*enterica*	*enterica*	≥ 99
Total		24			19	

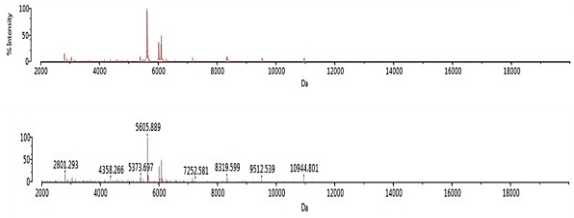

Figure 1 MALDI-TOF MS mass spectra for *Salmonella* isolates.

4. DISCUSSION

The results of this study revealed identification of 19/24 *Salmonella* isolates to a genus and species-level with proper identification accuracy of over 99 % (Table 1). The MALDI-TOF system took less than 20 min. from colony to identification, compared with ≥24 h by the conventional method.

The current results of the MALDI-TOF MS identification were consistent with the reports of Clark et al. (2013) and Public Health England (2021) that viewed identification of *Salmonella* to species level using MALDI-TOF MS. Additionally, Weller et al. (2011) and Bell et al. (2016) reported that after 24 hours of incubation, MALDI-TOF MS of clinical samples could identify 98% of *Salmonella* that had previously been identified by conventional methods. The mass spectrum range agrees with the findings of Leuschner et al. (2004), Dieckmann and Malorny (2011), and Shell et al. (2017).

The conventional biochemical method may identify *Salmonella* spp. in 6–18 hours. However, with the MALDI-TOF MS technology, the identification procedure only takes five to thirty minutes. The ribosomal protein-based protein spectra of every bacterium, which are pre-recorded in the system database, serve as the basis for the MALDI-TOF approach for detecting bacteria (Wieser et al., 2012). Therefore, MALDI-TOF technologies are intrinsically unable to distinguish between closely related species or strains, such as *Salmonella* spp., due to the similarity of their ribosomal proteins.

MALDI-TOF MS systems proved to be more accurate, faster, and much less expensive than phenotypic identification techniques, providing laboratories with an improved identification tool (Martiny et al., 2012). MALDI-TOF-MS and PCR are both valuable techniques in microbial detection. MALDI-TOF-MS has emerged as a rapid and robust method for accurate microbial identification in clinical microbiology laboratories (Clark et al., 2013). It is easily implementable for the routine identification of bacteria, offering a promising alternative to conventional methods (Veen et al., 2010; Croxatto et al., 2012). MALDI-TOF-MS provides advantages such as broad detection capabilities if the microorganism is present in the database used for identification (Sy et al., 2020). Additionally, MALDI-TOF-MS has a comprehensive database of clinically important microorganisms, making it a cost-effective and accurate method for microbial identification (Rahi et al., 2016). On the other hand, PCR has its strengths, particularly in the detection of specific genetic material. A study comparing PCR and MALDI-TOF-MS for identifying bacteria found that both methods were equivalent in characterizing of bacteria (Kaleta et al., 2011). While MALDI-TOF-MS is favored for its rapid and cost-effective microbial identification capabilities (Doan et al., 2012), PCR is highly specific in detecting particular genetic material (Kaleta et al., 2011). The choice between MALDI-TOF-MS and PCR for microbial detection may depend on the need for broad detection capabilities and rapid identification (favoring MALDI-TOF-MS) versus the requirement for specific genetic material detection (favoring PCR).

The results of the current research demonstrated that MALDI-TOF MS could be conducted for routine identification of *Salmonella* from food of animal origins, as compared to traditional techniques; it has a more significant species-level discerning ability. Further extension of the VITEK-MS instrument's database would improve identification accuracy and species diversity, which this promising technology can quickly identify.

To ascertain the sensitivity, reliability, and performance of *Salmonella* identification, more samples would be needed in further studies. Because this is a pilot study to test this strategy in food analysis labs in Egypt, the sample size used in this research is limited. Therefore, more investigation is required to confirm the efficacy of the MALDI-TOF MS approach for direct colony detection as well as to identify ways to get over some of its drawbacks.

5. CONCLUSIONS

Food safety and public health organizations continue to be extremely concerned about *Salmonella* with the most common source of salmonellosis being contaminated animal-derived food. By identifying tainted foods, quick and effective detection methods can significantly improve food safety while also preventing and controlling *Salmonella* illness. The majority of foodborne pathogens can potentially be identified using MALDI-TOF MS systems in place of the current techniques. MALDI-TOF MS is a rapid, easy, cost-effective, and straightforward to use that requires less time to identify the bacteria and a high-throughput proteomic technique for the identification of significant foodborne pathogens, which can be implemented in a routine, conventional food analysis setting which is crucial for maintaining a high standard of food safety.

DATA AVAILABILITY

The corresponding authors provide the additional data upon reasonable request.

ACKNOWLEDGMENT

The Research Support-Youth Grant (STDF-RSGY), Project No. 43021, from the Science and Technology Development Fund (STDF), Ministry of Higher Education and Scientific Research, Egypt, is acknowledged by the authors for financing this research.

COMPETING INTERESTS

The authors claim to have no conflicting agendas.

6. REFERENCES

1. Abd El-basit, M. R., Abd El-Azeem, M. W., Sultan, S., and Nasef, S. A. 2019. Molecular characterization of biofilm producing genes in Salmonellae Isolated from chicken. Journal of Advanced Veterinary Research, 9,2 , 39–44.
2. Bell, R. L., Jarvis, K. G., Ottesen, A. R., McFarland, M. A., and Brown, E. W. 2016. Recent and emerging innovations in Salmonella detection: a food and environmental perspective. Microbial Biotechnology, 9,3, 279–292. https://doi.org/10.1111/1751-7915.12359
3. Clark, A. E., Kaleta, E. J., Arora, A., and Wolk, D. M. 2013. Matrix-Assisted Laser Desorption Ionization–Time of Flight

Mass Spectrometry: a Fundamental Shift in the Routine Practice of Clinical Microbiology. Clinical Microbiology Reviews, 26,3 , 547–603. https://doi.org/10.1128/CMR.00072-12

4. Croxatto, A., Prod'hom, G., and Greub, G. 2012. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. FEMS Microbiology Reviews, 36,2 , 380–407. https://doi.org/10.1111/j.1574-6976.2011.00298.x
5. Dieckmann, R., and Malorny, B. 2011. Rapid Screening of Epidemiologically Important Salmonella enterica subsp. enterica Serovars by Whole-Cell Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. Applied and Environmental Microbiology, 77,12 , 4136–4146. https://doi.org/10.1128/AEM.02418-10
6. Diep, B., Barretto, C., Portmann, A.-C., Fournier, C., Karczmarek, A., Voets, G., Li, S., Deng, X., and Klijn, A. 2019. Salmonella Serotyping; Comparison of the Traditional Method to a Microarray-Based Method and an in silico Platform Using Whole Genome Sequencing Data. Frontiers in Microbiology, 10,1 , 2550–2554. https://doi.org/10.3389/fmicb.2019.02554
7. Doan, N., Hoorde, K., Cnockaert, M., Brandt, E., Aerts, M., Thanh, B., and Vandamme, P. 2012. Validation of maldi-tof ms for rapid classification and identification of lactic acid bacteria, with a focus on isolates from traditional fermented foods in northern vietnam. Letters in Applied Microbiology, 55,4 , 265-273. https://doi.org/10.1111/j.1472-765x.2012.03287.x
8. Edris, S. N., Hamad, A., Awad, D. A. B., and Sabeq, I. I. 2023. Prevalence, antibiotic resistance patterns, and biofilm formation ability of Enterobacterales recovered from food of animal origin in Egypt. Veterinary World, 20,1 , 403–413. https://doi.org/10.14202/vetworld.2023.403-413
9. ISO. 2017. ISO 6579-1: 2017 Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Detection of Salmonella spp. ISO, Geneva, Switzerland.
10. Kaleta, E., Clark, A., Cherkaoui, A., Wysocki, V., Ingram, E., Schrenzel, J., and Wolk, D. 2011. Comparative analysis of pcr–electrospray ionization/mass spectrometry ,ms and maldi-tof/ms for the identification of bacteria and yeast from positive blood culture bottles. Clinical Chemistry, 57,7 , 1057-1067. https://doi.org/10.1373/clinchem.2011.161968
11. Leuschner, R. G. K., Beresford-Jones, N., and Robinson, C. 2004. Difference and consensus of whole cell Salmonella enterica subsp. enterica serovars matrix-assisted laser desorption/ionization time-of-flight mass spectrometry spectra. Letters in Applied Microbiology, 38,1 , 24–31. https://doi.org/10.1046/j.1472-765X.2003.01436.x
12. Martiny, D., Busson, L., Wybo, I., El Haj, R. A., Dediste, A., and Vandenberg, O. 2012. Comparison of the Microflex LT and Vitek MS Systems for Routine Identification of Bacteria by Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry. Journal of Clinical Microbiology, 50,4 , 1313–1325. https://doi.org/10.1128/JCM.05971-11
13. Mesureur, J., Ranaldi, S., Monnin, V., Girard, V., Arend, S., Welker, M., O'Callaghan, D., Lavigne, J.-P., and Keriel, A. 2016. A Simple and Safe Protocol for Preparing Brucella Samples for Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Analysis. In C.-A. D. Burnham ,Ed. , Journal of Clinical Microbiology. 54,2 , 30-45. https://doi.org/10.1128/JCM.02730-15
14. Neuschlova, M., Vladarova, M., Kompanikova, J., Sadlonova, V., and Novakova, E. 2017. Identification of Mycobacterium Species by MALDI-TOF Mass Spectrometry. In M. Pokorski ,Ed. , Pulmonary Care and Clinical Medicine ,pp. 37–42 . Springer, Cham. https://doi.org/10.1007/5584_2017_26
15. Panda, A., Kurapati, S., Samantaray, J. C., Myneedu, V. P., Verma, A., Srinivasan, A., Ahmad, H., Behera, D., and Singh, U. B. 2013. Rapid identification of clinical mycobacterial isolates by protein profiling using matrix assisted laser desorption ionization-time of flight mass spectrometry. Indian Journal of Medical Microbiology, 31,2 , 117–122. https://doi.org/10.4103/0255-0857.115217
16. Public Health England. 2021. Identification of Salmonella Species. UK Standards for Microbiology Investigations. ID 24 Issue 4. Public Health England, 1,1 , 19-31. https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories.
17. Rahi, P., Prakash, O., and Shouche, Y. 2016. Matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry ,maldi-tof ms based microbial identifications: challenges and scopes for microbial ecologists. Frontiers in Microbiology, 7. https://doi.org/10.3389/fmicb.2016.01359
18. Sabeq, I., Awad, D., Hamad, A., Nabil, M., Aboubakr, M., Abaza, M., Fouad, M., Hussein, A., Shama, S., Ramadan, H., and Edris, S. 2022. Prevalence and molecular characterization of foodborne and human-derived Salmonella strains for resistance to critically important antibiotics. Transboundary and Emerging Diseases, 69,5 , 2153–2163. https://doi.org/10.1111/tbed.14553
19. Shell, W. S., Sayed, M. L., Allah, F. M. G., Gamal, F. E. M., Khder, A. A., Samy, A. A., and Ali, A. H. M. 2017. Matrix-assisted laser desorption-ionization-time-of-flight mass spectrometry as a reliable proteomic method for characterization of Escherichia coli and Salmonella isolates. Veterinary World, 10,9 , 1083–1093. https://doi.org/10.14202/vetworld.2017.1083-1093
20. Singhal, N., Kumar, M., Kanaujia, P. K., and Virdi, J. S. 2015. MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Frontiers in Microbiology, 6,1 , 780–791. https://doi.org/10.3389/fmicb.2015.00791
21. StataCorp. 2021. Stata Statistical Software: Release 17. ,17 . College Station, TX: StataCorp LLC. https://www.stata.com/
22. Sy, I., Margardt, L., Ngbede, E., Adah, M., Yusuf, S., Keiser, J., and Becker, S. 2020. Identification of adult fasciola spp. using matrix-assisted laser/desorption ionization time-of-flight ,maldi-tof mass spectrometry. Microorganisms, 9,1 , 82. https://doi.org/10.3390/microorganisms9010082
23. Veen, S., Claas, E., and Kuijper, E. 2010. High-throughput identification of bacteria and yeast by matrix-assisted laser desorption ionization-time of flight mass spectrometry in conventional medical microbiology laboratories. Journal of Clinical Microbiology, 48,3, 900-907. https://doi.org/10.1128/jcm.02071-09
24. Weller, U., Sparbier, K., Boogen, C., Fremlin, L., and Kostrzewa, M. 2011. Rapid detection of salmonella from clinical specimen by MALDI-TOF MS. Pathology, 43,1 , 69–74. https://doi.org/10.1016/S0031-3025,16 33251-2
25. Wieser, A., Schneider, L., Jung, J., and Schubert, S. 2012. MALDI-TOF MS in microbiological diagnostics—identification of microorganisms and beyond ,mini review . Applied Microbiology and Biotechnology, 93,3 , 965–974. https://doi.org/10.1007/s00253-011-3783-4
26. Xiong, D., Song, L., Tao, J., Zheng, H., Zhou, Z., Geng, S., Pan, Z., and Jiao, X. 2017. An Efficient Multiplex PCR-Based Assay as a Novel Tool for Accurate Inter-Serovar Discrimination of Salmonella Enteritidis, S. Pullorum/Gallinarum and S. Dublin. Frontiers in Microbiology, 8,1 , 400–420. https://doi.org/10.3389/fmicb.2017.00420
27. Yang, S.-M., Kim, E., Kim, D., Baek, J., Yoon, H., and Kim, H.-Y. 2021. Rapid Detection of Salmonella Enteritidis, Typhimurium, and Thompson by Specific Peak Analysis Using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry. Foods, 10,5 , 933-942. https://doi.org/10.3390/foods10050933