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.

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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 MALDI-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, MALDI-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),...
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 (BioMérieux, 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 mL of formic acid (BioMérieux, France) and left to dry. Afterward, they were covered with 1 µL of α-cyano-4-hydroxycinnamic 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 enterica ssp. enterica by a 99% confidence value, compared to ATCC Salmonella enterica ssp. enterica 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 enterica ssp. enterica 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>
<thead>
<tr>
<th>Sample No.</th>
<th>Colony morphology</th>
<th>Food Source</th>
<th>MALDI-TOF VITEK-MS</th>
<th>Confidence value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>Salmonella</td>
<td>Raw chicken breast</td>
<td>Salmonella enterica</td>
<td>≥ 99</td>
</tr>
<tr>
<td>N2</td>
<td>Salmonella</td>
<td>Raw chicken breast</td>
<td>Salmonella enterica</td>
<td>≥ 99</td>
</tr>
<tr>
<td>N3</td>
<td>Salmonella</td>
<td>Raw chicken breast</td>
<td>Salmonella enterica</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>N4</td>
<td>Salmonella</td>
<td>Raw chicken breast</td>
<td>Salmonella enterica</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>N5</td>
<td>Salmonella</td>
<td>Raw chicken breast</td>
<td>Salmonella enterica</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>N6</td>
<td>Salmonella</td>
<td>Raw chicken breast</td>
<td>Salmonella enterica</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>N7</td>
<td>Salmonella</td>
<td>Raw chicken breast</td>
<td>Salmonella enterica</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>N8</td>
<td>Salmonella</td>
<td>Raw chicken breast</td>
<td>Salmonella enterica</td>
<td>&lt; 10</td>
</tr>
<tr>
<td>N9</td>
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<td>Raw chicken breast</td>
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<td>&lt; 10</td>
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<tr>
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<td>Breaded chicken product</td>
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<td>Salmonella</td>
<td>Breaded chicken product</td>
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<td>Salmonella</td>
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<tr>
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</tr>
<tr>
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<td>Mugil fillet</td>
<td>Salmonella enterica</td>
<td>≥ 99</td>
</tr>
<tr>
<td>N24</td>
<td>Salmonella</td>
<td>Mackerel fish</td>
<td>Salmonella enterica</td>
<td>≥ 99</td>
</tr>
</tbody>
</table>

Total 24

19
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

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COMPETING INTERESTS

The authors claim to have no conflicting agendas.

6. REFERENCES

Hamad et al. (2024)


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