A novel short-term high-lactose culture approach combined with a matrix-assisted laser desorption ionization-time of flight mass spectrometry assay for differentiating *Escherichia coli* and *Shigella* species using artificial neural networks

Jin Ling¹,²,³, Hong Wang¹,², Gaomin Li¹,², Zhen Feng⁴, Yufei Song⁵, Peng Wang⁶, Hong Shao¹,², Hu Zhou⁷, Gang Chen¹,²*

¹ Department of Biochemical Drugs and Biological Products, Shanghai Institute for Food and Drug Control, Shanghai, China, ² NMPA Key Laboratory for Quality Control of Therapeutic Monoclonal Antibodies, Shanghai Institute for Food and Drug Control, Shanghai, China, ³ Department of Pharmacy, Zhejiang Jinhua Guangfu Hospital, Jinhua, China, ⁴ Department of Antibiotics and Microbiology, Shanghai Institute for Food and Drug Control, Shanghai, China, ⁵ Department of Gastroenterology, Lihuili Hospital of Ningbo Medical Center, Ningbo, China, ⁶ Shanghai Key Laboratory of Intelligent Information Processing, School of Computer Science, Fudan University, Shanghai, China, ⁷ Department of Analytical Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China

* chengang@sifdc.org.cn

Abstract

Background

*Escherichia coli* is currently unable to be reliably differentiated from *Shigella* species by routine matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis. In the present study, a reliable and rapid identification method was established for *Escherichia coli* and *Shigella* species based on a short-term high-lactose culture using MALDI-TOF MS and artificial neural networks (ANN).

Materials and methods

The *Escherichia coli* and *Shigella* species colonies, treated with (Condition 1)/without (Condition 2) a short-term culture with an in-house developed high-lactose fluid medium, were prepared for MALDI-TOF MS assays. The MS spectra were acquired in linear positive mode, with a mass range from 2000 to 12000 Da and were then compared to discover new biomarkers for identification. Finally, MS spectra data sets 1 and 2, extracted from the two conditions, were used for ANN training to investigate the benefit on bacterial classification produced by the new biomarkers.

Results

Twenty-seven characteristic MS peaks from the *Escherichia coli* and *Shigella* species were summarized. Seven unreported MS peaks, with *m/z* 2330.745, *m/z* 2341.299, *m/z* 2350.837, *m/z* 2358.963, *m/z* 2361.680, *m/z* 2367.678, and *m/z* 2370.860, were identified and used for ANN training.
Conclusions

In summary, adding a short-term high-lactose culture approach before the analysis enabled a reliable and easy differentiation of *Escherichia coli* from the *Shigella* species using MALDI-TOF MS and ANN.

Introduction

Matrix assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a fast and cost-effective method for bacterial identification, and it is used routinely in many laboratories and clinical testing organizations[1–3]. Although standard mass spectrum database-based MALDI-TOF MS can identify thousands of bacterial species, *Escherichia coli* (*E. coli*), the most common bacteria in clinical practice, is currently unable to be reliably differentiated from Shigella by routine MALDI-TOF MS analysis [4]. Because the *E. coli* and *Shigella* species are closely related and they both belong to the family Enterobacteriaceae, their MS spectra are very similar to each other. The MALDI-TOF MS identification results can be only reported as *E. coli/Shigella* species, which challenges the entity separation and rapid identification in epidemiology and clinical diseases[5].

*E. coli* and *Shigella* species are classified as separate species based on their biochemical characteristics and clinical relevance[6,7]. Assessing the biochemical characteristics and serotyping are commonly used for the identification of *E. coli* and *Shigella* species to obtain accurate results. These approaches are classical and reliable, but may have a suboptimal diagnostic performance.

The accurate identification of *E. coli* and *Shigella* species isolated from the clinical sample is urgently required for clinical diagnostics and public health. In this study, we present a novel short-term culture approach that is combined a MALDI-TOF MS analysis method to realize the accurate and reliable identification of *E. coli* and *Shigella* species.

Materials and methods

Bacterial strains

A total of 23 bacterial strains identified by a consensus approach of biochemical and 16S rRNA gene sequencing was selected for the experiment, which covered all the common *Escherichia* and *Shigella* species (See supporting information).

Culture and sample preparation

The strains were grown on commercial tryptic soy (Huankai microbial, Guangzhou, China) at 35˚C for 24 h to obtain fresh colonies. The strain colonies were inoculated into our in-house developed high-lactose fluid medium and were incubated at 35˚C for 2 h (Fig 1). The
colonies on tryptic soy agar and the bacterial suspension in the fluid medium were prepared before the MALDI-TOF MS analysis (See supporting information).

**MALDI-TOF MS data acquisition**

The MS analyses and MS/MS analyses were performed using a 4800 Plus MALDI-TOF MS (AB Sciex, Redwood City, US) and an Autoflex maX MALDI-TOF/TOF system (Bruker Daltonik GmbH, Bremen, Germany). The MS spectra were acquired in linear positive-ionization mode. Afterwards, the target MS peak was selected for the MS/MS analysis in a reflector mode. The MS/MS spectra were interpreted primarily with the FlexAnalysis™ software (Bruker Daltonics, Bremen, Germany) (See supporting information).

**Protein identification**

The protein database searches were performed with MASCOT version 2.5 (Matrix Science, London, UK) and BioTools 3.2 software (Bruker Daltonics, Bremen, Germany) against the Swiss-Prot database for protein identification (See supporting information).

**Bacterial identification using artificial neural networks**

Back propagation neural networks (BPNN), a frequently used artificial neural network, was employed to recognize the classification of the target bacteria, and this was performed in Matlab™ software R2015b (MathWorks, Redick, USA) (See supporting information).
Results

Comparison of the spectra in the different culture conditions

As shown in Fig 2, the spectra from E. coli strains obtained from the tryptic soy agar culture condition contained 27 fully conserved MS peaks, which were also shared with most of the E. coli strains. Analogously, the 27 MS peaks described above were also observed in the spectra of most of the Shigella strains. There was no significant differential peak between the spectra from the E. coli strains and the Shigella strains, leading an inability to identify them using MALDI-TOF MS (S1 Fig). In addition, compared with the spectra from the E. coli and Shigella species, the spectra from other 4 Escherichia species did not contain MS peaks of m/z 2547, m/z 4165, m/z 4186, m/z 4440, m/z 4873, m/z 5100, m/z 5474, m/z 7594, m/z 9078 and m/z 9756 simultaneously (Fig 2). The spectrum from E. hermannii showed special MS peaks of m/z 2717, m/z 3120, m/z 3631, m/z 4594, m/z 4757, m/z 5440, m/z 5757, m/z 6261 and m/z 9524, which were not observed in the spectra of any of the other strains and could be used as biomarkers for identification. The MS peaks of m/z 3793 and m/z 7282 were only observed in the spectrum from Shimwellia blattae, which could be used as biomarkers for identification. Based on the characteristic peaks described above, the other Escherichia species was easily identified using MALDI-TOF MS.

The spectra from the E. coli strains changed after culture in the in-house developed high-lactose fluid medium for 2 hours. In addition to the 27 fully conserved MS peaks, seven new peaks of m/z 2330.745, m/z 2341.299, m/z 2371.581, m/z 2401.038, m/z 3794.851, m/z 3824.839 and m/z 3852.548 were obviously observed in the spectra from the E. coli strains (S2 Fig). In contrast, these differential MS peaks were not observed in either the Shigella or the other Escherichia species, as shown in S2 Fig. Therefore, these seven newly discovered characteristic MS peaks could be used as novel biomarkers to differentiate the E. coli from Shigella species.

https://doi.org/10.1371/journal.pone.0222636.g002

Fig 2. Coloured heat map of the characteristic MS peaks. Coloured heat map showing the characteristic MS peaks in the spectra from the E. coli, Shigella and other four Escherichia strains. The relative intensity of the MS peak was categorized using Hierarchical Clustering Explorer 3.0. Blocks, with a relative intensity equal to 0 shown in black; blocks with a relative intensity less than 10% are shown in dark green; blocks with a relative intensity between 10% to 100% are shown in bright green. E., Escherichia; Sh., Shigella.
Identification of new characteristic MS peaks

As shown in Table 1, six of the seven new characteristic MS peaks were successfully identified both by the amino acid sequence and by the type of protein. By searching the protein databases, these amino acid sequences belonged to acid shock protein, which indicated that the new characteristic peaks were fragments of acid shock protein. Because of the acid shock protein mutation, the molecular weights of the matched protein were different, and the new characteristic MS peaks had similar mass-to-charge ratios.

Adding a short-term high-lactose culture approach benefits back propagation neural networks classification modelling

Data sets 1 and 2, with the same matrix size of 12350×12396, were processed with the isomap algorithm to reduce their dimensionality from 12396 to 2048. As shown in Fig 3A, after the dimensionality reduction, Data set 1 obviously separated into two clusters, with *E. coli* group and *Shigella* group labels. Whereas Data set 2 was not clustered with satisfaction. The results

| Precursor ion (m/z) | Fragment ion (m/z) | Amino acid sequence | Mascot score | Protein hits | Molecular weight | Source |
|---------------------|--------------------|---------------------|--------------|--------------|------------------|--------|
| 2330.288            | 110.023, 297.132, 481.225, 618.253, 792.443, 1016.466, 1153.556, 1281.567, 1409.626, 1538.686, 1705.813, 1794.886, 2073.129, 2241.410, 2330.293 | AAKKHAGKSHHQPAKPAQQPA | 160 | Acid shock protein | 10470 | Escherichia coli (strain SE11) |
| 2341.174            | 129.095, 266.173, 403.227, 518.331, 645.380, 801.433, 986.471, 1114.576, 1227.525, 1314.648, 1442.673, 1540.671, 1641.820, 1805.906, 1942.970, 2070.947, 2195.317 | AAKKHKNAKAEQKAPEQKAQ | 144 | Acid shock protein | 10525 | Escherichia coli O6:K15:H31 (strain S36 / UPEC) |
| 2371.207            | 129.066, 266.125, 355.106, 518.263, 645.311, 801.376, 999.411, 1144.520, 1227.462, 1344.574, 1472.574, 1570.542, 1671.747, 1835.845, 1972.790, 2225.229 | AAKKHHKNTKAEQKAPEQKAQ | 139 | Acid shock protein | 10585 | Escherichia coli (strain K12 / MC4100 / BW2952) |
| 2401.201            | 129.035, 266.007, 368.102, 518.469, 1144.500, 1224.586, 1352.602, 1480.758, 1672.278, 1776.871, 1865.676, 2003.033, 2144.293, 2367.983 | AAKKHAKHSHHQPAKPAQQPA | 114 | Acid shock protein | 10585 | Escherichia coli (strain K12 / MC4100 / BW2952) |
| 3794.908            | 265.917, 394.219, 636.342, 1049.585, 1433.869, 1570.899, 1707.990, 1910.008, 2485.891, 2205.954, 2741.652, 3216.909 | AETATTPAPTATTTKTAAAPKTHKQHKAAAPQAQKAQ | 173 | Acid shock protein | 10555 | Escherichia coli O17:K52:H18 (strain UMN026 / ExPEC) |
| 3824.807            | 266.038, 394.245, 522.368, 929.552, 1049.620, 1433.860, 1570.967, 1708.022, 1910.160, 2206.128, 2348.200, 2516.133, 2908.195, 3035.718 | AETTTTPAPTATTTKTAAAPKTHKQHKAAAPQAQKAQK | 101 | Acid shock protein | 10585 | Escherichia coli (strain K12 / MC4100 / BW2952) |
| 3852.973            | 154.393, 266.032, 522.326, 929.495, 1305.738, 1433.849, 1570.936, 1707.997, 1910.457, 2038.033, 2206.097, 2347.966, 2415.776, 2671.798, 2909.116, 3065.072, 3418.344, 3674.481 | No matched result | - | No matched result | - | - |

https://doi.org/10.1371/journal.pone.0222636.t001
indicated that Data set 1 contained prominent characteristics of dimensionality for distinguishing spectrum from the *E. coli* and *Shigella* species. BPNN Model I and II were trained basing Data set 1 and 2, with identical parameters. The overview of the receiver operating characteristic curves for the two BPNN models, achieved by the synchronously optimizing BPNN model I and II, are shown in Fig 3B. The areas under curve value were 0.99 and 0.72 for the BPNN model I and II, respectively. The classification accuracies of BPNN model I and II were 97.71 ± 0.16% and 74.39 ± 0.34% (n = 5). There was an extremely remarkable difference between two accuracy results, which were compared with a *t*-test (*p* < 0.001) (Fig 3C). These results suggested that BPNN model I was significantly improved when a short-term high-lactose culture approach was added before the MS analysis.

**Discussion**

Most *E. coli* strains are part of the normal gut flora, whereas the *Shigella* species are considered to be pathogenic bacteria. The *Shigella* species has been separated from *E. coli* strains, as a requirement for clinical diagnostics, using biochemical methods and serological techniques, since the first *Shigella* species were discovered in 1898[8]. A series of distinct phenotypic and biochemical characteristics-based laboratory methods are commonly used to distinguish them, which are currently not efficient enough to satisfy the diagnostic requirements. By comparing genomes and housekeeping genes, the *E. coli* and *Shigella* species are be considered to be part of the same phylogenetic continuum rather than clearly distinct species. The nuances, at the molecular level, between the *E. coli* and *Shigella* species lead to an indistinguishableness by routine sequencing of the 16S rRNA gene and MALDI-TOF MS-based identification[8].
The MALDI-TOF MS technique is a highly cost-effective and time-efficient way to identify bacteria[9–11]. Due to their close relatedness, the spectra of *E. coli* and *Shigella* species have a high degree of similarity. In our investigation, the *E. coli* and *Shigella* species shared 27 main characteristic MS peaks and had no significantly differentiated MS peaks. A routine MALDI--TOF MS analysis for the identification of *E. coli* and *Shigella* species is difficult. A recent study showed that a specialized automated system, based on an analysis of selective biomarker MS peaks in the spectra, using Biotype software followed by an analysis with FlexAnalysis and ClinProTools software for the identification of *E. coli* and *Shigella* species, was better than traditional techniques, including an automated microbiology identification system and serotyping[12]. Paauw et al. created a high-resolution reference library for implementation in Biotype software in order to reflect the genetic diversity of the *E. coli* and *Shigella* species and to rapidly distinguish the *Shigella* species from *E. coli*[13]. However, the reliability of the identification largely depended on the quality and resolution of the spectra, especially the sample spectra. In routine MALDI-TOF MS analysis, the quality of the sample spectra is usually affected by a series of factors, including the type of sample, bacterial state, culture medium recipe, sample pretreatment method and operation and the parameters of the MS analysis[14].

Even if a high-quality spectrum is acquired, the detection and recognition of low intensity biomarkers for the identification of the *E. coli* and *Shigella* species is still difficult. To provide a reliable and efficient identification method for the *E. coli* and *Shigella* species for clinical diagnostic purposes, the biochemical method and the MALDI-TOF MS technique are combined. A novel high-lactose fluid medium is prepared on the optimized prescription. We added a short-term culture approach, using an in-house developed high-lactose fluid medium, before the routine MALDI-TOF MS assay, which enabled a reliable distinction between the *E. coli* and *Shigella* species. Seven MS peaks were newly observed in the spectra of the *E. coli* species only, which can be used as new biomarkers to distinguish *E. coli* and *Shigella* using MALDI-TOF MS.

The limited strains involved in our study and the unknown biomarkers may cause methodological limitations of its application. The distinct mechanism needs to be understood to determine whether the present approach successfully applies to any of the *E. coli* and *Shigella* strains. The proposed biomarkers are identified as fragments of acid shock protein, which makes the distinct mechanism clarified. Acid shock protein is encoded by the *asr* gene in *E. coli*, which is strongly induced by a high acid environment (pH < 5.0)[15]. Acid shock protein is subject to an N-terminal cleavage of the signal peptide, yielding an 8-kDa polypeptide, which is detected in acid-shocked bacteria. These results indicated that the new biomarkers emerged because of *E. coli* was under an acid shock condition. To our knowledge, *E. coli* strains can ferment lactose, by which organic acids are produced [16]. These organic acids are dissolved in the liquid medium, leading a pH reduction of the culture condition, and thus, the *E. coli* species are stressed to produce many acid shock proteins to protect themselves against the low pH survival condition made by the lactose fermentation [15]. After 2 h incubation, the pH value of liquid medium in *E. coli* group was 5.2±0.5, which support our hypothesis. Whereas the *S. flexneri, S. sonnet, S. bogdii E. hermannii, Shimwella blatta*, *E. fergusonii* and *E. albertii* strains do not ferment lactose, while some *S. dysenteriae* strains ferment lactose slowly (delayed lactose fermentation), which leads to no significant change of pH, as well as the expression of acid shock protein when they survive in a high-lactose condition for a short time. Therefore, our method can be widely applied to differentiate all lactose-fermenting *E. coli* from *Shigella* species and other non-lactose-fermenting *Escherichia* species.

The classification algorithm plays a crucial role in the MALDI-TOF MS-based bacterial identification method[17–19]. BPNN, as an artificial intelligence algorithm, has to be well-trained using high-quality data with a good separation[20,21]. This gives a satisfying...
classification result and achieves automatic bacterial identification. Although the new biomarkers are obvious and easily recognized by an analyst, the BPNN model test on the new biomarkers is required. The aim of the BPNN model establishment is to determine whether the new biomarkers provide a high feature separation of the data and further improve the classification efficiency and accuracy. BPNN model I and II were synchronously optimized to investigate the influence of adding a short-term high-lactose approach. The results indicated that the feature separation of data set 1 was higher than that of data set 2, and BPNN model I performed better than BPNN model II. The E. coli and Shigella species were correctly classified, with 97.71±0.16% accuracy, using the BPNN model I. This encourages us to recommend this method for the identification of E. coli and Shigella species in the clinical laboratory. The short-term culture, combined with the MALDI-TOF MS assay, described here provides a novel bacteria identification strategy for allied bacteria groups, which are unable to be identified using the routine MALDI-TOF MS approach.

Conclusion

In the present study, we provide a novel short-term culture approach, with optimized high-lactose fluid medium components, before the MS analysis in order to induce the expression of new biomarkers to distinguish E. coli and Shigella using a MALDI-TOF MS. The new biomarkers increase the data separation, which significantly improves the classification efficiency and accuracy of artificial neural networks.

Supporting information

S1 Fig. The MALDI-TOF MS spectra of the E. coli, Shigella and other Escherichia strains cultured on tryptic soy agar for 24 h. The MALDI-TOF MS spectra of the E. coli, Shigella and other Escherichia strains acquired from the linear positive mode, with a mass range from 2000 to 12000 Da. All the experimental strains were cultured on tryptic soy agar for 24 h followed by sample preparation and MALDI-TOF MS analysis.

(TIF)

S2 Fig. The MALDI-TOF MS spectra of the E. coli, Shigella and other Escherichia strains treated with an additional 2 h culture in the in-house developed high-lactose fluid medium. The MALDI-TOF MS spectra of the E. coli, Shigella and other Escherichia strains acquired from the linear positive mode, with mass range from 2000 to 12000 Da. All the experimental strains were cultured on tryptic soy agar for 24 h, with an additional 2 h culture in the in-house developed high-lactose fluid medium followed by sample preparation and MALDI-TOF MS analysis. Newly discovered MS peaks, as identification biomarkers, are marked with the respective mass-to-charge ratio.

(TIF)

S1 File. Supporting information. The detailed methods of experiments in research work. The bacterial strains for experiments, culture conditions, sample preparation method, MALDI-TOF MS data acquisition method, protein identification method and artificial neural networks for bacterial identification were detailed.

(DOCX)

Acknowledgments

This study was supported by Shanghai Municipal Food and Drug Administration Research Projects (No. KT-201708210037).
Author Contributions
Formal analysis: Jin Ling, Hong Wang.
Funding acquisition: Jin Ling.
Investigation: Jin Ling, Gaomin Li, Peng Wang.
Methodology: Jin Ling, Peng Wang, Gang Chen.
Resources: Zhen Feng, Yufei Song.
Validation: Hong Shao.
Writing – original draft: Jin Ling.
Writing – review & editing: Hu Zhou, Gang Chen.

References
1. Buchanan R, Ball D, Dolphin H, Dave J. Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry for the identification of Neisseria gonorrhoeae. Clin Microbiol Infect. 2016; 22: 815.e815–815.e817.
2. Mari-Almiraill M, Cosgaya C, Higgins PG, Van Assche A, Telli M, Huys G, et al. MALDI-TOF/MS identification of species from the Acinetobacter baumannii (Ab) group revisited: inclusion of the novel A. seifertii and A. dijkshoorniae species. Clin Microbiol Infect. 2017; 23: 210.e211–210.e219.
3. Yu J, Liu J, Li Y, Yu J, Zhu W, Liu Y, et al. Rapid detection of carbapenemase activity of Enterobacteriaceae isolated from positive blood cultures by MALDI-TOF MS. Ann Clin Microbiol Antimicrob. 2018; 17: 22. https://doi.org/10.1186/s12941-018-0274-9 PMID: 29776363
4. Dallagassa CB, Huergo LF, Stets MI, Pedrosa FO, Souza EM, Cruz LM, et al. Matrix-assisted laser desorption ionization-time of flight mass spectrometry analysis of Escherichia coli categories. Genet Mol Res. 2014; 13: 716–722. https://doi.org/10.4238/2014.January.29.2 PMID: 24615036
5. Devanga Ragupathi NK, Muthuirulandi Sethuvel DP, Inbananthan FY, Veeraraghavan B. Accurate differentiation of Escherichia coli and Shigella serogroups: challenges and strategies. New Microbes New Infect. 2018; 21: 58–62. https://doi.org/10.1016/j.nmni.2017.09.003 PMID: 29204286
6. Chattaway MA, Schaefer U, Tewolde R, Dallman TJ, Jenkins C. Identification of Escherichia coli and Shigella Species from Whole-Genome Sequences. J Clin Microbiol. 2017; 55: 616–623. https://doi.org/10.1128/JCM.01790-16 PMID: 27974538
7. Campilongo R, Di Martino ML, Marcocci L, Pietrangeli P, Leuzzi A, Grossi M, et al. Molecular and functional profiling of the polyamine content in enteroinvasive E. coli: looking into the gap between commensal E. coli and harmful Shigella. PLoS One. 2014; 9: e106589. https://doi.org/10.1371/journal.pone.0106589 PMID: 25192335
8. Ud-Din A, Wahid S. Relationship among Shigella spp. and enteroinvasive Escherichia coli (EIEC) and their differentiation. Braz J Microbiol. 2014; 45: 1131–1138. https://doi.org/10.1590/s1517-8382201400040002 PMID: 25763015
9. Schultness B, Brodner K, Bloemberg GV, Zbinden R, Bottger EC, Hombach M. Identification of Gram-positive cocci by use of matrix-assisted laser desorption ionization-time of flight mass spectrometry: comparison of different preparation methods and implementation of a practical algorithm for routine diagnostics. J Clin Microbiol. 2013; 51: 1834–1840. https://doi.org/10.1128/JCM.02654-12 PMID: 23354198
10. Harju I, Lange C, Kostrzewa M, Maier T, Rantakokko-Jalava K, Haanperä M. Improved Differentiation of Streptococcus pneumoniae and Other S. mitis Group Streptococci by MALDI Biotyper Using an Improved MALDI Biotyper Database Content and a Novel Result Interpretation Algorithm. J Clin Microbiol. 2017; 55: 914–922. https://doi.org/10.1128/JCM.01990-16 PMID: 28053215
11. Schultness B, Bloemberg GV, Zbinden R, Bottger EC, Hombach M. Evaluation of the Bruker MALDI Biotyper for identification of Gram-positive rods: development of a diagnostic algorithm for the clinical laboratory. J Clin Microbiol. 2014; 52: 1089–1097. https://doi.org/10.1128/JCM.02399-13 PMID: 24452159
12. Khot PD, Fisher MA. Novel approach for differentiating Shigella species and Escherichia coli by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2013; 51: 3711–3716. https://doi.org/10.1128/JCM.01526-13 PMID: 23985919
13. Paauw A, Jonker D, Roeselers G, Heng JM, Mars-Groenendijk RH, Trip H, et al. Rapid and reliable discrimination between Shigella species and Escherichia coli using MALDI-TOF mass spectrometry. Int J Med Microbiol. 2015; 305: 446–452. https://doi.org/10.1016/j.ijmm.2015.04.001 PMID: 25912807

14. Veloo AC, de Vries ED, Jean-Pierre H, Justesen US, Morris T, Urban E, et al. The optimization and validation of the Biotype MALDI-TOF MS database for the identification of Gram-positive anaerobic cocci. Clin Microbiol Infect. 2016; 22: 793–798. https://doi.org/10.1016/j.cmi.2016.06.016 PMID: 27404365

15. Seputiene V, Motiejunas D, Suziedelis K, Tomenius H, Normark S, Melefors O, et al. Molecular characterization of the acid-inducible asr gene of Escherichia coli and its role in acid stress response. J Bacteriol. 2003; 185: 2475–2484. https://doi.org/10.1128/JB.185.8.2475-2484.2003 PMID: 12670971

16. Bergey DH. Bergey’s manual of systematic bacteriology 2nd Edition. Springer New York Dordrecht Heidelberg London; 2010.

17. Chen YD, Zheng S, Yu JK, Hu X. Artificial neural networks analysis of surface-enhanced laser desorption/ionization mass spectra of serum protein pattern distinguishes colorectal cancer from healthy population. Clin Cancer Res. 2004; 10: 8380–8385. https://doi.org/10.1158/1078-0432.CCR-1162-03 PMID: 15623616

18. Fangous MS, Mougari F, Gouriou S, Calvez E, Raskine L, Cambau E, et al. Classification algorithm for subspecies identification within the Mycobacterium abscessus species, based on matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2014; 52: 3362–3369. https://doi.org/10.1128/JCM.00788-14 PMID: 25009048

19. Huang B, Zhang L, Zhang W, Liao K, Zhang S, Zhang Z, et al. Direct Detection and Identification of Bacterial Pathogens from Urine with Optimized Specimen Processing and Enhanced Testing Algorithm. 2017; 55: 1488–1495. https://doi.org/10.1128/AEM.02549-16 PMID: 28249997

20. Lasch P, Beyer W, Nattermann H, Stammier M, Siegbrecht E, Grunow R, et al. Identification of Bacillus anthracis by using matrix-assisted laser desorption ionization-time of flight mass spectrometry and artificial neural networks. Appl Environ Microbiol. 2009; 75: 7229–7242. https://doi.org/10.1128/AEM.00657-09 PMID: 19767470

21. Feng L, Zhu S, Lin F, Su Z, Yuan K, Zhao Y, et al. Detection of Oil Chestnuts Infected by Blue Mold Using Near-Infrared Hyperspectral Imaging Combined with Artificial Neural Networks. Sensors (Basel). 2018; 18: E1944