Clinica Chimica Acta 473 (2017) 180–185

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Review

Traditional approaches versus mass spectrometry in bacterial identification and typing

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ARTICLE INFO

Keywords:
Mass spectrometry
Bacterial identification, serotyping

ABSTRACT

Biochemical methods such as metabolite testing and serotyping are traditionally used in clinical microbiology laboratories to identify and categorize microorganisms. Due to the large variety of bacteria, identifying representative metabolites is tedious, while raising high-quality antisera or antibodies unique to specific biomarkers used in serotyping is very challenging, sometimes even impossible. Although serotyping is a certified approach for differentiating bacteria such as *E. coli* and *Salmonella* at the subspecies level, the method is tedious, laborious, and not practical during an infectious disease outbreak. Mass spectrometry (MS) platforms, especially matrix assisted laser desorption and ionization-time of flight mass spectrometry (MALDI-TOF-MS), have recently become popular in the field of bacterial identification due to their fast speed and low cost. In the past few years, we have used liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based approaches to solve various problems hindering serotyping and have overcome some insufficiencies of the MALDI-TOF-MS platform. The current article aims to review the characteristics, advantages, and disadvantages of MS-based platforms over traditional approaches in bacterial identification and categorization.

1. Introduction

1.1. Identification of bacteria

Traditionally, identification of bacteria has been a complicated process. When a patient presents with a suspected bacterial infection, epidemiological information, symptoms, and signs are collected by a clinician, and a clinical sample (e.g. blood, urine, sputum, etc.) is sent to a laboratory for confirmatory testing. Efforts to try to isolate the bacterium or check its components are then made, using available morphological, biochemical, serological, and molecular methods [1]. Several examples are described here: (i) Gram staining not only determines whether the bacteria are Gram positive or negative, but is also used to observe the shape of the bacteria; (ii) *E. coli* O157:H7 isolates can be identified based on their inability to ferment sorbitol after overnight culture; (iii) Pulse field gel electrophoresis (PFGE), a method used to observe whole genome DNA restriction patterns, can be applied to differentiate O157 positive from O157 negative *E. coli* isolates [2]; (iv) Antisera to somatic antigens, such as O antigens (lipopolysaccharides, LPS) and H antigens (flagella), can be used to categorize *Listeria* or *Campylobacter* into many subtypes [3–5]. Of these approaches, we will describe serotyping, a traditional approach of categorizing pathogenic bacteria, in the following section.

1.2. Serotyping of bacteria

Two bacteria routinely categorized using bacterial serotyping are *E. coli* and *Salmonella* [6,7]. Here, O antigens and H antigens are exploited in a series of agglutination reactions. Validated serum will bind to antigens of specific strains of *E. coli* or *Salmonella* and not others. However, multiple strains may express the same antigen, so additional antigens must then be used to differentiate them [6,7]. Since serotyping of these bacteria has been performed extensively over the past half century [8,9], the method has become standardized among World Health Organization (WHO) coordinated reference centers for both organisms. Complications arise, however, due to the fact that there are more than

Abbreviations: ACN, acetonitrile; BAL, bronchoalveolar lavage; CHCA, cyano-4-hydroxy-cinnamic acid; CLSI, Clinical and Laboratory Standards Institute; DHB, 2,5-dihydroxybenzoic acid; FA, formic acid; FDA, Food and Drug administration; HPLC, high performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LOS, lipooligosaccharides; LPS, lipopolysaccharides; MALDI-TOF, matrix-assisted laser desorption ionization-time-of-flight; *m/z*, mass to change ratio; MRM, multiple reaction monitoring; MS, mass spectrometry; MS-H, mass spectrometry-based H typing; PCR, polymerase chain reaction; PFGE, pulse field gel electrophoresis; PMF, peptide mass fingerprinting; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TFA, trifluoroacetic acid

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http://dx.doi.org/10.1016/j.cca.2017.08.035
Received 30 June 2017; Received in revised form 28 August 2017; Accepted 29 August 2017
Available online 31 August 2017
0009-8981/ © 2017 Published by Elsevier B.V.

150 O and 53 H antigens among *E. coli*, and 60 O and 114 H antigens among *Salmonella*. Hence, despite their usefulness, these conventional antibody-based assays can be costly and laborious to perform due to the wide range of antibodies (i.e. antiserum) and the multiple antibody/agglutination reactions needed to assign a final classification [6,7]. High costs are consequently associated with the purchase, quality control, and conservation of these antisera. In Canada, provincial public health laboratories will perform serotyping for common types of *E. coli* and *Salmonella* (e.g. *E coli* O157: H7, *Salmonella enteritidis*), while difficult strains are serotyped at the National Microbiology Laboratory (NML). H typing is more time-consuming than O typing, especially in cases when the bacteria have little or no motility and require motility induction to increase flagella production [8–10]. At the NML, H typing for an *E. coli* isolate is usually completed within 2–12 days after receipt of the isolate. For *Salmonella*, serotyping of H antigens is further complicated due to frequent diphasic or even multi-phasic flagella expression. In such cases, a procedure called "phase inversion" must be applied whereby the production of one type of flagella is suppressed with antisera while the other is identified. After serotyping, each *Salmonella* serovar is then designated sequentially by antigens O, H1, H2 (and sometimes H3), separated by colons, with their individual factors represented by numbers and letters (e.g. diphasic serovar Typhimurium is written as "4,5,12:i:1,2"). For monophasic *Salmonella* serovars, the non-existing H antigen (either H1 or H2) will be written as a hyphen (e.g. serovar Typhi is written as "9,12:d:-"). Expression of diphasic flagella does not exist in *E. coli*, so *E. coli* are annotated using O and H antigen numbers directly (e.g. O157:H7). The inherent limitations of polyclonal antisera production make it difficult to obtain antisera for testing rare and emerging strains [11,12]. Currently there are more than 2500 serovars among *Salmonella* species according to WHO collaborative centers, who update serovars formulae regularly [13]. A technical report of multi-center quality assurance in Europe indicated that there are more inconsistencies in *Salmonella* H typing than there are in O typing [14]. An interesting phenomenon is that in serotyping, especially for *E. coli*, when LPS become lipooligosaccharides (LOS) for unknown reasons or due to long-term storage, the bacteria turn "rough", and although the strain remain motile and possess flagella, neither O nor H serotyping can be performed due to the resulting change in cell surface. Consequently, the strain is labeled "undetermined". This is why in literature we often see "undetermined", "rough", and "NM" (non-motile) describing *E. coli* strains or isolates.

Due to the time-consuming nature of serotyping, along with its difficulty in differentiating close serotypes and its inability to type "rough" and emerging strains, several alternative approaches have been investigated for faster and more accurate typing. Among these approaches, mass spectrometry (MS) is the fastest and most promising.

1.3. MS-based bacterial identification

MS has become a well-known approach for bacterial identification, especially after the US Food and Drug Administration (FDA)'s approval of two matrix assisted laser desorption-time of flight-mass spectrometry (MALDI-TOF-MS)-based platforms [15,16]. Recently, the Clinical and Laboratory Standards Institute (CLSI) published microbiology guideline methods for the identification of cultured microorganisms using MALDI-TOF-MS (M58-Ed) [17]. From the available literature, we see that from January 2012 to December 2016, MALDI-TOF-based applications dominated the field with the most usage. They have become routine microbiological procedures in various clinical laboratories to reduce turnaround times, costs, and overall labor. Although it seems MALDI-TOF-MS application in bacterial identification in general has plateaued, we can see that MS application through blood culture is still very active, and LC-MS/MS (liquid chromatography-tandem mass spectrometry)-based application is also growing (Fig. 1).

The following sections will explain commonly used MS platforms and their use in bacterial identification and categorization. Some important aspects of MS, such as sample preparation and database creation, will be emphasized. Our unique experience in this area will also be introduced.

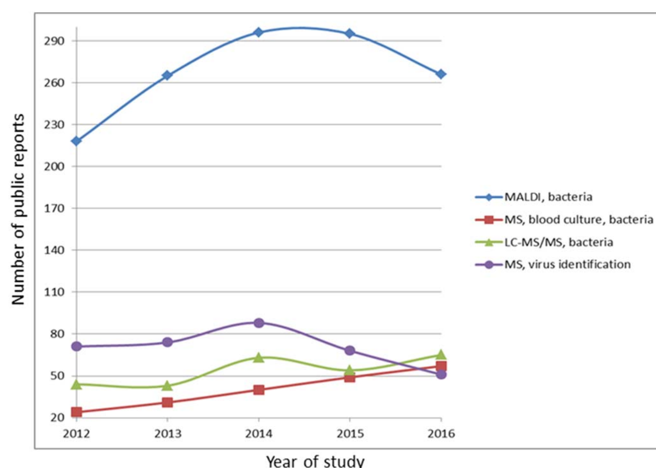

Fig. 1. Number of MS-based microbial identification studies reported in PubMed from Jan. 01, 2012 to Dec. 31, 2016.

2. Common platforms in MS-based bacterial identification

2.1. Mass pattern match

MALDI Biotyper and VITEK MS are both MALDI-TOF-MS-based instruments. Bacteria culture is treated with a strong solvent such as 1% trifluoroacetic acid (TFA) in 50% acetonitrile (ACN) [18] or 70% formic acid (FA) followed by 50% ACN [19] and spun down. Extracted molecules are mixed with matrices, often cyano-4-hydroxy-cinnamic acid (CHCA), and loaded onto a MALDI plate for MS detection. The sample spots are shot by laser energy and mass spectra, represented by mass to change ratios (m/z), are obtained. Stored mass fingerprints of verified bacteria in the database are compared with the spectra obtained from the sample. The identity of the microorganism is based upon which set in the database provides the best match with the accumulated spectra. Bacteria culture can also be directly smeared onto the MALDI plate and then covered by matrix [20]. Each set of matching spectra results is a potential identification and is given a confidence score. Generally, a score equal or greater than 2.0 is considered a confident and correct identification. The scoring can be further categorized: any score below 1.7 being considered unreliable, from 1.7 to 1.9 indicating probable genus identification, 2.0 to 2.29 indicating confident genus identification, and 2.3 to 3.0 indicating highly confident species identification [21]. Some laboratories have also performed bacterial identification straight from positive blood culture by spinning down the blood cells first at low speed and then collecting the bacteria from the supernatant at higher speed. The bacterial pellet was then washed with water and treated with a solvent, such as 70% FA, to extract molecules for MALDI-TOF-MS analysis [22–24].

Since Biotyper and VITEK-MS-based spectral pattern comparisons do not involve any fractionation and protease treatment of samples, the composition of the spectra very likely represents the most abundant components of the microbes, such as ribosomal proteins [25]. Even so, the spectra are often very unique for most bacteria, especially at the genus level [26–28]. Although pure cultures produce more stable and consistent results [27–28], different culture conditions, such as temperature and culture media, may also affect the quality of the spectra and corresponding identification [26].

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