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Commentary on clinical utility of whole genome sequencing and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

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Abstract

The present study is commentary and aims to evaluate the practical application of Whole Genome Sequencing (WGS) and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in clinical microbiology. The MALDI-TOF MS method has been replaced cultural and biochemical tests for species identification in most laboratories worldwide. Moreover, WGS has elevated the discriminatory power by detection of single nucleotide polymorphisms (SNPs), and helped with the identification of microorganisms that were misidentified by previous culture and biochemical tests.

Moreover, WGS can be used in epidemiological studies (e.g. clinical outbreaks) by tracking of phylogenetic maps and analyzing SNP distance; antimicrobial resistance studies such as the study of antimicrobial resistance genes, e.g. CTX-M, NDM, KPC, or OXA; and the study of recombination of plasmid or insertion sequence (IS) elements to elucidate the mechanism of antimicrobial resistance.

MALDI-TOF technology is used for bacterial or fungal species identification in most laboratories. VITEK MS (bioMérieux) and Biotyper (Bruker Daltonics) are examples of commercially available MALDI-TOF MS systems. The technique has been used for direct identification of organisms from blood culture bottles, markedly shortening the identification time. Beta-lactamases or carbapenemases have been detected by analyzing specific protein peaks using MALDI-TOF MS. Shiga toxin-producing Escherichia coli, Salmonella serotype, and Vibrio phenotypes can also be detected.

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Introduction

The present study aims to evaluate the practical application of Whole Genome Sequencing (WGS) and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in clinical microbiology. The use of Whole Genome Sequencing (WGS) in clinical microbiology has become increasingly popular in recent times [1-3]. WGS is an accurate and sensitive method for identifying pathogenic organisms that, if properly applied, could improve infection control. WGS can be used to determine the association between asymptomatic commensal and pathogenic organisms. For instance, diverse organisms are present in the respiratory tract (RT) and gastrointestinal tract (GIT). Asymptomatic commensal organisms, such as Neisseria species in the RT, can obtain virulence factors via plasmids or insertion sequences (IS) from nearby virulent strains and become virulent. WGS can be used to elucidate these processes and mechanisms. Furthermore, although 16S rRNA sequencing is the gold standard for species identification, it has low discriminatory power and sensitivity when distinguishing between closely related species, whereas WGS provides more information with higher accuracy and sensitivity. WGS occasionally reveals previous misidentifications assigned by conventional methods, which leads to taxonomic changes [4,5]. In epidemiological studies, the discriminatory power of WGS is higher than that of pulsed-field gel electrophoresis (PFGE) or multilocus sequence typing (MLST). Bacterial evolution, lineage, or clonality can be determined using a phylogenetic map based on WGS data [6]. Antimicrobial resistance by organisms that produce extended spectrum beta-lactamases (ESBLs) such as cefotaximase (CTX-M), or carbapenamases such as KPC carbapenamase (KPC) and New Delhi metallo-beta-lactamase (NDM-1) can be elucidated by WGS [7-9]. In infection control, the route of transmission of multi-drug resistant (MDR) strains such as methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococci (VRE), MDR Escherichia coli, MDR Klebsiella pneumoniae, and MDR Pseudomonas, can also be tracked [10]. Owing to the effect of selective pressure on pathogenic strains following vaccination, non-susceptible strains can become predominant and serotype or sequence type switch or transformation is possible, which leads to the development of new MDR clones [11]. WGS can be used to determine this serotype or sequence type switch or transformation. In addition, WGS databases (e.g., http://www.mlst.net and http://pubmlst.org) can be used to develop new polymerase chain reaction (PCR) assays that require a target sequence and newly designed primers [12].

Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a standard in mass spectrometry, especially in protein analysis. Its advantages include high sensitivity, tolerance to buffers, fast data acquisition, and simple and robust instrumentation. MALDI-TOF was first used for bacterial identification 10 years ago, and nearly all identifications in North America and Europe are now carried out using this technique [13,14]. Although setting up bioinformatics software can be costly and time consuming, and complicated computing infrastructure is a major limitation, once set up, MALDI-TOF can be more efficient in terms of

cost and workload than other techniques are. Philippe et al. reported on the direct identification of microorganisms in blood culture vials using MALDI-TOF, which considerably shortened the identification procedure [15]. Moreover, beta-lactamases or carbapenamases can be detected using this technique [16].

General procedure for WGS

Genomic DNA is extracted from isolates and whole-genome sequencing is carried out. Processing is required before analysis owing to unassembled short read sequences. Trimmed, filtered sequencing reads are mapped to the genome of the reference strain for single nucleotide polymorphism (SNP) analysis. The target insert size and paired end reads are determined and assembled using bioinformatics tools for phylogenetic and population structure analyses.

Clinical Applications of WGS

Communication between asymptomatic commensal and pathogenic strains

Asymptomatic commensal organisms such as Neisseria spp. and Clostridium difficile are present in the RT and GIT, respectively [3]. An avirulent strain colonizing the RT or GIT could acquire a virulence gene or an antimicrobial resistance gene and become virulent. For example, avirulent *C. difficile* in the GIT can acquire the *C. difficile* toxin B (TcdB) gene or other virulent genes from neighboring pathogens, and become virulent. Moreover, it can acquire antimicrobial resistance genes from neighboring bacteria of the same or other species, and become antimicrobial resistant, with the potential to harm people and environment. The mechanism of acquiring resistance genes can be elucidated by WGS.

Species identification

16S rRNA sequencing is the gold standard for species identification. This method has remarkably increased sensitivity and specificity compared to the previous standards of culturing and biochemical tests. However, it still has low discriminatory power and sensitivity when distinguishing between closely related species. In contrast, WGS provides more information with higher accuracy and sensitivity. A large number of genomic sequences are represented in the Bacterial Isolate Genome Sequence Database (BIGSdb), and WGS occasionally reveals previous misidentifications obtained by conventional methods, which leads to taxonomic changes [4]. For example, species previously misidentified as *S. aureus* have been changed to *S. argenteus* or *S. schweitzeri* following WGS [5].

Epidemiological studies

In epidemiological studies, the discriminatory power of WGS is higher than that of PFGE or MLST. Bacterial evolution, lineage, or clonality can be determined using a phylogenetic map based on WGS data [6]. The Seqsphere+ bioinformatics software (Ridom GmbH) analyzes core genome (cg) MLST, which uses more housekeeping genes than MLST alone does [7,8]. Furthermore, horizontal transmission and mutation or recombination events can be detected by WGS [9]. Mellmann et al. have developed a surveillance program for control of prospective ESBLs, carbapenemases, and colistin-resistant mcr-1 strains [10].

Infection control

In infection control, the route of transmission of MDR strains such as MRSA, VRE, MDR E. coli, MDR K. pneumoniae, and MDR Pseudomonas can be tracked [10]. A software can be compiled for prospective infection control in a clinical setting.

Differentiation between vaccine-targeted and non-targeted strains

Owing to selective pressure following vaccination, strains not targeted by vaccines can become predominant, and serotype or sequence type switch or transformation is possible, thus leading to the development of new MDR clones [11]. WGS can be used to determine the serotype, sequence type switch, or transformation, and to detect the development of new MDR clones. Following vaccination, it is now possible to determine whether a fever is due to a vaccine-targeted strain or infection by a new wild-type microorganism.

Design of new PCR methods

WGS databases (e.g., http://www.mlst.net and http://pubmlst.org) can be used to develop new PCR assays, which require a target sequence and designed primers [12].

For example, real-time PCR can be used to differentiate between *Campylobacter jejuni* and *C. coli.* Best et al. [12] evaluated this method by analyzing more than 1,700 Campylobacter genomes extracted from the PubMLST database. The primer and probe sequences of mapA and ceuE, which are PCR targets for *C. jejuni* and *C. coli*, respectively, were analyzed in silico. As a result, a real-time PCR assay identified 99.7% of the isolates accurately. However, the reduced specificity of *C. coli* identification was determined to be due to the introgression in mapA or sequence diversity in ceuE. This demonstrates how a WGS database can be used for re-evaluation of the results of previous PCR experiments.

Preparation of samples for MALDI-TOF MS experiments

For the identification of bacteria, single colonies are usually suspended in 70% ethanol, vortexed, and concentrated by centrifugation. The supernatant is discarded, the cells are resuspended in 50 µL of 70% formic acid, and an equal volume of acetonitrile is added. The mixture is vortexed and then centrifuged. An aliquot of the supernatant (1 µL) is spotted on the target plate, allowed to evaporate, and then overlaid with a matrix of α-cyano-4-hydroxy-cinnamic acid. If the solution contains at least 5-10 × 10^6 cells/µL, sufficient spectra are obtained for identification.

Clinical applications of MALDI-TOF MS

Species identification

MALDI-TOF has completely replaced biochemical methods for species identification worldwide. VITEK MS (bioMérieux) and Biotyper (Bruker Daltonics) are examples of commercially available MALDI-TOF systems. A recent report revealed that an assay based on MALDI-TOF had high correspondence with culture-based and biochemical tests [13]. Moreover, MALDI-TOF shares high result correspondence with 16S rRNA analysis, proving that it is an accurate technique [14]. Skin diphtheroids had previously been regarded as contaminants until MALDI-TOF enabled species identification. Reports of these bacilli behaving pathogenically have since then increased.

Direct species identification from blood culture vials

Recently, MALDI-TOF has been directly applied to positive blood culture bottles for the rapid identification of pathogens. Since this lead to the reduction of turnaround time, the technique has potential beneficial impact for patients [15]. The development of a commercially available extraction kit (Bruker Sepsityper) for use with the Bruker MALDI BioTyper has facilitated the processing required for identification of pathogens directly from blood cultures.

Detection of beta-lactamases and carbapenemases

ESBLs and carbapenemases can be detected using MALDI-TOF by analysis of the different mass peaks. Alongside using another method for the detection of carbapenemases, isolates were incubated with ertapenem; the results were considered positive, i.e., carbapenem hydrolysis occurred, when the characteristic carbapenem peak (m/z 475) completely disappeared [16].

Other methods

Virulent clones or sequence types, specific antimicrobial resistance types, Shiga toxin-producing *E. coli*, Salmonella serotype, and Vibrio phenotypes have also been identified using MALDI-TOF MS.

Nevertheless, MALDI-TOF MS has some limitations. The interpretation of m/z peaks is subjective, the method is prone to technical variations, and identification of rare species is difficult because the current databases are still being updated [17].

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