

Review Article

Overview of typing techniques as molecular epidemiology tools for bacterial characterization



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ABSTRACT

The main purpose of microbial typing is to evaluate the relationships between microbial isolates. Microbial typing can use for identifying the source of infection by detecting a clonal link between the strains. Moreover, it can analyze outbreaks, antimicrobial-resistant strains, and evaluate the effectiveness of control measures so, the efficiency of monitoring systems would increase. HAIs can affect hospitalized patients in all age ranges with any clinical situation, and lead to death. Molecular epidemiology is useful to determine genetic relatedness between isolated pathogens from patients, and design proper prevention plans to prevent infection through the hospital and community. Nowadays, typing methods for a wide range of bacterial strains are known as essential epidemiological tools to prevent and control infections in hospitals and communities. Although basic typing methods were more focused on phenotypic techniques like antibiogram and serotyping, new methods are based on molecular techniques including PCR-based methods and sequencing-based methods. Due to the high frequency of methods, choosing the right one for research applications seems difficult and requires basic knowledge about all of them. In this review, we aim to introduce the most useful and practical molecular typing techniques. Also, their utilization, advantages, and disadvantages were compared.

1. Introduction

The classification of specific microorganisms based on macromolecules such as fatty acids, proteins, and DNA is termed molecular typing [1]. The term "molecular epidemiology" first appeared in the 1970s and early 1980s in three distinct areas: cancer epidemiology, environmental epidemiology, and infectious disease epidemiology [2]. Based on valid health organizations' reports, molecular epidemiology principles could be helpful in both clinical and non-clinical research such as epidemiologic, molecular, biochemical,

genetics, and human diseases studies. The knowledge of molecular epidemiology is striking to prognosis, detection, and prevention of infectious outbreaks [3].

Studies of molecular epidemiology are essential for identifying the source of infection, developing preventive strategies, and transmission of pathogens among patients [4]. HAIs are one of the main threatening challenges for patients and health center staff [5]. For example, catheters, surgical equipment, and ventilators could turn into a source of infection. Also, HAIs can affect

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not only a single hospitalized patient but also the community, which could be the first step of spreading multidrug-resistant pathogens through the communities [6, 7]. In addition, The application of a molecular typing system facilitates are useful for the understanding of clonal relationships between both infection-causing isolates and antibiotic-resistance strains [8]. Owing to this, typing systems can be used as a tool to study molecular epidemiology. Traditional phenotype-based typing methods, such as serotyping, biotyping, bacteriophage, and antibiogram, have been used for many years [9]. However, new methods detect the relationship of isolates at the molecular level and can increase the ability to distinguish between different bacterial subspecies. Since Human pathogens belong to a variety of microorganisms, typing techniques must have a very high ability to distinct species [10]. Molecular typing methods are mostly DNA-based, such as plasmid profiling, chromosomal DNA profiling using PFGE. Some of the methods are based on amplification like PCR-based techniques [11]. Despite the early development of Sequencing technology in the 1970s, it was not used in clinical trials in the early days due to some limitations like high cost and use of radioactive and toxic substances [12]. Also, because of the availability of a wide range of methods and several possibilities, such as location and workspace, amount of information store space, the difficulty of the protocol, cost, and speed it seems difficult to choose the right method. Finally, according to the sample size, the most appropriate method is to use a combination of several methods to perform [13].

In this review article, the most widely used and practical phenotypic and molecular typing techniques are gathered. Also, their applications and their advantages and disadvantages are compared.

2. Materials and methods

2.1 phenotypic typing

Phenotypic typing embraces several methods like antibiogram-based techniques which are used by clinical microbiology laboratories to guide drug therapy [14].

The next phenotypic typing tool is serotyping which is used to classify most members of human or animal pathogens like *Pasteurellaceae* [15]. Serotyping systems owing to distinguish variables are reliable methods for typing *Salmonella* spp. and *Escherichia coli* isolates. Therefore, This typing method is used in reference laboratories as the standard method [16].

One of the phenotypic techniques is ribotyping, in this technique bacterial chromosomal DNA is extracted and cut with a restriction endonuclease enzyme. The fragments are separated by agarose gel electrophoresis and transferred to a membrane. Those fragments which are complementary to the probe are highlighted by radioactivity or an enzyme-substrate reaction [17].

The other useful phenotypic method is mass spectrometry which initially was used to analyze the structure of complex chemical or organic compounds and macromolecules [18]. This method allows the identification and characterization of the molecules that make up a substance in the form of complex spectra which can be compared with mass spectrum databases. Accordingly, this method has been recently approved as a fast and reliable tool for identifying bacterial species, such as *E. coli* O157: H7 and *Legionella pneumophila* [19, 20].

2.2 molecular typing methods

We categorized molecular typing methods into four main classes.

The first class is band-based methods which PCR plays an integral role. PCR is the most common method of nucleic acid amplification and identification, has had a significant impact on the epidemiological diagnosis and investigation of infectious diseases [21, 22]. This method is encompassing several techniques, like PCR fingerprinting which investigate duplication of DNA sequences scattered in the bacterial genome. In addition, the amplification of short genomic fragments between repeating elements using PCR is called rep-PCR, which is an efficient method for bacterial genotyping. The integral advantages of this technique are flexibility, simplicity, and technical speed.

However, one of the drawbacks of rep-PCR is the difference between laboratories which is due to the effect of variable amplifying components. Generally, this system is fully standardized, and reproducible, but relatively expensive [23, 24]. RAPD-PCR is a typing method based on the use of random short primers. This method has a sufficient affinity for chromosomal DNA sequencing at low temperatures so this vantage can be used as the starting point for bacterial genome replication regions. On the other hand, this method does not require former knowledge of specific DNA target sequences and is flexible with general application. For instance, this method has been used in the study of *Enterococcus* species, a common microflora of the human intestine that causes a variety of HAIs until today. Identification of the source of these pathogens infections at the beginning of HAIs could prevent former outbreaks and protect more people to get infected by them [19]. Previous studies have used the mentioned method for molecular typing to emphasize the necessity of adding molecular epidemiological studies in every hospital [16, 25, 26]. Moreover, MLVA is a PCR-based method that involves the determination of the amount of repeat copy units of multiple loci for size analysis of repeat regions by using multiplex PCR. To do so, resolution systems like agarose electrophoresis can also be used. In another word, this strategy is of particular interest for prime resolution genotyping of pathogens with a high genome homogeneity, such as *Bacillus anthracis* and *Mycobacterium tuberculosis* [27]. Last in order is ERIC-PCR that the target regions of central inversion are 126 highly conserved pairs located outside the gene regions of the *Enterobacteriaceae* family. The location of these elements in the genomes of different *Enterobacteriaceae* species and other strains is varied so, it is used as a genetic marker. As an example, ERIC-PCR has been very useful as a typing method for antibiotic-resistant *Enterobacteriaceae* spp. and rapid detection of *E. coli* ST131 epidemic clone [28].

The second class of molecular typing methods is hybridization-based methods and notably, the advantage of this class is the use of nucleotide probes. The first technique is direct hybridization, in this method, microbial

DNA is immobilized on support or probe and finally will be detected by using specific DNA fragments. The other hybridization technique is DNA microarray typing. As microbiology enters the post-genomic era, bacterial typing is increasingly inclined to whole-genome analysis. Although whole-genome sequencing requires advanced, expensive, and limited equipment, the presence of microarrays provides extensive information about the entire content of genes. The most important benefit of this technique is its widespread application and ability to detect several bacteria simultaneously. Examples of bacterial pathogens that microarrays in terms of epidemiological typing have been used successfully for, are *Salmonella enterica*, *Staphylococcus aureus*, *E. coli*, and *Campylobacter jejuni* [22, 25, 29].

The third class is sequencing-based methods, which study the polymorphism of DNA sequences. The sequencing methods could be divided into two main classes based on using the PCR technique. That is PCR-based sequencing such as MLST, and non-PCR-based sequencings like SLST and WGS. MLST was originally designed to identify pathogens of *Neisseria meningitidis*. Also, MLST can detect variations in DNA sequences (size 400-500 bp) in five to ten housewife genes. Nowadays, MLST is known as the "gold standard" as a result of defining the population structure of the genetic subspecies of many microorganisms [30]. Yet, the focus of SLST is on the computational method to develop the SLST typing scheme. Thus, the resulting design can be used to type bacterial species directly. Additionally, a fundamental advantage of sequencing-based methods over band-based techniques is that the sequence data is unambiguous and transferable between laboratories. The SLST method due to the analysis of an over-variable region of the protein A gene (*spa*) in *A. aureus* typing is well validated. The advantages of this technique are speed, repeatability, ease of use, and portability [31]. SNP is a change in a single nucleotide of a sequence relative to its wild type owing to a random nucleotide mutation, horizontal gene transfer, or intragenic recombination [32]. For instance, SNP genotypic is primarily used to explain the association between highly homologous

pathogen isolates such as *E. coli* O157: H7, or *Bacillus anthracis* [33]. WGS is increasingly competing with other diagnostic technologies, including traditional bacterial culture methods. Today, the hypothesis of eliminating all intermediate typing approaches such as MLST and SNP analysis and replacing it with WGS has become popular. According to some researchers, within a few years, WGS could become the only diagnostic and molecular epidemiology tool for genetic identification and drug susceptibility testing. Although the cost of sequencing is still high, the interpretation is reliable [34, 35]. The process of WGS in a clinical laboratory is shown in Figure 1.

Lastly, the fourth class is enzymatic restriction-based methods. This class includes PFGE and RFLP. RFLP was the first generation of bacterial genotypic typing in the 1980s. This technology had several disadvantages such as time-consuming and laborious work, preparation of high concentration and quality DNA, and accurate standardization of electrophoresis protocols for in vitro comparison [21]. In Pulse PFGE the restriction pattern of the whole bacterial genome is analyzed without the use of probes. Due to the compatibility of various restriction enzymes with PFGE, it is considered the golden standard for subtyping of bacteria and is the

currently used method in the PulseNet USA network, the molecular surveillance network for foodborne infections in the United States [36].

A summary of the advantages and disadvantages of some of the most widely used typing methods is shown in Table 1.

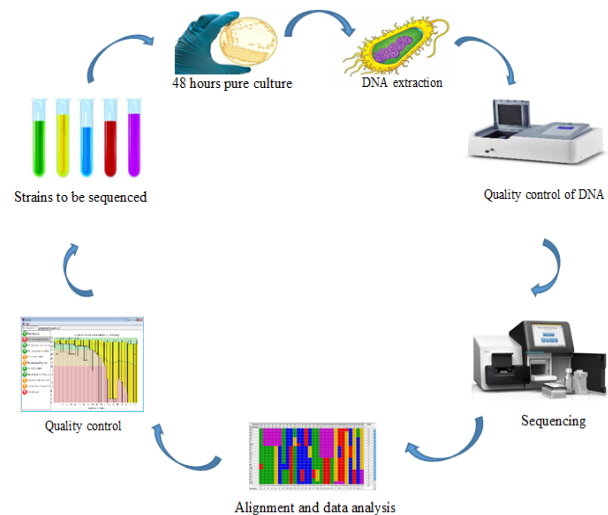


Fig. 1. The process of whole-genome sequencing (WGS) in a clinical laboratory.

Table 1. Advantages and disadvantages of widely used phenotypic and molecular typing methods.

Method	Advantages	Disadvantages
Ribotyping	Good reproducibility and discriminatory power	Complex, requiring 3-4 days to complete a test
MLS	Available data and the possibility of easy comparison of data between different laboratories and different countries.	Expensive
REP – PCR	Ability to accurately detect strains	A complex method that requires training and expertise.
ERIC – PCR	Easy working procedure	Relatively low reproducibility
RAPD-PCR	High turnover of the strains	Non-portable
Arrays	Portable and reproducibility	Low reproduction carpet
WGS	Portable and reproducibility	Average producibility, discriminatory power, and approximately 80% typability
PFGE	High discriminatory power	Lack of online database
RFLP	Cheap and high sensitivity for detecting bacterial strains	Average cost
		Expensive
		Cost and reproducibility of results across different laboratories, requiring 3-5 days to complete a test
		consuming

3. Discussion

HAIs are responsible for a significant percentage of death in infants, children, and adults annually throughout the world. The mortality rate in neonates associated with HAIs is more than 20% [37]. Nosocomial infections should be controlled particularly about immunocompromised patients, these patients could be vulnerable to being infected by bacterial isolates, and spread them through overall the hospital. The spread of resistant bacteria among patients in a hospital could be effective in increasing their length of stay and imposing costs on the health care systems and the patients [38]. According to the WHO, 15% of hospitalized patients have been infected by hospital-acquired infections, and this percentage will increase every year. 3.2% of hospitalized patients in the USA are exposed to HAIs annually, this percentage is twice higher in Europe by 6.5%, and much higher around the world. An effective surveillance system can monitor HAIs, design controlling programs, and prevent rising these severe infections worldwide [39]. It is necessary to design infection control strategies to encounter these deadly infections, identify the causes and sources of contamination that have caused the outbreaks [34]. Source tracking is the basic and integral step for HAIs control, which should be screened in each health care center, based on the type of center, the type of patients, and the type of dominant HAIs causing microorganisms reported from each health care center. However, the point is to interrupt the transmission cycle of these microorganisms and identify the transmission route of infection between patients [40].

Molecular epidemiology could provide a new opportunity for the prevention and treatment of common infections in this regard. Today, typing techniques are used as a tool for molecular epidemiology and identification of the clonal relationship of microorganisms [35].

We suggest that in each medical center, according to the specialized staff and available equipment, one of the molecular epidemiological methods be used to stop the transmission of microorganisms causing HAIs.

In conclusion, source tracking of HAIs could decrease the length of stay and mortality rate among hospitalized patients. Molecular typing methods are known as effective strategies for identification and detecting clonal relationships between bacterial isolates. The work process of large clinical and public health laboratories is towards sequencing and analyzing high throughput and standard data, so the next step in using this technology is to use and synchronize sequencing simultaneously with high throughput analysis for efficient clinical diagnoses, schedule effective infection control plans and accurate screening of public health through the entire world.

4. Conclusion

In conclusion, source tracking of HAIs could decrease the length of stay and mortality rate among hospitalized patients. Molecular typing methods are known as effective strategies for identification and detecting clonal relationships between bacterial isolates and finding source of infection in health care setting. The work process of large clinical and public health laboratories is towards sequencing and analyzing high throughput and standard data, so the next step in using this technology is to use and synchronize sequencing simultaneously with high throughput analysis for efficient clinical diagnoses, schedule effective infection control plans and accurate screening of public health through the entire world.

Abbreviation

HAIs: Hospital-acquired infections

PFGE: pulse-field gel electrophoresis

PCR: polymerase chain reaction

Rep-PCR: Repetitive element palindromic PCR

RAPD: Random Amplified Polymorphic DNA

MLVA: Multilocus Variable number of tandem repeat Analysis

ERIC: Enterobacterial Repetitive Intergenic Consensus

MLST: Multi Locus Sequencing Typing

SLST: Single Locus Sequencing Typing

WGS: Whole Genome Sequencing

SNP: Single Nucleotide Polymorphism

RFLP: Restriction Fragment Length Polymorphism

WHO: World Health Organization

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The authors declare that no conflict of interest

Consent for publications

The author read and proved the final manuscript for publication.

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Authors' Contribution

All authors had an equal role in study design, work, statistical analysis, and manuscript writing.

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No human or animals were used in the present research. This is a study on methodology and techniques.

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