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Multilocus Sequence Typing Characterization of *Pseudomonas Aeruginosa*

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Abstract

Pseudomonas aeruginosa is a common Gram-negative opportunistic bacterium that causes acute and chronic infections in humans, especially the immunocompromised ones and in hospitalized patients. Its exceptional adaptability coupled with inherent resistance to various antibiotics as well as its capacity to develop other determinants of resistance has placed it among the main global concerns in public health. Pathogenic success of *P. aeruginosa* is explained by a combination of virulence factors, such as outer membrane proteins, lipopolysaccharides, biofilm formation, secretion systems, toxins, lytic enzymes, siderophores, antioxidant enzymes, and quorum-sensing networks all of which contribute to the increase of colonization, immune evasion and tissue damage. Molecular typing procedures have been of paramount importance in attempting to explain the epidemiology and population structure of this pathogen. Of these, multilocus sequence typing (MLST) has become one of the standardized, reproducible, and globally comparable methods, which rely on the study of shared housekeeping genes. This review summarizes the existing information about virulence determinants and pathogenicity of P. aeruginosa and critically analyzes the advantages and drawbacks of MLST and the corresponding genomic typing methods. Dynamics between virulence, resistance, and population structure must provide a better understanding of the contribution to enhancing surveillance, treatment regimens, and transmission among patient of high-risk P. aeruginosa clones in healthcare settings.

Keywords: *Pseudomonas aeruginosa*, Virulence factors, Multilocus sequence typing.

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توصيف بكتريا الزائفة الزنجارية باستخدام تقنية التنميط التسلسلي متعدد المواضع

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المستخلص

الزائفة الزنجارية (*Pseudomonas aeruginosa*) بكتيريا انتهازية شائعة سالبة الغرام، تُسبب عدوى حادة ومزمنة لدى البشر، وخاصةً ذوي المناعة الضعيفة والمرضى المُنوَّمين في المستشفيات. وقد جعلتها قدرتها الاستثنائية على التكيف، إلى جانب مقاومتها المتأصلة للعديد من المضادات الحيوية، فضلاً عن قدرتها على تطوير عوامل مقاومة أخرى، من بين أهم المخاوف العالمية في مجال الصحة العامة. ويُعزى نجاح الزائفة الزنجارية في إحداث المرض إلى مجموعة من عوامل الضراوة، مثل بروتينات الغشاء الخارجي، والليبوسكاريد، وتكوين الأغشية الحيوية، وأنظمة الإفراز، والسموم، والإنزيمات المحللة، والسيدروفورات، والإنزيمات المضادة للأكسدة، وشبكات استشعار النصاب، وكلها تُسهم في زيادة الاستعمار، والتهرب المناعي، وتلف الأنسجة، وقد كانت إجراءات التنميط الجزيئي ذات أهمية بالغة في محاولة فهم وبائيات هذا العامل الممرض وبنيته السكانية. من بين هذه الطرق، أصبح تحديد النمط التسلسلي متعدد المواقع (MLST) أحد الأساليب المعيارية والقابلة للتكرار والمقارنة عالميًا، والتي تعتمد على دراسة الجينات الأساسية المشتركة. تلخص هذه المراجعة المعلومات المتوفرة حول محددات الضراوة وقدرة بكتيريا الزائفة الزنجارية على إحداث المرض، وتحلل بشكل نقدي مزايا وعيوب تقنية MLST وطرق تحديد النمط الجينومي المقابلة لها. يجب أن توفر ديناميكيات العلاقة بين الضراوة والمقاومة وبنية التجمعات البكتيرية فهمًا أفضل لمساهمة تعزيز المراقبة وبروتوكولات العلاج

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معلومات البحث
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وانتقال سلالات الزائفة الزنجارية عالية الخطورة بين المرضى في مرافق الرعاية الصحية.

الكلمات المفتاحية: الزائفة الزنجارية، عوامل الضراوة، تحديد النمط التسلسلي متعدد المواقع

Introduction

Pseudomonas aeruginosa is a commonly encountered opportunistic pathogen in hospital settings that can cause both acute and chronic infections in immunocompromised individuals, such as those with chronic obstructive pulmonary disease, cystic fibrosis, cancer, wound, burns, sepsis, and ventilator-associated pneumonia [1].

Pseudomonas aeruginosa is a Gram-negative bacillus that grows in a variety of conditions, including water and soil. Human activities, such as sewage disposal and pollution with hydrocarbons and pesticides, have influenced the occurrence of this bacterium [2]. *P. aeruginosa* possesses several virulence factors, including quorum-sensing systems, secreted enzymes, toxins, and the ability to form biofilms, which increase its potential to cause severe infections [3]. *Pseudomonas aeruginosa* is a multidrug-resistant bacterial infection that can make people very sick and even kill them when it infects the respiratory tract, circulatory system, urinary tract, and central nervous system. The strains of *Pseudomonas aeruginosa* have become the most common cause of infections that people get in hospitals. This is a big problem for individuals with weak immune systems who are in the hospital. [4] It is a common organism that can cause serious opportunistic infections, especially in those with weak immune systems. This organism is very bad for health care institutions because it breaks through the body's first line of defense and penetrates through the skin, causing nosocomial infections especially in hospital intensive care units (ICUs). Because there are several ways for *P. aeruginosa* to resist most antibiotics, its pathogenesis is complex, leading to the formation of a wide range of cellular structures and substances outside of cells that are important for increasing pathogenicity [5]. The aim of this review is to provide a comprehensive overview of *Pseudomonas aeruginosa* as an opportunistic and multidrug-resistant pathogen, with particular emphasis on its major virulence factors, mechanisms of pathogenicity, and molecular epidemiology. This review will also aim to evaluate the usefulness of multilocus sequence typing (MLST) and its various forms in the characterization of P. aeruginosa isolates, the clarification of population structure, the occurrence of high-risk clones and enable infection control and epidemiology monitoring within hospitals.

2. Pseudomonas aeruginosa: Virulence factors Magor and Genicity

Initially discovered in wounds, has become an important pathogen, which has complex mechanisms of disease causation, including outer membrane proteins (OMPs). The functions of these proteins are to carry amino acids and peptides, absorb antibiotics, carry carbon, adhere to bacteria, liberate virulence factors, and identify hosts.[6]

Lipopolysaccharides (LPS): This is a structural component of bacterial cell wall which shields the outer membrane and is toxic to host cells. It leads to tissue damage, adhesion, and the recognition of host receptors, which influences antimicrobial resistance and biofilm formation [7] Biofilm formation: P. aeruginosa biofilm formation consists of flagella, pili, adhesins, and other elements, which contribute to its colonization,

adhesion, swimming, swarming, and chemotactic signals, The secreted toxins alter host cell signaling, destabilize the extracellular matrix, cause tissue injury and alter the local microbiota.

Exopolysaccharides (EPS): EPSs of alginate, Psl and Pel stimulate the formation of biofilm, bacterial aggregation and microcolony development during pneumonia. The anionic matrix prevents phagocytosis, antibodies and complement [8] Toxin: Toxins produced by Pseudomonas include T3SS-delivered ExoU, ExoT, ExoS and ExoY which modify the intracellular environment. The exotoxin A disrupts the formation of host proteins, pyocyanin produces oxidative disease, and a range of toxins affects immune reaction and tissue destruction [9].

Lytic enzymes: Elastases LasA and LasB, alkaline protease (AprA), lipases, and esterase A destroy epithelial cells by degrading lung surfactant and junction disruption [10]. Siderophores: pyoverdine and pyochelin siderophores assist in the absorption of iron and the formation of virulence factors including biofilms [11]. Antioxidant enzymes: Catalases, hydroperoxide reductases alkyl, superoxide dismutase do not allow elimination of phagocytes by neutralising Reactive Oxygen Species (ROS) [11]. Quorum Sensing (QS): To initiate infection by Pseudomonas, the QS, comprising of Ls, Rhl and Pqs pathways, mediate gene expression and activity. The synergy promotes survival and subdues the immunity [12].

3. MLST & bacterial isolate characterization

A major source of the success of MLST is its design, whereby the aims were to enhance the knowledge of bacterial population structure and evolutionary biology specifically the role and influence of lateral gene transfer [13], The idea of studying multiple housekeeping genes, which are thought to belong to the core genome and usually are under neutral or near-neutral selection, was first reported and applied in multilocus enzyme electrophoresis (MLEE) [14].

As compared to MLEE which assumed allelic variation as a result of variations in protein mobility during starch gel electrophoresis, MLST was a welcome development as it directly indexed nucleotide sequence variation in these genes, which provided much greater accuracy. The reproducibility and portability of nucleotide sequence data was also an added benefit of MLST as facilitated by the presence of curated online reference databases [15]. Another issue that is dealt with by MLST is the question of using phylogenetic approaches to recombining bacteria. MLST also circumvents the complexities associated with the different nature of point mutations, which usually occur at a single nucleotide position and recombination events, which typically create many polymorphisms. In MLST, treatment of mutation types is the same as each one leads to a new allele, therefore having equal weight in the analysis process [16].

In addition, MLST schemes using as few as seven housekeeping genes have been shown to have high discriminatory information in a set of bacteria species [15], There is a staggering level of diversity in allelic profiles which usually constitute less than 0.2 percent of the genome in question, based on an evaluation of the existing publicly accessible MLST techniques. This highlights the need to have a simple, yet highly scaled system of comparing and summarizing bacterial diversity, with standard definitions of alleles and sequence types (STs). Because ST and allele designations are arbitrary, there is no need to revise the underlying nomenclature. Instead, these identifiers

can be flexibly organized into higher-order groupings as new insights into the biological significance of the observed diversity emerge.

MLST data are particularly well suited for analyses of population structure, as the presence of shared alleles across loci allows inference of ancestral lineages using clustering methods and Bayesian approaches such as BAPS [17], Structure [18] and ClonalFrame [19]. In many bacterial species, the number of identified STs exceeds the number of alleles per locus by more than an order of magnitude, reflecting extensive genetic exchange [20], Despite this diversity, analysis of seven-locus MLST data frequently reveals distinct "clonal complexes." Even in highly recombining species, certain genotypes—represented by specific STs—tend to dominate. Some STs occur frequently and persist over time and across geographic regions, whereas the majority are rare and transient [15]. [21] suggested that these high-frequency STs represent stable consensus genotypes, often referred to as "central genotypes," though terms such as "founder" or "ancestor" may be misleading, as there is limited evidence to support them in this context. This example of the spread of carbapenem-resistant P. aeruginosa ST-235 all over Eastern Europe illustrates the persistence of such genotypes [22]. These consensus genotypes have been associated with some STs that sometimes are used to designate high risk clones. Multidrug resistance is specifically linked to ST-235, ST-111 and ST-175 of P. aeruginosa within healthcare settings [23]. Such correlations can be of therapeutic importance even in the absence of detailed knowledge on specific chromosomal mechanisms of resistance.

4. Amplification of seven housekeeping genes of *P. aeruginosa*:

The multilocus sequence typing system developed by [24] on Pseudomonas aeruginosa formed the basis of the choice of seven housekeeping genes; acsA, aroE, guaA, mutL, nuoD, ppsA, and trpE. They were selected because of these two reasons; they play critical roles in fundamental metabolic processes and the rate of their evolution is relatively steady so as to serve as good indicators of genetic diversity and population structure.

The MLST methodology based on the investigation of the allelic profiles of these conserved genes is an efficient high-resolution technique to characterize P. aeruginosa isolates and monitor their epidemiological entanglements in the various environments and clinical settings. The Pseudomonas aeruginosa MLST scheme works with internal segments of the following seven housekeeping genes:

- acsA (An acetyl-coenzyme A synthetic enzyme) is located in the mitochondrion.
- aroE (Shikimate dehydrogenase)
- guaA (GMP synthase)
- mutL -DNA mismatch repair protein.
- nuoD (NADH dehydrogenase I chain C, D)؛
- ppsA (Phosphoenolpyruvate synthase
- trpE component 1anthralinate synthase

5. Types of MLST (Multilocus Sequence Typing)

- In a molecular epidemiological study of *Pseudomonas aeruginosa* using MLST (Multilocus Sequence Typing), different types of MLST approaches can be applied [25].
- The traditional type of MLST is the sequencing of seven housekeeping genes of Pseudomonas aeruginosa cacsA, aroE, guaA,

mutL, nuoD, ppsA, and trpE. A set of unique allele combinations make up a Sequence Type (ST), allowing comparison on a global scale, a study of the population structure and monitoring of important clonal lineages such as ST235 and ST111. Whole-genome MLST (wgMLST) looks at hundreds or thousands of loci throughout the genome providing more resolution than classical MLST. It is especially effective in the investigation of the outbreak and the differentiation of closely related strains.

- The middle ground is core genome MLST (cgMLST), which focuses on genes that are common to every strain of a species. It can be used in comparisons on the species level and epidemiological studies on a large scale. Conserved ribosomal protein genes are targeted by ribosomal MLST (rMLST), which can be used in high-resolution typing as well as powerful phylogenetic analysis, applicable in evolutionary studies and the separation of deep lineage relationships.
- Custom MLST schemes involve custom gene panels dependent on the local epidemiology or characteristics such as resistance. They are particularly helpful when there is a desire to investigate strain diversity in a specific region, e.g. with respect to hospital settings in Brazil, and may also assist in the detection of new or emerging lineages.

6. Comparison with other techniques

One of the most widely used methods to type Pseudomonas aeruginosa is Multilocus Sequence Typing (MLST), the principle of which is based on the sequencing of seven housekeeping genes. It is standardized, reproducible and applicable to large epidemiological studies although it has moderate resolution and might fail to differentiate closely related outbreak strains. Whole genome MLST is an expansion on the concept, using hundreds to thousands of genes on the core and accessory genome, which gives it far greater resolution and discrimination of closely related strains.

It is increasingly being adopted into clinical settings, but is more time consuming and resource intensive. The highest resolution is found in the typing technique of single nucleotide variants (SNV) which detects a single nucleotide variant across genomes. They perform best when it comes to the investigation of outbreaks in detail and need excellent sequencing and bioinformatics skills. Repetitive element PCR (rep-PCR) was once quite popular because of speed and low cost but has poor resolution and reproducibility thus is not very reliable these days in a clinical application.

No longer regarded as the gold standard of bacterial strain typing, Pulsed-field gel electrophoresis (PFGE) provides high-resolution but is being gradually replaced by genome-based techniques because it is labor-intensive and inter-laboratory standardization is lacking [26].

7. Comparison between MLST and WGS

Multilocus Sequence Typing (MLST) is a popular molecular typing method applied to the study of bacterial pathogenic organism diversity and structure of their populations including Pseudomonas aeruginosa. This technique relies on sequencing a collection of well-conserved housekeeping genes which are typically seven loci to designate isolates to specific sequence types (STs). MLST is a very reproducible and standardized methodology that aids in global comparison of bacterial strains using publicly available databases making it especially useful in

epidemiological surveillance and population studies [27].

Conversely, Whole Genome Sequencing (WGS) examines the complete genome of a bacterium and gives a far greater amount of genetic resolution than MLST. WGS can be used to determine single-nucleotide polymorphisms (SNPs), antimicrobial resistance genes, virulence factors, and mobile genetic elements. It has, therefore, become a crucial instrument in the field of molecular epidemiology in terms of tracking the spread of bacteria and studying outbreaks with a more precise focus [28], Even though MLST is still useful in a large-scale epidemiological study where its relative simplicity and standardized methodology have its limited discriminatory power since it only analyzes a small section of the bacterial genome. WGS, in contrast, allows a broader genomic view that allows a more detailed phylogenetic analysis and a more detailed understanding of how bacteria evolve and develop resistance to different methods. Nevertheless, the use of WGS would involve more sophisticated sequencing methods, bioinformatics, and increased costs of operation over MLST, hence the popularity of MLST in most laboratories [29].

8. Advantages and Disadvantages of MLST

Due to its high resolution, reliability, and ability to generate data that can be compared on a global basis within pre-defined schemes, Multilocus Sequence Typing (MLST) is a widely used methodology of typing pathogens. It employs conventional sequencing methods, and it can be easily automatable to large and small sample sizes**.** The technique is also very movable as it needs only DNA or dead cells that may be transported and manipulated securely throughout the laboratories. Also, MLST data may be exchanged on an international scale in curated online databases and may be useful in population genetics and epidemiological modeling. Nevertheless, there are weaknesses of MLST. It relies on reference genomes in the selection of appropriate housekeeping genes, and genetic characteristics differ across species and therefore, there are no universal gene sets, so each new pathogen requires specific schemes to be designed.

Also, certain pathogens, such as Mycobacterium tuberculosis and Yersinia pestis, have poor genetic diversity with which MLST is unlikely to succeed. Interpretation of data remains an issue even in the context of automation and the analysis of data manually may require a significant amount of time when numerous loci are at hand.[30]

9. Limitations of MLST:

Multilocus sequence typing (MLST) has become a trusted population genetic technique, offering a standardized reproducible methodology of characterizing isolates of bacteria. Nevertheless, it possesses some significant shortcomings as well. Its cost is also considered one of the main disadvantages, especially when individual locus sequencing is needed as was historically the case [31], which limits its application in detailed epidemiological investigations.

In order to overcome the problem of strain differentiation, an alternative method has been developed under the name of multi-virulence-locus sequence typing. The method targets virulence genes and was first used on Listeria monocytogenes and is more discriminatory at the strain level because virulence factors are typically more polymorphic than housekeeping genes and, more importantly, have high rates of recombination that traditional MLST frameworks do not effectively handle [32]. With the advent of

next-generation sequencing technologies, some of the limitations of MLST have disappeared. Whole genome sequencing (WGS) can nowadays be used to obtain MLST data directly out of genome assemblies without the use of locus-specific sequencing [33]. In addition, WGS gives a considerably more comprehensive and more detailed picture of bacterial genomes, reflecting the entire genome and not the 0.1 percent contained in MLST loci. This improved resolution has allowed distinctions of strains within one lineage of MLST on finer scales. As an illustration, a WGS study showed that the ST258 sequence type of the Klebsiella pneumoniae strain is made up of two genetic clades, which defies previous beliefs of its clonal origin [34].

In spite of these, MLST is still popular especially with its strong and maintained online databases. These resources present reference allele sequence, types of sequence, and related epidemiological data, and consolidated resources to query and analyze sequences of the users. MLST is, therefore, still an asset in microbial typing in addition to research and the public health setting.

Conclusions

In conclusion, Pseudomonas Aeruginosa is a significant opportunistic pathogen with various virulence factors, and high ability to resist most drugs, making the pathogen persistent in clinical practices. The pathogenicity of the organism is also associated with the close relations with such mechanisms as biofilm formation, toxin production, secretion systems, and quorum sensing. The multilocus sequence typing (MLST) has been demonstrated to be a stable and standardized way of characterizing P. aeruginosa isolates and learning their population composition. MLST makes it possible to identify common types of sequences and high-risk clones of antimicrobial resistance. In spite of some drawbacks, MLST is an effective instrument of epidemiological research and comparison. In general, this study has demonstrated that the combination of knowledge of virulence determinants and MLST information can contribute to better knowledge of the genetic diversity and clinical importance of P. aeruginosa.

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