"Next Generation Sequencing (NGS) technology as a cutting-edge tool for the characterization of pathogens of interest in health.".

Authors

Diana Stefanía Gutiérrez Andrés Ricardo Carrazco Montalvo María Gabriela Echeverría Garcés Cristina Jeanneth Rodríguez Pólit

National Reference Center for Genomics, Sequencing, and Bioinformatics. National Institute of Public Health on Research - INSPI Quito, Ecuador

#### Introduction

### 1. Role of Microbiology in the Identification of Pathogenic Microorganisms

Various diseases associated with pathogens have been reported worldwide by a large number of people. Some of the most common have been *Escherichia coli* (*E. coli*), *Campylobacter jejuni* (*C. jejuni*), *Listeria monocytogenes* (*L. monocytogenes*), *Shigella spp.*, and *Salmonella spp.*, which cause dysentery, food poisoning, chronic diarrhea, and outbreaks originating in hospitals. This is a significant public health concern (Marušić, A. 2011).

These microorganisms constitute an important part of the microflora in various environments and ecological niches. They possess the ability to survive in extreme conditions or adapt to different external factors such as temperature, humidity, and limited nutrient availability. In this context, some pathogens are difficult to cultivate due to their demanding energy and nutrient requirements. Their genetic characteristics and biochemical capabilities determine their pathogenicity, affecting both the industry and public health (Franco-Duarte, R et al. 2019).

It is estimated that approximately 1,400 pathogens have the capacity to cause human diseases, with bacteria being the etiological agents in 350 million cases of foodborne illnesses (Mendez-Vilas, A. 2012). Clinical microbiology plays an important role for the pathogen identification through biochemical techniques, microscopic examinations, and cultures on selective media. These methods have enabled the management of infectious diseases and epidemiological surveillance at both local and global levels.

The adoption of new methodologies has facilitated the transition from conventional microbiology techniques to automated technologies such as mass spectrometry and genomics. These advancements have enabled the characterization of difficult-to-cultivate isolates and the identification of outbreaks (Váradi, L., et al.2017).

In a clinical setting, the primary challenge is to identify a possible infection caused by pathogenic microorganisms that could lead to hospital outbreaks.

Several factors contribute to the emergence of outbreaks,, including the interaction between the host and the infectious agent, which influences both the course and transmission of the disease. Therefore, there should be a close collaboration between clinical microbiology laboratories and physicians to guide a preliminary clinical diagnosis of individuals with suspected infections, aiming to provide efficient antibiotic treatment (Tang, Y.-W., & Stratton, C. 2012).

Thus, precise identification of microorganisms is essential in healthcare, applied research, and industry, encompassing areas from clinical microbiology to food manufacturing. Various criteria are considered when choosing identification methods, ranging from traditional techniques to cutting-edge technologies (Prakash, O., et al. 2007).

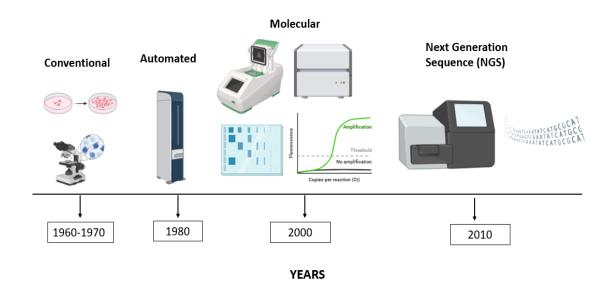
### 2. Evolution from Classical Microbiology to Molecular Microbiology and Its Limitations

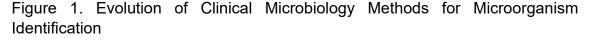
Since World War II, clinical microbiology has been characterized as a discipline in constant motion, evolving steadily with the advent of new technologies that have revolutionized the diagnosis of multiple infectious diseases (Vanbelkum, A. 2003). During this period and for many years, phenotypic classification was considered the only viable approach for the identification of microorganisms, even though this methodology often led to uncertain results and analytical difficulties.

Numerous reports describe inaccuracies in strain identification based on phenotypic methods or discordant results, highlighting the need technologies that generate consistent and precise results (Donelli, G., Vuotto, C., & Mastromarino, P. 2013). Consequently, the evolution of clinical microbiology has emerged in response to clinical needs. The adoption of innovative technologies over conventional methods has improved the identification of microbial etiologies associated with various infectious diseases, leading to more precise and effective therapeutic treatments (Ainsa, J. 2002a).

Since classic methods based on cultivation have constituted the first step in microbiological diagnosis, they depend on the growth of an infectious or pathogenic microorganism and subsequent its isolation to obtain a pure culture. Over time, other general procedures have been incorporated, such as staining,

the application of selective growth media, biochemical tests, and the development of more precise assays (Isenberg, H. 2003) (Figure 1).





The evolution of conventional microbiology, from automated plate readers to molecular identification of organisms cultured in solid media, offers potential advantages in terms of time, cost, and precision of increasingly specialized diagnostic tests, bypassing the need for cultivation (Fairfax, M., Bluth, M. H., & Salimnia, H. 2018). On the other hand, biochemical characterization has enabled the establishment of phenotypic profiles for different microorganisms This serves as the basis for taxonomic identification, with cultivation serving as a preliminary requirement for the development of phenotypic antimicrobial susceptibility tests, which are considered as a preliminary diagnosing an infection (Tang, Y.-W., & Stratton, C. 2012)

Molecular tests utilizing nucleic acid amplification, such as PCR (Polymerase Chain Reaction), are considered highly successful due to their exceptional sensitivity and specificity in detecting new pathogens through broad-spectrum DNA amplification methods. These tests have become routine, employing universal primer sites associated with conserved genes such as the 16S region in bacteria, which can be amplified via PCR (Deiman, B., van Aarle, P., & Sillekens, P. 2002).

However, the evolution of this test to qPCR (real-time PCR) along with commercial kits, has automated these technologies and expanded their potential applications (Gilbert, G. 2002). Similarly, commercial kit providers have significantly expanded the number of molecular diagnostic assays for the detection of infectious diseases.

Advancements in these types of tests have enabled real-time results that extend beyond mere detection, providing a semi-quantitative assessment of the quantity of bacterial cells present in the clinical sample. Consequently, these data serve as an indicator of the severity of an infectious disease, allowing for the identification various genes associated with antimicrobial resistance (Grisol, A et al., 2002).

Despite ongoing advancements, there are still cases of human diseases without a defined microbial cause. Their epidemiological and clinical characteristics are often associated with potential infections resulting from population growth, which increases the risk of exposure to zoonotic agents or bacterial pathogens carrying acquired antimicrobial resistance genes (Sibley, D., Peirano, G., & Church, D. 2012).

In conclusion, both conventional and molecular methods present limitations in pathogen diagnosis (refer to Table 1). The applicability of each of these methodologies largely depends on the type of sample, availability of resources, and infrastructure. This will facilitate the transition towards new technologies aimed at improving microorganism identification, thereby providing useful tools to physicians with the goal of benefiting patients.

Table 1. Limitations of Conventional Microbiology Methods vs. MolecularMethods in pathogen diagnosis.

Aspect	Conventional Microbiology Methods	Molecular Methods
Sensitivity	Lower	Higher
Specificity	Lower	Higher
Analysis Time	Longer	Shorter
Process Complexity	High	Low

Requires Culturing	Yes	No	
Pathogen Detection	Limited	Broad	
Precise Identification	Not always precise	High precision	
Cost	Relatively low	Can be higher	
<b></b>			

Source: Sibley, D., Peirano, G., & Church, D. 2012.

# 3. Genomics: The New Era of Identifying and Characterizing Pathogens of Interest in Health

With the growing concern about the spread of infectious diseases with pandemic potential and the increasing attention to outbreaks of communicable diseases, new technologies for identifying pathogens of interest in health are playing an increasingly important role. These tools are crucial for preventing and mitigating infections associated with a variety of diseases, including microbial organisms (Ellwanger et al., 2021; Tang, Croxen, Hasan, Hsiao, & Hoang, 2017).

Recently, advances in DNA sequencing have revolutionized epidemiology, allowing for a detailed analysis of pathogen genomes. These methods, faster and more precise that traditional techniques, are gradually replacing older methods and are expected to better guide public health practices and infection control more effectively (Tang et al., 2017). In this context, rapid detection and precise identification of microorganisms are crucial challenges in the field of health.

Since the early identification of microorganisms, scientists have developed classification schemes with the aim to systematically characterize species in an evolutionary and phylogenetic context (Emerson, Agulto, Liu, & Liu, 2008). This identification has proven more challenging for bacteria than for other organisms. Due to their size, bacteria have a limited range of morphological attributes (Emerson et al., 2008).

Additionally, bacteria exhibit enormous biochemical diversity in both their metabolism and cellular structure. While this diversity is useful for the taxonomic identification of some bacterial groups, it is not comprehensive for all bacteria (Emerson et al., 2008). Classical methods for identifying microorganisms rely on approaches that are time-consuming and labor intensive.

For decades, clinical microbiology laboratories and public health authorities have relied on phenotypic methods to identify bacterial pathogens. However,

these approaches prove inadequate for bacteria that are challenging to cultivate, exhibit slow growth, or possess uncommon phenotypes, making identification difficult and time-consuming (Franco-Duarte et al., 2019; Winand et al., 2019).

Recently, metagenomics has played a pivotal role in identifying new species and strains, as well as monitoring outbreaks and complex diseases (Miller, Montoya, Gardy, Patrick, & Tang, 2013). With the rapid advancement of nextgeneration sequencing (NGS) technologies and the decreasing costs associated with them, metagenomic methods are expected to become increasingly prevalent in microbiological and public health laboratories for investigating infectious diseases. This is particularly likely due to recent technical improvements that enable the detection of pathogens at very low concentrations and allow testing directly from clinical samples or even from individual cells (Miller et al., 2013).

Alternatives such as genotypic methods for microbial identification are classified into two categories: (1) techniques based on features and (2) techniques based on sequences. Feature-based techniques use a structured procedure to obtain a series of fragments of an organism's chromosomal DNA. These fragments are separated by size to generate a unique profile of that organism and identify relationships with its close relatives. Utilizing this information, databases with profiles of known organisms can be created, allowing for comparison with other known organisms (Emerson et al., 2008).

On the other hand, techniques based on genomic sequences focus on determining the sequence of a specific fragment of DNA, sometimes associated with a particular gene (Emerson et al., 2008). This approach is similar to that used in genotyping, where a database of specific DNA sequences is used, and then a sequence of interest is compared (Emerson et al., 2008). The degree of similarity between the sequences indicates how closely related these organisms are. Additionally, computational algorithms enable the comparison of multiple sequences and construction of phylogenetic trees describing relationships between species across various taxonomic groups (Emerson et al., 2008; Godini & Fallahi, 2019).

Another alternative is the sequencing of the 16S rRNA gene, a wellestablished method used to identify bacteria regardless of their ability to be cultured or their phenotype (Muhamad Rizal et al., 2020; Winand et al., 2019). The 16S rRNA gene spans approximately 1500 base pairs and comprises nine regions (V1 to V9), interspersed with more conserved regions. This gene is present in all bacteria and exhibits varying evolutionary rates depending on the region of the gene considered (Church et al., 2020; Winand et al., 2019). For roughly the past 30 years, clinical microbiology laboratories have employed targeted partial cyclic sequencing of the 16S gene to identify and classification of human bacterial pathogens (Church et al., 2020).

Some experts argue that although sequencing of the 16S gene has proven to be a reliable genetic marker, unequivocal identification is not always achievable due to similarities in the 16S rRNA gene sequence among some bacterial species. Therefore, they advocate for sequencing analysis methods that cover the entire 16S-23S rRNA region to enhance species-level identification resolution (Sabat et al., 2017). Additionally, some specialists suggest that whole-genome sequencing (WGS) using next-generation sequencing (NGS) has the potential to replace 16S sequencing for routine diagnostic analysis (Church et al., 2020).

Next-generation sequencing is increasingly being used in the diagnosis of infectious diseases. There are studies where molecular panels are utilized to identify bacteria, viruses, and parasites through Illumina and Oxford Nanopore technologies. These platforms have the capability to sequence small fragments to identify pathogens in complex samples (Stefan, Hall, Graham, & Minogue, 2022). However, they are deemed complementary tests aimed at obtaining reliable results within a relatively short period of time.

Currently, whole-genome sequencing (WGS) has become accessible and cost-effective for genotyping bacteria. Analyzing the entire genome of these pathogens offers crucial information for the identification, characterization, and evolutionary lineages. Furthermore, this method enables the detection of genes resistant to specific antibiotics and facilitates tracing the origin of outbreaks (Franco-Duarte et al., 2019). Despite some reservations among healthcare professionals about this new technique, its potential in identifying pathogens and antimicrobial resistance in the clinical field is being evaluated. For instance, in one study, the transmission dynamics of a vancomycin-resistant *Enterococcus faecium* outbreak in an intensive care unit were detected using WGS. Additionally,

it revealed the presences of resistance genes in the analyzed bacterial isolates (Franco-Duarte et al., 2019; McGann et al., 2016).

Advances in whole-genome sequencing (WGS) technologies and analysis processes have rapidly increased production and analysis speed while reducing overall costs (Quainoo et al., 2017). However, one of the challenges facing clinical and research laboratories with these technologies is implementing the capacity to transform large amounts of genomic sequencing data into practical, understandable, and useful information for clinical interpretation. This involves not only identifying microorganisms and analyzing their genomic profile but also interpreting these data in terms of diseases and public health implications. Overcoming this challenge will require interdisciplinary collaboration and individuals with training and competence in bioinformatics to ensure that genomic sequencing becomes an effective tool in clinical practice (Church et al., 2020), thereby transforming decision-making for public health policies.

### 4. Genomic Data Analysis as a Tool for Pathogen Studies

Genomic information provides a discriminatory capacity that aids in distinguishing closely related organisms and enables surveillance of antimicrobial resistance. (Comas, 2017) (Deng et al., 2016).

Bioinformatics plays a vital role in the identification and characterization of pathogens, proving indispensable in the analysis of data from next-generation sequencing. Through bioinformatics tools, taxonomic characterization of microorganisms, determination of virulence factors, antimicrobial resistance analysis, and microbiome studies are achievable. Furthermore, deeper insights into transcriptomic, proteomic, and metabolomic analyses contribute to understanding pathogen behavior and enhancing future microbiological studies. Additionally, in the realm of public health, it enables effective genomic surveillance and containment of emerging outbreaks, epidemics, and pandemics. (Genetics and Biotechnology Department, Strategic Center for Diabetes Research, College of medicine, King Saud University, KSA & Saeb, 2018) (Hogeweg, 2011).

There are several reference databases that facilitate the identification of bacterial organisms, with Greengenes being one of the prominent ones. Particularly notheworthy is its recent update, Greengenes2, which contains 21,074,442 sequences comprising complete genomes and 16S rRNA records (McDonald et al., 2024) Additionally SILVA, harbors 11,387,745 SSU/LSU sequences of bacteria, archaea, and eukaryotes (https://www.arb-silva.de/) (Genetics and Biotechnology Department, Strategic Center for Diabetes Research, College of medicine, King Saud University, KSA & Saeb, 2018).

Whole Genome Sequencing (WGS) analysis has supplanted traditional molecular techniques for subtyping bacteria and fungi in outbreak surveillance. This approach, now routine in various countries such as the UK, France, Denmark, Canada, and the United States, has surpassed previously common yet limited methods like Pulsed-Field Gel Electrophoresis (PFGE) and Multi-Locus Sequence Typing (MLST). Advanced techniques for determining genetic similarity between bacteria, such as Single Nucleotide Polymorphism (SNP)-based Typing or whole genome Multi-Locus Sequence Typing (wgMLST), offer greater resolution in this regard (Álvarez-Molina et al., 2021) (Jagadeesan et al., 2019).

Genome reconstruction is achieved through de novo assembly, which involves assembling sequences without using a reference genome. The most common tool for this task for this task is SPAdes, a toolkit designated to facilitate assembly and analysis of short-read sequencing data. Specifically tailored for small genomes like those of bacteria, viruses, and fungi, SPAdes is not recommended for use with large genomes (Prjibelski et al., 2020).

ΤοοΙ	Utility				Reference
SPAdes	General genome assembler.			(Prjibelski et al., 2020)	
metaSPAdes	For metagenomes.			(Nurk et al., 2017)	
plasmidSPAdes	Extraction plasmids.	and	assembly	of	(Antipov et al., 2016)
metaplasmidSPAdes	Extraction plasmids in		assembly genomes.	of	(Antipov et al., 2019)

Table 1. Utility of multiple SPAdes tools.

metaviralSPAdes	Assembly of metaviromes.	(Antipov et al., 2020)	
rnaSPAdes	Assembly of transcriptomes	(Bushmanova et al.,	
	from RNA-seq data.	2019)	
biosintheticSPAdes	Assembly of biosynthetic genes.	(Meleshko et al., 2019)	
coronaSPAdes	Assembly of SARS-CoV-2.	(Meleshko et al., 2021)	

Once the genome is assembled, gene annotation can be performed using tools such as Prokka (Seemann, 2014), PGAAP (Tatusova et al., 2016) and PATRIC (Davis et al., 2019) to understand the biological function of genes and their contribution to cellular processes and phenotypic traits. Analysis of bacterial drug resistance can be conducted by using tools like ResFinder (Florensa et al., 2022) and its most recent update ResFinderFG v2.0 (Gschwind et al., 2023), CARD (Comprehensive Antibiotic Resistance Database) (Alcock et al., 2023) or RGI (Resistance Gene Identifier) which are among the most commonly used. Prediction of pathogenicity and virulence can be achieved using tools like PaPrBag (Deneke et al., 2017) (Genetics and Biotechnology Department, Strategic Center for Diabetes Research, College of medicine, King Saud University, KSA & Saeb, 2018).

Table 2. Tools for annotating bacterial genomes and analyzing antimicrobial resistances.

ΤοοΙ	Analysis	Link	Reference
Prokka	Genome annotation	https://github.com/vdejager/prokka	(Seemann, 2014)
PGAAP	Genome annotation	https://www.ncbi.nlm.nih.gov/genome/annotation_prok/	(Tatusova et al., 2016)
PATRIC	Genome annotation	https://www.patricbrc.org	(Davis et al., 2019)

ResFinderFG	Antimicrobial	http://genepi.food.dtu.dk/resfinder	(Gschwind	et al.,
v2.0	resistance		2023)	
CARD	Antimicrobial	https://card.mcmaster.ca/	(Alcock	et al.,
	resistance		2023)	
RGI	Antimicrobial	https://card.mcmaster.ca/analyze/rgi	(Alcock	et al.,
	resistance		2023)	
PaPrBaG	Pathogenicity	https://github.com/crarlus/paprbag	(Deneke	et al.,
	and virulence		2017)	

In addition to these bioinformatics resources, there are web applications that perform the entire analysis in an automated and user-friendly manner, especially for those with limited bioinformatics experience. Among these is MG-RAST (Meyer et al., 2008) which consists of over 150,000 datasets and enables users to analyze sequences from FASTQ files (Keegan et al., 2016). Similarly, the Viral and Bacterial Bioinformatics Resource Center is also available (BV-BRC) (Olson et al., 2023), which is a compilation of databases from PATRIC, the Influenza Research Database (IRD) (Zhang et al., 2017) and the Virus Pathogen Database and Analysis Resource (ViPR) (Pickett et al., 2012). BV-BCR is highly useful for analyses of both bacteria and viruses, enabling antimicrobial resistance analysis and the determination of pathogenicity and virulence factors in both types of microorganisms (Olson et al., 2023).

Similarly, these analyses are applicable to clinically relevant and diagnostically challenging fungi. For this purpose, databases like the Pathogen-Host Interactions database (PHI-base) are available, which includes a database of approximately 290 species of clinically relevant fungi (Cuzick et al., 2023). FungiDB is also available (https://fungidb.org/fungidb/app) FungiDB is a computer database and web application exclusively dedicated to fungi and oomycetes. It comprises 674 datasets and encompasses 300 genomes across various taxa such as Ascomycota, Basidiomycota, Chytridiomycota, Albuginales, Peronosporales, among others. This database contains a wide array of tools

facilitating user-friendly characterization and resistance analyses with robust data (Basenko et al., 2024).

These tools enable precise identification of microorganisms, delving into their antimicrobial resistance and virulence. Furthermore, collaboration across various disciplines has forged a robust framework for early detection of infectious diseases, containment of outbreaks, epidemics, and pandemics, and the implementation of effective public health strategies. This comprehensive approach propels research towards new frontiers, fostering a deeper understanding of the microbiological and epidemiological challenges we face.

## 5. Global Genomic Surveillance of Antimicrobial Resistance-Associated Infectious Diseases

The importance of genomics in antimicrobial resistance surveillance has become relevant since the COVID-19 pandemic. The emergence of this disease marked an unprecedented milestone worldwide. Surveillance of SARS-CoV-2 variants has allowed governments, especially middle- and low-income countries that previously lacked genomic laboratories and bioinformatics human resources, to develop this infrastructure and technology. The pandemic has also demonstrated the critical need for genomic laboratories in health systems to improve responses. Many countries are now incorporating genomics into their national strategic plans for antimicrobial resistance surveillance and securing funding through donors or national resources (World Health Organization.,2020).

The implementation of genomic surveillance to address antimicrobial resistance provides detailed information that enables in-depth characterization of disease outbreaks and facilitates the implementation of control and prevention measures (Jauneikaite et al., 2023). According to the World Health Organization document entitled "GLASS Whole-genome sequencing for surveillance of antimicrobial resistance," potential uses of genomics in public health include (World Health Organization.,2020):

• Identification of high-risk clones or antimicrobial resistance at regional and global levels.

- Identification of antimicrobial resistance mechanisms and their transmission to human populations, animals, and environmental sources ("One Health" approach).
- Identification and monitoring of the outbreaks.
- Identification of new antimicrobial and vaccine targets.
- Development of point-of-care tests for antimicrobial resistance.

Genomic surveillance provides new insights into disease transmission and virulence, and the dynamics of antimicrobial resistance when combined with epidemiological, clinical, and phenotypic microbiological information. This knowledge is useful for risk assessment and the design of effective interventions, enabling the rapid and accurate identification and characterization of pathogens. It also facilitates the early detection of outbreaks, tracing of transmission chains, and identification of sources of infection, which can generate significant savings for public health (World Health Organization.,2020).

In disease outbreak investigations, there are three levels of linkage between cases that increase resolution. Identifying exact transmission pairs and chains of transmission is essential for investigating outbreaks related to antimicrobial resistance. Genomics offers the highest resolution, allowing epidemiologists to guide reactive and proactive measures by tracing transmission routes and discovering outbreak origins (Harris et al., 2013). The use of genomics to infer transmission may be limited by the diversity of the circulating pathogen populations. Two genetically similar bacteria may indicate genuine transmission between hosts, resulting from different introductions of a third host, or arise from independent lineages with similar mutational events, although the latter is unlikely when significant there are а number of unique differences ( Senghore et al,2023).

Genomics provides a level of detail that is superior to previous methods and streamlined data processing. This allows pathogen surveillance from lineage levels to granular identification of identical isolates. In addition, it facilitates the detection of genetic determinants of antimicrobial resistance, virulence, and other relevant phenotypic markers previously explored using molecular techniques such as PCR. The electronic nature of genomic data allows for the sharing and analysis of results between laboratories and bioinformatics centers, favoring comparability and external quality assessment (Jauneikaite et al., 2023).

The use of whole-genome sequencing (WGS) improves standardization and reproducibility, providing greater comparability between laboratories than phenotypic testing. Digital sequence data allow retrospective analyses and ensure backward compatibility between new and old analyses (World Health Organization.,2020).

Strengthening research and development of new diagnostics and treatments is crucial because of the paucity of products against available treatment-resistant strains. Although genomics presents some limitations and practical challenges to its widespread application, it is vital that countries strengthen their surveillance systems and develop the capacity to respond to results, with effective response teams to stop the emergence and spread of antimicrobial resistance (Bankevich et al.,2012)

The document produced by the World Health Organization entitled "Global genomic surveillance strategy for pathogens with pandemic and epidemic potential 2022-2032" indicates that the vision for the future of genomic surveillance is to ensure scalability, sustainability, and integration into healthcare systems and surveillance networks. Although the infrastructure requires significant investment, genomics has the potential to transform healthcare and response through specialized surveillance, accurate diagnostics, vaccine design, and personalization of treatments (World Health Organization., 2023)

# 6. Future Perspectives of Genomics in Microorganism Characterization

The genomics field has revolutionized our understanding of microorganisms, providing a deeper characterization at the genetic level. This has opened new possibilities for studying microbial diversity, evolution, and function, as well as their potential applications in fields such as medicine, agriculture, environmental sciences, and biotechnology (Rogers, Y. H., & Zhang, C., 2016). With the progress of genomic technologies, a complete and detailed overview of microbial genomes is expected, including the analysis of metagenomes, identification of

new microbial species, and emergence of resistance mechanisms (De Abreu, V. A., Perdigão, J., & Almeida, S., 2021) (Danko et al.,2021).

In addition, genomics will play a crucial role in identifying and understanding the molecular mechanisms of emerging diseases and their epidemiology, resulting in improved diagnostics, vaccines, and therapeutic strategies (McCarthy, J.., McLeod, H. L., & Ginsburg, G.., 2013). This will contribute to the development of personalized medicine and effective treatments for infectious diseases. In addition, the integration of genomics with other omics technologies, such as proteomics and metabolomics, will provide a more complete understanding of the interactions and functions of microorganisms in complex ecosystems (Singh et al., 2020). Overall, the prospects for genomics in microorganism characterization are broad and promising. There is great potential to advance the knowledge and applications of microbiology, which will improve human health, environmental sustainability, and biotechnological innovations. In the future, different scientific and applied fields will continue to be transformed in this discipline. The following are some of the key perspectives.:

Human health and diagnosis disease

Genomics will facilitate more accurate and rapid diagnosis of infections caused by microorganisms (Sun, C., & Medvedev, P. 2019) Next-generation sequencing (NGS) and bioinformatics will help rapidly detect pathogens in clinical samples, including the presence of previously unrecorded antibiotic-resistant strains, and will improve clinical outcomes with the development of more specific and effective treatments (Köser, C.et al, 2012). Whole-genome sequencing of microorganisms in real time will support the detection and monitoring of infectious disease outbreaks, biosafety analysis, pathogen evolution, and discovery of unusual resistance mechanisms (Gardy, J. & Loman, N. J. 2018)

### • Microbiota

The use of genomic tools to understand how the microbiota affects health and its mechanism of action in illnesses will further contribute to the development of personalized therapies, specific probiotics, and diets to promote healthy microbial balance Gupta, A., Saha, S., & Khanna, S. 2020). Therapies to modulate gut microbiota: Past, present and future. World journal of gastroenterology, 26(8), 77

Environment

Through microorganism genomic characterization, we will characterize genes or genetic regions for biotechnological applications, such as biofuel production, pollutant elimination, and the manufacture of new microbes with beneficial potential Keasling, J., 2021)

• Food safety

Genomics plays an important role in food security. By studying genes related to desirable traits such as disease resistance or increased yield, genetic profiling could create stronger and more nutritious plant varieties (Hoisington, D.,1991). This not only enhances productivity, but also reduces the need for pesticides and chemical fertilizers, thereby encouraging more environmentally friendly agricultural practices. In the near future, genomics will continue to be an invaluable tool to ensure food security for future generations (Hoisington, D.,1991).

In conclusion, countries should make great efforts to implement sustainable genomic surveillance systems, where the use of sequencing technologies could be transversal and applied to different fields of study such as medicine, biotechnology, agriculture and ecology. Only in this way will we have new tools and approaches to address global challenges related to health and the struggle against antimicrobial resistance.

### **Bibliographic References**

- Ainsa, J. A. (2002a). Stuart B. Levy: The antibiotic paradox. How the misuse of antibiotics destroys their curative powers (Vol. 353). Perseus Publishing.
- Alcock, B. P., Huynh, W., Chalil, R., Smith, K. W., Raphenya, A. R., Wlodarski, M. A., Edalatmand, A., Petkau, A., Syed, S. A., Tsang, K. K., Baker, S. J. C., Dave, M., McCarthy, M. C., Mukiri, K. M., Nasir, J. A., Golbon, B., Imtiaz, H., Jiang, X., Kaur, K., ... McArthur, A. G. (2023). CARD 2023: Expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Research, 51(D1), D690-D699. https://doi.org/10.1093/nar/gkac920
- Álvarez-Molina, A., De Toro, M., Alexa, E. A., & Álvarez-Ordóñez, A. (2021). Applying Genomics to Track Antimicrobial Resistance in the Food Chain. En Comprehensive Foodomics (pp. 188-211). Elsevier. https://doi.org/10.1016/B978-0-08-100596-5.22700-5
- Antipov, D., Hartwick, N., Shen, M., Raiko, M., Lapidus, A., & Pevzner, P. A. (2016). plasmidSPAdes: Assembling plasmids from whole genome sequencing data. Bioinformatics, 32(22), 3380-3387. https://doi.org/10.1093/bioinformatics/btw493
- Antipov, D., Raiko, M., Lapidus, A., & Pevzner, P. A. (2019). Plasmid detection and assembly in genomic and metagenomic data sets. Genome Research, 29(6), 961-968. https://doi.org/10.1101/gr.241299.118
- Antipov, D., Raiko, M., Lapidus, A., & Pevzner, P. A. (2020). METAVIRAL SPADES: Assembly of viruses from metagenomic data. Bioinformatics, 36(14), 4126-4129. https://doi.org/10.1093/bioinformatics/btaa490
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A. A., Dvorkin, M., Kulikov, A. S., Lesin, V. M., Nikolenko, S. I., Pham, S., Prjibelski, A. D., Pyshkin, A. V., Sirotkin, A. V., Vyahhi, N., Tesler, G., Alekseyev, M. A., & Pevzner, P. A. (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology: A Journal of Computational Molecular Cell Biology, 19(5), 455–477. https://doi.org/10.1089/cmb.2012.0021

- Basenko, E. Y., Shanmugasundram, A., Böhme, U., Starns, D., Wilkinson, P. A., Davison, H. R., Crouch, K., Maslen, G., Harb, O. S., Amos, B., McDowell, M. A., Kissinger, J. C., Roos, D. S., & Jones, A. (2024). What is new in FungiDB: A web-based bioinformatics platform for omics-scale data analysis for fungal and oomycete species. GENETICS, 227(1), iyae035. https://doi.org/10.1093/genetics/iyae035
- Bushmanova, E., Antipov, D., Lapidus, A., & Prjibelski, A. D. (2019). rnaSPAdes: A de novo transcriptome assembler and its application to RNA-Seq data. GigaScience, 8(9), giz100. https://doi.org/10.1093/gigascience/giz100
- Church, D. L., Cerutti, L., Gürtler, A., Griener, T., Zelazny, A., & Emler, S. (2020). Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. Clinical Microbiology Reviews, 33(4). <u>https://doi.org/10.1128/cmr.00053-19</u>
- Comas, I. (2017). Genomic Epidemiology of Tuberculosis. En S. Gagneux (Ed.), Strain Variation in the Mycobacterium tuberculosis Complex: Its Role in Biology, Epidemiology and Control (Vol. 1019, pp. 79-93). Springer International Publishing. https://doi.org/10.1007/978-3-319-64371-7\_4
- Cuzick, A., Seager, J., Wood, V., Urban, M., Rutherford, K., & Hammond-Kosack, K. E. (2023). A framework for community curation of interspecies interactions literature. eLife, 12, e84658. https://doi.org/10.7554/eLife.84658
- Danko, D., Bezdan, D., Afshin, E. E., Ahsanuddin, S., Bhattacharya, C., Butler, D. J., Chng, K. R., Donnellan, D., Hecht, J., Jackson, K., Kuchin, K., Karasikov, M., Lyons, A., Mak, L., Meleshko, D., Mustafa, H., Mutai, B., Neches, R. Y., Ng, A., ... Zubenko, S. (2021). A global metagenomic map of urban microbiomes and antimicrobial resistance. *Cell*, *184*(13), 3376-3393.e17. https://doi.org/10.1016/j.cell.2021.05.002
- Davis, J. J., Wattam, A. R., Aziz, R. K., Brettin, T., Butler, R., Butler, R. M., Chlenski, P., Conrad, N., Dickerman, A., Dietrich, E. M., Gabbard, J. L., Gerdes, S., Guard, A., Kenyon, R. W., Machi, D., Mao, C., Murphy-Olson, D., Nguyen, M., Nordberg, E. K., ... Stevens, R. (2019). The PATRIC Bioinformatics Resource Center: Expanding data and analysis

capabilities. Nucleic Acids Research, gkz943. https://doi.org/10.1093/nar/gkz943

- 15. de Abreu, V. A. C., Perdigão, J., & Almeida, S. (2021). Metagenomic approaches to analyze antimicrobial resistance: An overview. Frontiers in Genetics, 11. https://doi.org/10.3389/fgene.2020.575592
- 16. Deiman, B., van Aarle, P., & Sillekens, P. (2002). Characteristics and applications of nucleic acid sequence-based amplification (NASBA).
  Molecular Biotechnology, 20(2), 163–180. https://doi.org/10.1385/mb:20:2:163
- 17. Deneke, C., Rentzsch, R., & Renard, B. Y. (2017). PaPrBaG: A machine learning approach for the detection of novel pathogens from NGS data. Scientific Reports, 7(1), 39194. https://doi.org/10.1038/srep39194
- 18. Deng, X., Den Bakker, H. C., & Hendriksen, R. S. (2016). Genomic Epidemiology: Whole-Genome-Sequencing–Powered Surveillance and Outbreak Investigation of Foodborne Bacterial Pathogens. Annual Review of Food Science and Technology, 7(1), 353-374. https://doi.org/10.1146/annurev-food-041715-033259
- 19. Donelli, G., Vuotto, C., & Mastromarino, P. (2013). Phenotyping and genotyping are both essential to identify and classify a probiotic microorganism. Microbial Ecology in Health and Disease, 24(0). <u>https://doi.org/10.3402/mehd.v24i0.20105</u>
- 20. Ellwanger, J. H., Veiga, A. B. G. da, Kaminski, V. de L., Valverde-Villegas, J. M., Freitas, A. W. Q. de, & Chies, J. A. B. (2021). Control and prevention of infectious diseases from a One Health perspective. Genetics and Molecular Biology, 44(1 suppl 1). <u>https://doi.org/10.1590/1678-4685-gmb-2020-0256</u>
- 21. Emerson, D., Agulto, L., Liu, H., & Liu, L. (2008). Identifying and characterizing bacteria in an era of genomics and proteomics. *Bioscience*, 58(10), 925–936. <u>https://doi.org/10.1641/b581006</u>
- 22. Fairfax, M. R., Bluth, M. H., & Salimnia, H. (2018). Diagnostic molecular microbiology. Clinics in Laboratory Medicine, 38(2), 253–276. <u>https://doi.org/10.1016/j.cll.2018.02.004</u>

- 23. Florensa, A. F., Kaas, R. S., Clausen, P. T. L. C., Aytan-Aktug, D., & Aarestrup, F. M. (2022). ResFinder an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. Microbial Genomics, 8(1). https://doi.org/10.1099/mgen.0.000748
- 24. Franco-Duarte, R., Černáková, L., Kadam, S., S. Kaushik, K., Salehi, B., Bevilacqua, A., Corbo, M. R., Antolak, H., Dybka-Stępień, K., Leszczewicz, M., Relison Tintino, S., Alexandrino de Souza, V. C., Sharifi-Rad, J., Melo Coutinho, H. D., Martins, N., & Rodrigues, C. F. (2019). Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms, 7(5), 130. <a href="https://doi.org/10.3390/microorganisms7050130">https://doi.org/10.3390/microorganisms7050130</a>
- 25. Gardy, J. L., & Loman, N. J. (2018). Towards a genomics-informed, realtime, global pathogen surveillance system. Nature Reviews. Genetics, 19(1), 9–20. https://doi.org/10.1038/nrg.2017.88
- 26. Genetics and Biotechnology Department, Strategic Center for Diabetes Research, College of medicine, King Saud University, KSA, & Saeb, A. T. M. (2018). Current Bioinformatics resources in combating infectious diseases. Bioinformation, 14(01), 031-035. https://doi.org/10.6026/97320630014031
- 27. Gilbert, G. L. (2002). Molecular diagnostics in infectious diseases and public health microbiology: cottage industry to postgenomics. Trends in Molecular Medicine, 8(6), 280–287. <u>https://doi.org/10.1016/s1471-4914(02)02349-3</u>
- 28. Godini, R., & Fallahi, H. (2019). A brief overview of the concepts, methods and computational tools used in phylogenetic tree construction and gene prediction. Meta Gene, 21(100586), 100586. <u>https://doi.org/10.1016/j.mgene.2019.100586</u>
- Grisold, A. J., Leitner, E., Mühlbauer, G., Marth, E., & Kessler, H. H. (2002). Detection of methicillin-resistant Staphylococcus aureus and simultaneous confirmation by automated nucleic acid extraction and realtime PCR. Journal of Clinical Microbiology, 40(7), 2392–2397. <u>https://doi.org/10.1128/jcm.40.7.2392-2397.2002</u>

- 30. Gschwind, R., Ugarcina Perovic, S., Weiss, M., Petitjean, M., Lao, J., Coelho, L. P., & Ruppé, E. (2023). ResFinderFG v2.0: A database of antibiotic resistance genes obtained by functional metagenomics. Nucleic Acids Research, 51(W1), W493-W500. https://doi.org/10.1093/nar/gkad384
- 31. Gupta, A., Saha, S., & Khanna, S. (2020). Therapies to modulate gut microbiota: Past, present and future. World Journal of Gastroenterology: WJG, 26(8), 777–788. https://doi.org/10.3748/wjg.v26.i8.777
- 32. Harris, S. R., Cartwright, E. J. P., Török, M. E., Holden, M. T. G., Brown, N. M., Ogilvy-Stuart, A. L., Ellington, M. J., Quail, M. A., Bentley, S. D., Parkhill, J., & Peacock, S. J. (2013). Whole-genome sequencing for analysis of an outbreak of meticillin-resistant Staphylococcus aureus: a descriptive study. The Lancet Infectious Diseases, 13(2), 130–136. https://doi.org/10.1016/S1473-3099(12)70268-2
- 33. Hogeweg, P. (2011). The Roots of Bioinformatics in Theoretical Biology.
   PLoS Computational Biology, 7(3), e1002021.
   https://doi.org/10.1371/journal.pcbi.1002021
- 34. Hoisington, D., Khairallah, M., Reeves, T., Ribaut, J.-M., Skovmand, B., Taba, S., & Warburton, M. (1999). Plant genetic resources: What can they contribute toward increased crop productivity? Proceedings of the National Academy of Sciences of the United States of America, 96(11), 5937–5943. https://doi.org/10.1073/pnas.96.11.5937
- 35. Isenberg, H. D. (2003). Clinical microbiology: Past, present, and future.
   Journal of Clinical Microbiology, 41(3), 917–918.
   <a href="https://doi.org/10.1128/jcm.41.3.917-918.2003">https://doi.org/10.1128/jcm.41.3.917-918.2003</a>
- 36. Jagadeesan, B., Gerner-Smidt, P., Allard, M. W., Leuillet, S., Winkler, A., Xiao, Y., Chaffron, S., Van Der Vossen, J., Tang, S., Katase, M., McClure, P., Kimura, B., Ching Chai, L., Chapman, J., & Grant, K. (2019). The use of next generation sequencing for improving food safety: Translation into practice. Food Microbiology, 79, 96-115. https://doi.org/10.1016/j.fm.2018.11.005
- Jauneikaite, E., Baker, K. S., Nunn, J. G., Midega, J. T., Hsu, L. Y., Singh,
   S. R., Halpin, A. L., Hopkins, K. L., Price, J. R., Srikantiah, P., Egyir, B.,
   Okeke, I. N., Holt, K. E., Peacock, S. J., & Feasey, N. A. (2023). Genomics

for antimicrobial resistance surveillance to support infection prevention and control in health-care facilities. The Lancet. Microbe, 4(12), e1040– e1046. https://doi.org/10.1016/s2666-5247(23)00282-3

- 38. Keasling, J., Garcia Martin, H., Lee, T. S., Mukhopadhyay, A., Singer, S. W., & Sundstrom, E. (2021). Microbial production of advanced biofuels. Nature Reviews. Microbiology, 19(11), 701–715. https://doi.org/10.1038/s41579-021-00577-w
- 39. Keegan, K. P., Glass, E. M., & Meyer, F. (2016). MG-RAST, a Metagenomics Service for Analysis of Microbial Community Structure and Function. En F. Martin & S. Uroz (Eds.), Microbial Environmental Genomics (MEG) (Vol. 1399, pp. 207-233). Springer New York. https://doi.org/10.1007/978-1-4939-3369-3\_13
- Köser, C. U., Ellington, M. J., Cartwright, E. J. P., Gillespie, S. H., Brown, N. M., Farrington, M., Holden, M. T. G., Dougan, G., Bentley, S. D., Parkhill, J., & Peacock, S. J. (2012). Routine use of microbial whole genome sequencing in diagnostic and public health microbiology. PLoS Pathogens, 8(8), e1002824. https://doi.org/10.1371/journal.ppat.1002824
- 41. Marušić, A. (2011). Food safety and security: ¿what were favourite topics for research in the last decade? Journal of global health, 1(1). <u>https://pubmed.ncbi.nlm.nih.gov/23198104/</u>
- 42. McCarthy, J. J., McLeod, H. L., & Ginsburg, G. S. (2013). Genomic medicine: A decade of successes, challenges, and opportunities. *Science Translational Medicine*, *5*(189).

https://doi.org/10.1126/scitransImed.3005785

- McDonald, D., Jiang, Y., Balaban, M., Cantrell, K., Zhu, Q., Gonzalez, A., Morton, J. T., Nicolaou, G., Parks, D. H., Karst, S. M., Albertsen, M., Hugenholtz, P., DeSantis, T., Song, S. J., Bartko, A., Havulinna, A. S., Jousilahti, P., Cheng, S., Inouye, M., ... Knight, R. (2024). Greengenes2 unifies microbial data in a single reference tree. Nature Biotechnology, 42(5), 715-718. https://doi.org/10.1038/s41587-023-01845-1
- 44. McGann, P., Bunin, J. L., Snesrud, E., Singh, S., Maybank, R., Ong, A. C., Kwak, Y. I., Seronello, S., Clifford, R. J., Hinkle, M., Yamada, S., Barnhill, J., & Lesho, E. (2016). Real time application of whole genome sequencing for outbreak investigation – What is an achievable turnaround

time? *Diagnostic Microbiology and Infectious Disease*, *85*(3), 277–282. https://doi.org/10.1016/j.diagmicrobio.2016.04.020

- 45. Meleshko, D., Hajirasouliha, I., & Korobeynikov, A. (2021). coronaSPAdes:
  From biosynthetic gene clusters to RNA viral assemblies. Bioinformatics, 38(1), 1-8. https://doi.org/10.1093/bioinformatics/btab597
- 46. Meleshko, D., Mohimani, H., Tracanna, V., Hajirasouliha, I., Medema, M. H., Korobeynikov, A., & Pevzner, P. A. (2019). BiosyntheticSPAdes: Reconstructing biosynthetic gene clusters from assembly graphs. Genome Research, 29(8), 1352-1362. https://doi.org/10.1101/gr.243477.118
- 47. Mendez-Vilas, A. (2012). Microbes in Applied Research: Current Advantages and Challenges. World Scientific.
- 48. Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R., Wilke, A., Wilkening, J., & Edwards, R. (2008). The metagenomics RAST server – a public resource for the automatic phylogenetic and functional analysis of metagenomes. BMC Bioinformatics, 9(1), 386. https://doi.org/10.1186/1471-2105-9-386
- Miller, R. R., Montoya, V., Gardy, J. L., Patrick, D. M., & Tang, P. (2013). Metagenomics for pathogen detection in public health. Genome Medicine, 5(9), 81. <u>https://doi.org/10.1186/gm485</u>
- 50.Nurk, S., Meleshko, D., Korobeynikov, A., & Pevzner, P. A. (2017). metaSPAdes: A new versatile metagenomic assembler. Genome Research, 27(5), 824-834. https://doi.org/10.1101/gr.213959.116
- 51. Olson, R. D., Assaf, R., Brettin, T., Conrad, N., Cucinell, C., Davis, J. J., Dempsey, D. M., Dickerman, A., Dietrich, E. M., Kenyon, R. W., Kuscuoglu, M., Lefkowitz, E. J., Lu, J., Machi, D., Macken, C., Mao, C., Niewiadomska, A., Nguyen, M., Olsen, G. J., ... Stevens, R. L. (2023). Introducing the Bacterial and Viral Bioinformatics Resource Center (BV-BRC): A resource combining PATRIC, IRD and ViPR. Nucleic Acids Research, 51(D1), D678-D689. https://doi.org/10.1093/nar/gkac1003
- 52. Pickett, B. E., Sadat, E. L., Zhang, Y., Noronha, J. M., Squires, R. B., Hunt, V., Liu, M., Kumar, S., Zaremba, S., Gu, Z., Zhou, L., Larson, C. N., Dietrich, J., Klem, E. B., & Scheuermann, R. H. (2012). ViPR: An open bioinformatics database and analysis resource for virology research.

Nucleic Acids Research, 40(D1), D593-D598. https://doi.org/10.1093/nar/gkr859

- 53. Prakash, O., Verma, M., Sharma, P., Kumar, M., Kumari, K., Singh, A., Kumari, H., Jit, S., Gupta, S. K., Khanna, M., & Lal, R. (2007). Polyphasic approach of bacterial classification — An overview of recent advances. Indian Journal of Microbiology, 47(2), 98–108. <u>https://doi.org/10.1007/s12088-007-0022-x</u>
- 54. Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A., & Korobeynikov, A. (2020). Using SPAdes De Novo Assembler. Current Protocols in Bioinformatics, 70(1), e102. https://doi.org/10.1002/cpbi.102
- 55. Quainoo, S., Coolen, J. P. M., van Hijum, S. A. F. T., Huynen, M. A., Melchers, W. J. G., van Schaik, W., & Wertheim, H. F. L. (2017). Wholegenome sequencing of bacterial pathogens: The future of nosocomial outbreak analysis. *Clinical Microbiology Reviews*, *30*(4), 1015–1063. <u>https://doi.org/10.1128/cmr.00016-17</u>
- Rogers, Y. H., & Zhang, C. (2016). Genomic technologies in medicine and health: past, present, and future. In Medical and health genomics (pp. 15-28). Academic Press.
- 57. Sabat, A. J., van Zanten, E., Akkerboom, V., Wisselink, G., van Slochteren, K., de Boer, R. F., Hendrix, R., Friedrich, A. W., Rossen, J. W. A., & Kooistra-Smid, A. M. D. (2017). Targeted next-generation sequencing of the 16S-23S rRNA region for culture-independent bacterial identification increased discrimination of closely related species. *Scientific Reports*, 7(1), 1–12. https://doi.org/10.1038/s41598-017-03458-6
- 58. Seemann, T. (2014). Prokka: Rapid prokaryotic genome annotation.
  Bioinformatics, 30(14), 2068-2069.
  https://doi.org/10.1093/bioinformatics/btu153
- Senghore, M., Read, H., Oza, P., Johnson, S., Passarelli-Araujo, H., Taylor, B. P., Ashley, S., Grey, A., Callendrello, A., Lee, R., Goddard, M. R., Lumley, T., Hanage, W. P., & Wiles, S. (2023). Inferring bacterial transmission dynamics using deep sequencing genomic surveillance data. Nature Communications, 14(1), 1–12. https://doi.org/10.1038/s41467-023-42211-8

- 60. Sibley, C. D., Peirano, G., & Church, D. L. (2012). Molecular methods for pathogen and microbial community detection and characterization: Current and potential application in diagnostic microbiology. Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases, 12(3), 505–521. <u>https://doi.org/10.1016/j.meegid.2012.01.011</u>
- 61. Singh, D., Geat, N., Mehriya, M., Rajawat, M. V. S., Prasanna, R., Kumar, A., ... & Jha, M. N. (2020). Omics (genomics, proteomics, metabolomics, etc.) tools to study the environmental microbiome and bioremediation. Waste to Energy: Prospects and Applications, 235-260.
- 62. Stefan, C. P., Hall, A. T., Graham, A. S., & Minogue, T. D. (2022). Comparison of illumina and oxford nanopore sequencing technologies for pathogen detection from clinical matrices using molecular inversion probes. The Journal of Molecular Diagnostics: JMD, 24(4), 395–405. <u>https://doi.org/10.1016/j.jmoldx.2021.12.005</u>
- 63. Sun, C., & Medvedev, P. (2019). Toward fast and accurate SNP genotyping from whole genome sequencing data for bedside diagnostics. Bioinformatics (Oxford, England), 35(3), 415–420. https://doi.org/10.1093/bioinformatics/bty641
- 64. Tang, P., Croxen, M. A., Hasan, M. R., Hsiao, W. W. L., & Hoang, L. M. (2017). Infection control in the new age of genomic epidemiology. *American Journal of Infection Control*, 45(2), 170–179. <u>https://doi.org/10.1016/j.ajic.2016.05.015</u>
- 65. Tang, Y.-W., & Stratton, C. W. (Eds.). (2012). Advanced Techniques in Diagnostic Microbiology (2a ed.). Springer.
- Tatusova, T., DiCuccio, M., Badretdin, A., Chetvernin, V., Nawrocki, E. P., Zaslavsky, L., Lomsadze, A., Pruitt, K. D., Borodovsky, M., & Ostell, J. (2016). NCBI prokaryotic genome annotation pipeline. Nucleic Acids Research, 44(14), 6614-6624. https://doi.org/10.1093/nar/gkw569
- Vanbelkum, A. (2003). Molecular diagnostics in medical microbiology: yesterday, today and tomorrow. Current Opinion in Pharmacology, 3(5), 497–501. <u>https://doi.org/10.1016/s1471-4892(03)00108-5</u>
- Váradi, L., Luo, J. L., Hibbs, D. E., Perry, J. D., Anderson, R. J., Orenga,
   S., & Groundwater, P. W. (2017). Methods for the detection and

identification of pathogenic bacteria: past, present, and future. Chemical Society Reviews, 46(16), 4818–4832. <u>https://doi.org/10.1039/c6cs00693k</u>

- 69. World Health Organization. (2020). GLASS whole-genome sequencing for surveillance of antimicrobial resistance. World Health Organization. License: CC BY-NC-SA 3.0 IGO.
- 70. World Health Organization. (2023). Global genomic surveillance strategy for pathogens with pandemic and epidemic potential 2022–2032: progress report on the first year of implementation
- 71. Winand, R., Bogaerts, B., Hoffman, S., Lefevre, L., Delvoye, M., Van Braekel, J., Fu, Q., Roosens, N. H. C., De Keersmaecker, S. C. J., & Vanneste, K. (2019). Targeting the 16S rRNA gene for bacterial identification in complex mixed samples: Comparative evaluation of second (Illumina) and third (Oxford Nanopore Technologies) generation sequencing technologies. International Journal of Molecular Sciences, 21(1), 298. https://doi.org/10.3390/ijms21010298
- 72. Zhang, Y., Aevermann, B. D., Anderson, T. K., Burke, D. F., Dauphin, G., Gu, Z., He, S., Kumar, S., Larsen, C. N., Lee, A. J., Li, X., Macken, C., Mahaffey, C., Pickett, B. E., Reardon, B., Smith, T., Stewart, L., Suloway, C., Sun, G., ... Scheuermann, R. H. (2017). Influenza Research Database: An integrated bioinformatics resource for influenza virus research. Nucleic Acids Research, 45(D1), D466-D474. https://doi.org/10.1093/nar/gkw857