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Bioinformatics approaches to the study of antimicrobial resistance

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ABSTRACT

Detection and monitoring of antimicrobial resistance are two pillars on which clinical microbiology will be based in the coming decades. The implementation of certain technologies such as whole genome sequencing (WGS) or mass spectrometry and the creation of national and international databases that include and gather data on antimicrobial resistance from around the world has allowed the application of bioinformatics in the study of antimicrobial resistance in microorganisms involved in human pathology.

Keywords: bioinformatics, antimicrobial resistance, whole genome sequencing.

INTRODUCTION

The development of antimicrobial resistance (AMR), intrinsic to the use of antibiotics, has growth over the last few decades, spreading fast due to their overuse and misuse in both humans and animals. AMR has become the main challenge facing global health. For some pathogens frequently involved in human infection, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* or the *Mycobacterium tuberculosis complex*, among others, high rates of resistance against antibiotics have been observed world-wide. Infections caused by these microorganisms are associated with high morbidity and mortality rates; in fact, a recent WHO report estimates that drug-resistant microorganisms will cause up to 10 million deaths each year by 2050 with its consequent social, political, and economic impact [1]. In this context, the WHO and several other institutions are developing action plans in order to improve the understanding of AMR, reduce the incidence of infectious diseases through implementing infection prevention measures, optimize the use of antimicrobial medicines and develop new drugs and treatment strategies. Despite huge efforts have been made within the framework of these plans to combat AMR, this is still an unavoidable problem that reduces the effectiveness of antibiotics for treating infections. A practical and feasible solution to this issue might be use of certain bioinformatic tools that permit healthcare systems worldwide to have a greater control on AMR. To this end, certain techniques such as MALDI-TOF mass spectrometry and whole genome sequencing, that are slowly becoming part of the daily routine of most microbiology laboratories, can help us not only to detect different mechanisms of antimicrobial resistance in bacteria and other pathogens but guiding us to a better understanding of those mechanisms from an epidemiological and molecular point of view. Moreover, with the aid of bioinformatics, we can analyze the vast amount of information generated, in order to implement certain strategies, tailored to a specific context, to improve prevention, control and treatment measures for infections caused by antimicrobial resistant microorganisms.

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BIOINFORMATIC TOOLS FOR WHOLE GENOME SEQUENCING ANALYSIS IN AMR

Phenotypic antimicrobial susceptibility testing (AST) is the classic method to detect AMR, but in the last few years Whole Genome Sequencing-based AST (WGS-AST) has emerged as a fast and accurate method for AMR detection. In some cases, there is a direct connection between genotype and phenotype, allowing the determination of certain AMR genes by applying molecular tests available in clinical practice, as for example occurs in the detection of the *mecA* gene, responsible for beta-lactam antibiotics resistance in *S. aureus*. However, most of the mechanisms of antimicrobial resistance involve multiple genes and cellular signaling pathways or simply are not well known yet, and therefore WGS presents as an alternative to understand these mechanisms by obtaining the whole genome of pathogens isolated in clinical

practice, and enabling comprehensive AMR detection. Nevertheless, there is one big problem concerning WGS: it provides large amounts of data, making difficult to predict the presence of unknown antibiotic resistance genes or gene variants related to antibiotic resistance. Fortunately, these data sets obtained from applying WGS to clinical isolates can be analyzed through free bioinformatic tools such as Resfinder (the first pipeline addressed to non-trained users), AMRfinder or the Comprehensive Antibiotic Resistance Database (CARD) among others, that are actively curated and can be employed to identify genetic elements involved in AMR (single-nucleotide polymorphism mutations, horizontal gene transfer, inversions, etc) without having in-depth bioinformatics skills. To identify and predict the presence of AMR genes or other AMR mechanisms in a certain clinical isolate, these tools rely on large, high-quality AMR gene databases. The differences between these tools are based on the algorithm these databases use and their data composition. Despite the fact that some studies have shown the utility of these bioinformatic resources for AMR detection, a recent inter-laboratory study by Doyle et al [2] found that there is high variability in the results between laboratories depending on what software and analytical pipeline are used for detecting and predicting the presence or absence of AMR genes when studying the same clinical isolates, resulting in poor concordance with phenotypic AST results. These discordant results may be due not only to the bioinformatic tool used but also to the quality of the sequences obtained by WGS (samples have to be sequenced to a sufficient depth) and to the interpretation of the data obtained after the bioinformatic analysis. We must also note that some authors, including a European Committee on Antimicrobial Susceptibility Testing (EUCAST) subcommittee comprising experts on WGS [3], have concluded that even though there are large amounts of data from studies focused on phenotypic-genotypic AST concordance, WGS is still a poor tool to accurately predict antimicrobial susceptibility. Furthermore, the EUCAST subcommittee recommends using epidemiological cut-off values (ECOFF) as comparators for WGS-based prediction of AST instead of clinical breakpoints. As of today, WGS-AST techniques are not routinely performed in most clinical microbiology laboratories, because of their high cost, the need of skilled personnel, the poor quality of the data obtained and the difficulty in interpreting those data. In the same way, and as previously discussed, it is understandable the urgent need for standardized, comprehensive resistance sequence databases, accessible online for free, as well as standardized recommendations on sequence data quality. Even so, in the next few years the development of new bioinformatic pipelines and their application for studying AMR will facilitate the translation of large amounts of data into apprehensible and indictable insights, allowing the detection of AMR genes in WGS data, which was unimaginable a few years ago. In our opinion, WGS will be the method of choice for the detection of AMR in clinical practice, as the cost of WGS continues to decrease and experience is gained in data analysis and interpretation.

BIOINFORMATIC TOOLS FOR MONITORING AMR

Monitoring of antimicrobial resistance is as important as its detection. In this era of globalization, AMR determinants can easily spread around the world, transcending geographical barriers and increasing the global prevalence of AMR. In the past few years, for example, there has been a rapid increase in the detection of carbapenemase-resistant Enterobacterales (CPE) [4], both in hospital and community settings. Likewise, the use of antibiotics in animals has grown substantially in the last decades, leading to the propagation of antibiotics in the environment and increasing the burden of AMR in zoonotic agents, which means that AMR surveillance using only clinical samples is not useful as an early warning system for detecting resistance mechanisms. Thus, the development of tools for tracking global AMR has gained importance, since these tools may provide relevant information to guide healthcare professionals in the development of stewardship programs and public health actions. Building a network and infrastructure to support this amount of information is necessary too. The largest AMR surveillance system in Europe, the European Antimicrobial Resistance Surveillance Network (EARS-Net), collects comparable, representative and accurate AMR data, analyzing temporal and spatial trends of AMR, providing evidence of unfavorable events and encouraging immediate countermeasures. The main problem regarding EARS-Net is the fact that it is based on routine clinical antimicrobial susceptibility data, obtained only form invasive clinical isolates (blood and cerebrospinal fluid) [5]. With that in mind, it is obvious that we need to improve ARM surveillance systems capable of gather information not only from clinical but environmental isolates. As we have seen, AMR detection is possible by applying WGS, which generates huge amounts of data that will be subsequently analyzed using bioinformatic pipelines, helping to define the microbial populations and establishing trends in antimicrobial resistance. WGS is being integrated into the clinical and public health settings, though the use of WGS has been centred primarily on outbreak identification and monitoring. In a recent survey on the current epidemic of carbapenem-resistant *Klebsiella pneumoniae* (CRKp) in Europe, David et al [6] demonstrated (by analyzing the genomic sequences and geographical distribution of 1,717 *K. pneumoniae* clinical isolates) that carbapenemase acquisition is the major cause of carbapenem resistance in *K. pneumoniae*, and that almost 70% of the CRKp isolates were concentrated in just four clonal lineages; the analysis of the genomic sequences even showed that the highest transmissibility in hospital settings is related with the degree of carbapenem resistance. Another example of applying WGS in AMR monitoring is the observational study carried out by Harris et al [7] on clinical isolates of *Neisseira gonorrhoeae* from the European Gonococcal Antimicrobial Surveillance Programme. They found that WGS is an optimal tool for molecular epidemiology, since it identifies mixed infections, predicts antimicrobial resistance, and allows rapid analysis of genomic-phylogenetic relationships, giving a realistic picture of the circulating *N. gonorrhoeae* strains. But the largest achievement of this study was

the creation of a database including epidemiological, phenotypic, and genotypic data, that can be used for AMR prediction, molecular typing and phylogenetic clustering. The creation of these kind of databases should be a priority for the control of the dissemination of AMR, as they allow the storage of multiple resistance profiles and genomic information from microorganisms of high epidemiological interest like methicillin-resistant *Staphylococcus aureus*, MDR and XDR *Pseudomonas aeruginosa* or *Acinetobacter baumannii*.

PROTEOMIC TOOLS IN AMR

Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a rapid, accurate method to routinely identify microorganisms from both clinical isolates and clinical samples, and it has recently emerged as a reliable method to detect antimicrobial resistance. Detection of antimicrobial resistance via MALDI-TOF MS can be done in one of three ways: detection of AMR by analysis of the peak patterns of pathogens, measuring antibiotic modifications due to the enzymatic activity of bacteria (e.g. detecting β-lactamases by measuring mass changes in the antibiotic), and quantification of bacterial growth in the presence of an antibiotic [8]. Although MALDI-TOF MS has showed in several studies its capacity to detect AMR from clinical isolates, there are two main limitations to its routinely use: a limited number of AMR mechanisms that can be identified by this method and its low sensitivity for direct AMR detection from clinical samples. However, machine learning methods have been recently used for detection of drug resistant pathogens using the profile spectra obtained via MALDI-TOF MS [9]. Despite the fact that some advances have been made, the analysis of hundreds of peaks is still a challenge when applying machine learning methods. In spite of being a promising method for AMR detection, more studies on MALDI-TOF MS are required before it is integrated into the normal workflow of microbiology laboratories.

CONCLUSION

Antimicrobial resistance is the biggest public health challenge of our time. Fighting it requires global strategies, focused on preventing AMR emergence by implementing infection prevention, improving antibiotic use and the development of new drugs, implementing surveillance systems to track resistance, and improving laboratories capacity to identify resistant pathogens. Microbiology laboratories play a key role in detecting and monitoring AMR. In this sense, we have moved forward towards new ways to detect AMR in clinical settings. Progress in mass spectrometry and whole genome sequencing technologies has allowed their application not only in pathogen identification but also in AMR detection, becoming potent systems that may change the paradigms of antimicrobial susceptibility testing. Since these methods provides a huge amount of data, sophisticated bioinformatic tools are needed to interpret the results. There is an urgent need for comprehensive, open access databases that include all known resistance genes/mutations to facilitate comparison between methods and bioinformatics tools; although some progress has been made, lack of standardization hold back their potential. In our opinion, the development and implementation of these technologies in microbiology laboratories will permit fast and accurate identification of AMR, facilitating personalized medicine and simplifying AMR surveillance.

CONFLICTS OF INTEREST

The authors declare no conflict of interests.

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