Quo vadis clinical diagnostic microbiology?

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Hospital-acquired infections caused by antibiotic-resistant opportunistic pathogens are on the rise worldwide and represent one of the greatest challenges to modern medicine. Infections due to antibiotic resistant pathogens cause suffering, incapacity and death, and impose an enormous financial burden on both, healthcare systems and on society in general. In 2019, the WHO estimated that each year 700,000 deaths are a direct consequence of multidrug-resistant infections [1].

The management of hospital-acquired infections caused by multidrug-resistant bacteria requires a multi-pronged strategy. This includes the development of new, and the rational use of current antimicrobials. However, also early diagnosis, followed by targeted treatment and the implementation of infection control measures, is a powerful weapon on the battlefield of multidrug-resistant infections.

Despite the clinical need, microbiological diagnostics has not changed fundamentally over the last decades. Identification of bacterial species and resistance testing still rely predominantly on culture-dependent methods. As a result, and certainly in comparison to other areas of laboratory medicine, clinical microbiology is labor intensive and slow. Future diagnostic microbiology faces two important challenges: it must have more impact on the management of infectious diseases, and it should accommodate the general drive for more cost-effective medicine.

It seems fascinating that the identification of biomarkers (such as genomic, transcriptomic, proteomic or metabolomic biomarkers), and thus objective measurement parameters, can increase diagnostic precision in many diseases and, based on this, a therapy can be developed that is tailored to the individual with improved efficacy and chances of cure. The transfer of this concept of individualized medicine to clinical microbiology means that with the decoding of the genetic imprint of the individual pathogen, the individual resistance (and also virulence) characteristics can be identified and thus, a therapy adapted to the individual pathogen profile becomes possible.

Innovations in diagnostics will create important prerequisites for the successful implementation of individualized medicine in dealing with antibiotic resistance. In this context, molecular methods based on gene detection will play a particularly important role. The ability to compare the genome of a newly sequenced bacterial strain with a reference has led to unprecedented discoveries in the genomic era. Whole genome sequencing (WGS) allowed genomic-informed high-resolution pathogen surveillance and was the basis for much work uncovering the molecular mechanisms of antibiotic resistance [2]. Today, relatively inexpensive next generation sequencing (NGS) technologies can produce large amounts of sequencing data, so that genomic analysis is no longer limited to comparing sequence variation between two or a few strains, but examines global genetic variation within bacterial species.

Taking advantage of the advances in sequencing technology and the associated cost reductions, an increasing number of studies are focusing on predicting antimicrobial resistance using genomic data [2-6]. The GEMARA-SEIMC/REIPI Pseudomonas study group aimed at systematically identifying the genetic markers that explain *Pseudomonas aeruginosa* resistance against five commonly used

antipseudomonal antibiotics [3]. *P. aeruginosa* can cause severe nosocomial infections and this pathogen is particularly feared due to commonly found multi-drug resistance. In the two-step approach, the Pseudomonas study group first determined natural genetic variation in 40 chromosomal resistance genes, and then combined this information with information on the presence of horizontally acquired resistance cassettes. In a second step, knowledge-based scores were assigned individually to each resistance marker in respect to its expected contribution to a resistance phenotype. This represents an essential step in the clinical implementation of a diagnostic assay, as transparency and traceability of how diagnostic decisions are made are inevitable for the establishment of a diagnostic tool.

The authors demonstrate that resistance phenotypes can be reliably predicted with very high accuracy based on the *P. aeruginosa* genomic sequence information. However, molecular prediction of antibiotic resistance and susceptibility can only be as good as the underlying genomic reference databases. This, in turn, depends on the current state of knowledge about which (combination of) genetic determinant(s) contribute to antimicrobial resistance. While today existing knowledge is able to reliably predict resistance phenotypes with high accuracy for some antibiotics in some species [5, 6], predicting resistance in *P. aeruginosa* and here particular against beta lactam antibiotics (such as meropenem) remains challenging [3-6]. Additional information on the expression status of individual genes (e.g. efflux pumps) is needed [4, 7, 8]. Nevertheless, while todays existing knowledge cannot explain all resistance phenotypes, it is clear that as more bacterial genomes are sequenced, more information on genetic determinants of resistance will become available. The use of machine learning for qualitative and quantitative phenotype prediction from genotype data thereby might facilitate the discovery of genomic mutations underlying transcriptional profiles that confer to a resistance phenotype. Furthermore, prediction of the evolution of antimicrobial resistance might become possible and thus might spare repetitive sampling of patients to monitor resistance development during antibiotic treatment.

Similar to the interpretation of sequence variants in molecular pathology [9], a need for standardized guidelines arises for clinical diagnostic microbiology to assist interpretation of genetic variation in bacteria. The scoring system developed by the GEMARA-SEIMC/REIPI Pseudomonas study group is a good start to establish a weighted decision matrix to meet this need. The authors highlight the fact that prediction of the *intermediate* phenotype classification (e.g. for meropenem) is a challenge that can hardly be mastered so far [3]. Nevertheless, WGS in combination with a scoring system for data interpretation holds the potential to resolve the ambiguity of results obtained by applying either the EUCAST or the CLSI system for resistance breakpoint determinations. The idea of using a scoring system to evaluate not only resistance markers but also markers for increased susceptibility phenotypes is an interesting attempt [3]. Furthermore, the approach of the GEMARA-SEIMC/REIPI Pseudomonas study group considers contributions of different markers and how these confer to clinically relevant resistance phenotypes singularly and in combination. This is an important point, since the genomic context of a resistance marker may influence to what extent a resistance phenotype is expressed and thus, influences the decision which antibiotic to choose. As mentioned by the authors, further studies on international cohorts will be essential to truly capture all genomic variations of a species, to adjust the scoring system and thus, to initiate the next step towards using WGS as a global standard.

The implementation of molecular based assay systems in clinical microbiology diagnostics will however, face additional challenges. While obtaining WGS information even from a large number of clinical isolates does not seem to be a major hurdle, the readout and interpretation of the individual genetic sequence will remain difficult. Due to the enormous complexity of genetic variation, bioinformatics analyses require appropriate expertise. Currently, capturing all genetic variations (i.e. gene-presence-absence, insertions/deletions, single nucleotide polymorphisms (SNPs)) is a labor intensive process requiring the use of different combination of tools, each of which has its strength and weaknesses [reviewed in 10]. In the future, high quality pan-genomic reference databases that capture the sequence variation landscape within the pan-genes of bacterial species promise to fulfil the needs for a high quality standard in data analysis [reviewed in 11, 12-14]. New tools that allow read mapping to those pan-genomic references will pave the way for a fast and easy read out of all

genetic variants over a plethora of genomes [15]. In the end, these tools need to be optimized for routine use without expert level knowledge, and they will have to fulfil the needs for a high quality standard in data analysis workflows in order to ensure reliability and patient safety.

It will be furthermore important to make the complex WGS data available to clinicians in the context of a more intuitively interpretable report as well as to discuss the many new possibilities with clinical colleagues. Close collaboration and mutual communication with the clinicians could reveal whether timely availability of information on the molecular mechanisms of resistance as well as pathogenicity profiles can influence clinical action to the benefit of patients. Moreover, deep diagnosing offers the unique opportunity to move from the search for a one-size-fits-all to an individualized antimicrobial therapy. Possibly, problematic infections characterized by the expression of particular pathogenicity traits or tolerance phenotypes might also become treatable, when a critical sub-group specific target is identified in a companion diagnostic approach.

In summary, the implementation of bacterial genome sequencing in routine practice promises more detailed clinical microbiology diagnostic reports, which are based on objective metrics. In an iterative process of exchange with clinical disciplines, genomic sequencing information can be understood in an individualized context. The association of genomic sequence information with a disease picture could become the basis for individualized management of severe infectious diseases. The application of WGS technologies may also aid in the development of more rapid and cost-effective diagnostic microbiology. The expected further developments in sequencing technologies, as well as sequencing of large amounts of bacterial DNA in centralized structures will further decrease costs, and e.g. direct sequencing of clinical samples using third generation sequencing technologies will allow faster availability of data. This would set the stage for fundamental changes in diagnostic microbiology.

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