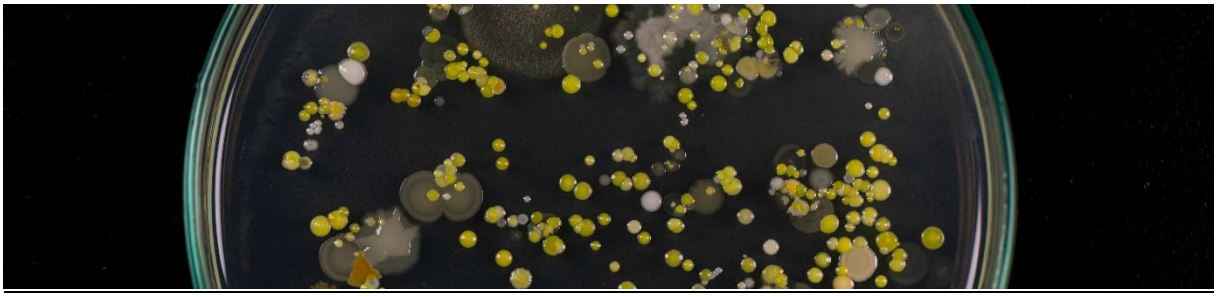
Opinion piece by Prof. dr. dr. Alex van Belkum



Antimicrobial resistance (AMR) is a global threat to human health and the rapid detection and quantification of antibiotic resistance, combined with antimicrobial stewardship, are key interventions to combat the spread of AMR (1). Antimicrobial susceptibility testing (AST) systems are the collective set of diagnostic processes that facilitate the phenotypic and genotypic assessment of AMR and antibiotic susceptibility. Several studies have established proof of principle for various innovative AST methods, including both molecular- and genome-based methods, which await clinical trials and regulatory review. Still, AST is mostly performed using growth-based techniques that essentially require viable bacteria (2). Technologies based on the measurement of bacterial density have evolved marginally in accuracy and rapidity in the 20th century, but assays for new combinations of bacteria and antimicrobials have been automated and made amenable to high-throughput turn-around. Recently, elevated AST detail and rapidity has been provided by nucleic acid-mediated amplification technologies, "omic" methodologies, and the use of next-generation sequencing. In rare cases, AST at the level of single-cell visualization was developed. This has not yet led to major changes in routine high-throughput clinical microbiological detection of antimicrobial resistance. Continuous improvement for existing systems is clearly still very much needed.

As an example of such an improvement, ETEST fosfomycin (ETEST FO, bioMérieux, France) clinical performance was evaluated by three study sites on 152 *Enterococcus faecalis*, 100 *Staphylococcus* spp. and 330 Enterobacterales in comparison with the CLSI and EUCAST agar dilution reference methods (3). Referring to FDA performance criteria, the ETEST FO achieved 91.0% of essential (EA) and 99.0% of categorical agreement (CA) for *Escherichia coli*. In addition, 98.0% EA and 93.4% CA were achieved for *E. faecalis*, with no very major errors (VME) or major errors (ME). According to EUCAST breakpoints for intravenous fosfomycin use, Enterobacterales and *Staphylococcus* spp. also met ISO acceptance criteria for EA and CA. A VME rate of 8.8% was observed for Enterobacterales but the MICs were within EA. A trend to predict lower MICs for *Citrobacter* spp., *E. coli* and *Salmonella enterica* and to predict higher MICs for *Klebsiella pneumoniae* MICs was observed, while ETEST FO should not be used for *Enterobacter cloacae*, because of low EA and a high VME rate. This very detailed study supports the efficiency of the novel ETEST FO and shows that expansion of the capabilities for existing AST systems are as much needed as the development of entirely new methods.

As a second example, recent years have also witnessed the development of bioinformatic-based approaches predicting phenotypic AMR traits on the basis of their whole-genome sequences (4). While often convincing in terms of predictive performance, the underlying models are not straightforward to interpret, the interplay between the actual genetic determinant and its translation to phenotypic markers being generally hard to decipher. The benefit of this approach on the task of predicting microbial antibiotic resistance profiles from a genome should be in the weighted linear combinations of genetic elements that can be identified as genuine antibiotic resistance determinants. While

antibiotic resistance usually is an important motivating factor, the method is generic and can be transposed to any other bacterial trait showing the diagnostic perspectives for genomic approaches.

To help improve the development of really new and innovative AST systems, target product profiles (TPP) have been developed to focus developers' attention on the key aspects of AMR diagnostic tests (5). It was noted that specific AMR-related TPPs could be extended by incorporating the interdependencies between the key characteristics associated with the development of such TPPs. This led to the identification of 46 characteristics associated with six main categories including intended use, the diagnostic question covered, test description, assay protocol, testing performance and commercial application. The interdependencies of these characteristics were identified and mapped against each other to generate new insights for use by AST stakeholders. Although it may not be possible to achieve all of the recommendations in every category of a TPP, the use of such guidance could lead to more efficient AMR diagnostics development.

The Kairos System developed by ShanX Medtech (Eindhoven, The Netherlands) fits the description of a rapid, cheap, portable and accurate new AST system (6). It fulfils many if not all of the criteria and requirements discussed above and provides a clear example of an efficient AMR-diagnostic tool.

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