"How inkjet printing technology can defeat multidrug-resistant pathogens"

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When a patient presents with a serious infection, clinicians will start empiric antimicrobial therapy, an informed prediction as to what will be a successful treatment. We know that when empiric therapy does not match the organism's antimicrobial susceptibility profile, there is suboptimal clinical outcome [1, 2]. The longer the time on inappropriate therapy, the worse the patient does. Unfortunately, with the emergence of multidrug resistant (MDR) pathogens, our empiric therapy predictions are increasingly wrong. This new reality is dramatically illustrated in patients with carbapenem-resistant Enterobacteriaceae bloodstream infections where delay in institution of appropriate therapy is associated with significantly increased mortality [3].

Therefore, to identify needed corrections to empiric therapy, patient specimens are cultured by a hospital-based clinical microbiology laboratory to isolate and identify the infecting pathogen, and to determine which antimicrobials are active against it. The gold standard methods for antimicrobial susceptibility testing (AST) are broth and agar dilution, which involve preparing a doubling dilution series of antimicrobials and determining the lowest concentration at which bacteria are inhibited (the minimal inhibitory concentration, or MIC). However, these methods are complex and laborious, precluding their use in hospital-based clinical laboratories. Therefore, AST is typically performed using automated platforms, a process which practically takes one day. We call the time between initiation of empiric therapy and the availability of the antimicrobial susceptibility profile the "antimicrobial susceptibility testing gap" (ATG). The gap may be two to three days, taking into account the time needed for isolation of the organisms and AST.
Unfortunately, the ATG may be particularly long for MDR bacteria, the type of organisms where our empiric therapy guesses are most likely to be wrong. Specifically, standard AST methods used by hospital-based laboratories often consist of commercially-produced, pre-fabricated, fixed panels of antimicrobials chosen to match hospital formularies. However, for MDR pathogens, we often find that the organism is either (1) resistant to all agents tested or (2) practically resistant because the patient is allergic to or cannot tolerate side effects from active antimicrobials.

We therefore need to test second line agents, leading to further delay and a longer ATG. In fact, we often need to test agents of last resort such as colistin and newly released drugs that are unavailable in either pre-made panels or surrogate methods such as disk diffusion. These drugs can only be tested by technically complex broth and agar dilution reference methods, which are unavailable in hospital-based clinical laboratories. Isolates are therefore commonly sent to a reference laboratory for such testing, extending the ATG up to 7 days, a clearly unacceptable delay for MDR pathogens with unpredictable susceptibility profiles.

We are therefore in desperate need for solutions to close the ATG and expand the capabilities of hospital-based clinical microbiology laboratories. Current trends in AST systems favor increased automation at the cost of reduced flexibility. However, we believe that such a tradeoff is unnecessary.

More specifically, we validated inkjet printing technology as a way to perform automated AST for any antimicrobial agent at will [4]. It turns out that with some engineering tweaks inkjet printers (i.e., the HP D300) can print out things other than ink - in our case, antimicrobial stock solutions. Instead of ink cartridges, the HP D300 utilizes cassettes that can be loaded with up to 8 different antimicrobial stock solutions which can then be printed out as a dilution series in any
desired format. Per manufacturer's specification, the D300 can print out droplets ranging in size from 11 picoliters to 10 microliters [5]. The size of the droplet determines the amount of antimicrobial in a microplate well, and a standard two-fold dilution series can thereby be created with a single pipetting step.

We recently verified the performance of the inkjet methodology in comparison with gold standard, reference broth microdilution AST using 7 antimicrobials including colistin against a large panel of clinical isolates [4]. The new inkjet technology performed just as accurately and with greater precision. Importantly, the flexibility of inkjet technology contrasts with automated AST methods in current use which are typically “locked down” to include only limited dilutions of specific antimicrobials and may also only provide an extrapolated rather than a true MIC. Moreover, because the inkjet printer is much more spatially precise than a human being, we were able to miniaturize testing to a 384-well plate format. This saving of microplate real estate has important implications as it enables performance of additional testing relevant to MDR pathogens.

Specifically, with limited options for treating MDR pathogens, novel solutions are needed to rescue the ability of available antimicrobials to serve as useful agents. This rescue may take two forms, both of which rely on highly accurate quantitative measures of antibacterial inhibitory levels. First, for many antimicrobials, therapeutic success is predicated on a balance between in vivo drug exposure and pathogen susceptibility (i.e., reflected in in vitro MIC measurements). Therefore, there may be room to rescue use of antimicrobials for treatment of relatively resistant organisms through augmented dosing. However, here we run into limitations of traditional MIC testing in its ability to help us reliably negotiate within the therapeutic safety window and avoid harmful side effects.
Standard MIC values are determined using a doubling dilution series. These values are used to categorize organisms as susceptible or resistant. However, we know that MIC values determined by standard doubling dilution testing may have significant error (± 1 dilution) and certainly may fall between concentrations tested. In 2015, the Clinical and Laboratory Standards Institute introduced a new MIC interpretive category, susceptible dose-dependent (SDD), for the antibiotic, cefepime [6]. This category lies between susceptible and resistant with the specific goal of giving clinicians a new ability to treat relatively resistant Enterobacteriaceae by either increasing the antimicrobial dose (MIC = 4 µg ml⁻¹) or increasing both the dose and frequency of dosing (MIC = 8 µg ml⁻¹). Unfortunately, the spacing of concentrations tested in standard doubling AST become larger at higher antibiotic concentrations with unfortunate consequences for MIC-based dosing.

For instance, an organism with a cefepime MIC of 10 potentially could still be treated based on pharmacokinetic principles alone. However, one with an MIC of 30 µg ml⁻¹ likely could not be. Yet, both, when tested by the classic doubling dilution scheme in current use, may show an MIC of 16 µg ml⁻¹ taking into account the fact that only 8, 16, and 32 µg ml⁻¹ are traditionally tested in this upper concentration range. Therefore, traditional practice and methods lack the requisite precision to provide a confident basis for SDD dosing regimens for cefepime and other antimicrobials for which SDD dosing regimens will likely be recommended in the future.

To accommodate changing AST standards and demand for increased precision, inkjet printing technology can be used to create a finer dilution series centering on critical clinical cutoffs (such as the SDD range). Using this technology, we can easily test concentrations between two-fold dilutions (e.g. 6, 8, 10, 12, . . . µg ml⁻¹) to know more precisely how a
particular dosing strategy may affect an organism, and gain further confidence that our MIC
determinations are accurate. The importance of this greater accuracy as a foundation for reliable
pharmacodynamics (treatment efficacy) studies is self-evident. The importance of this finer
precision in defining the appropriate use of antibacterials with a small safety margin (e.g.,
colistin) also seems compelling.

The second potential salvage strategy is antimicrobial synergy. Synergy for our
discussion means that two agents, considered ineffective when tested individually, fully inhibit
growth of a pathogen at clinically relevant concentrations (i.e., within susceptible range) when
tested in combination. Unfortunately, synergy testing is even more complicated than reference
laboratory dilution testing, because it essentially squares the amount of work involved.
Therefore, synergy testing is essentially never performed outside of a research setting. However,
by loading two antimicrobials into the HP D300, a synergy grid can easily be created, allowing
for rapid determination of synergistic activity between two (or potentially more) antimicrobials
within a clinically actionable time frame. We have used this technology to identify potential
double and triple synergistic combination therapies for *Legionella pneumophila* and anticipate
that this methodology will be applicable to a wide variety of pathogens [7].

We predict the conceptually simple automation provided by inkjet printing technology is
poised to have significant impact on antimicrobial testing. It will open up new options for
treatment by enabling precise MIC-dependent dosing, and spur both research into and use of new
synergy-based combinations. Importantly, it will also provide flexibility to immediately
incorporate newly approved antimicrobials developed for MDR pathogens into hospital-based
laboratory testing, drugs that otherwise might not appear in clinical panels for several years. At
present, the HP D300 can be used in so-called "laboratory-developed tests" validated by
individual clinical laboratories. We have found in our hands that inkjet printer-based AST meets a verification standard recommended by the FDA and the AST community [4]. We therefore envision that inkjet technology could form the foundation of a future clinical platform that addresses unmet needs in AST diagnostics and significantly shortens the antimicrobial testing gap.
References


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