Editorial

Advances in laboratory diagnostic technologies in clinical microbiology and what this means for clinical practice

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"...antimicrobial resistance continues to increase to this day and the impact on patient care is enormous in some clinical settings."

The treatment of infectious diseases has become more complicated over the past two decades due to increasing antimicrobial resistance. Antimicrobial resistance is not new and was recognized shortly after the introduction of penicillin into clinical practice; penicillinase-producing *Staphylococcus aureus* clinical isolates were quickly identified. In 1945, Alexander Fleming wrote, "But I would like to sound a rate of warning … it is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentration not sufficient to kill them and the same thing has occasionally happened in the body." Later, Fleming was quoted as saying, "… the greatest possibility of evil in medication is the use of too small doses so that instead of clearing up infection the microbes are educated to resist penicillin and a host of penicillin-fast (resistant?) organism is bred out, which can be passed to other individuals and from them to others, until they reach someone who gets septicaemia or pneumonia which penicillin cannot save" (New York Times, 26 June 1945).

Clearly, the early recognition of the potential for antimicrobial resistance and its potential association with mortality should have been sufficient warning for ongoing antimicrobial use. Unfortunately, antimicrobial resistance continues to increase to this day and the impact on patient care is enormous in some clinical settings. Multidrug-resistant organisms are common globally and are now seen in organisms associated with community and hospital-acquired infections.

In 1957, a group of leading experts from Europe and North America convened in London, UK, to discuss "Drug Resistance in Micro-organisms, Mechanisms of Development." Sir Charles Harington, in his opening address to attendees said, "In spite of the great advances that have been made in recent years in the chemotherapeutic

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treatment of infectious diseases – advances that have brought under some measure of control, the majority of protozoal and bacterial infections and some helminthic infections – the subject of chemotherapy remains distressingly empirical" [1].

Empiric, in its simplest definition, means 'without the knowledge of', and in today's environment is consistent with a syndromic approach to treating infection. A syndromic approach means that therapy is directed at treating pneumonia or urinary tract infection and not specifically against the causative organism but that is obviously implied with drug selection approved for that specific clinical indication. Such an approach has been facilitated by the development of broader spectrum antimicrobial agents or combinations of agents that have a spectrum suitable for covering the most frequent pathogens associated with a specific infection (e.g., pneumonia or urinary tract infection). Indeed, various different antimicrobial therapy guidelines have been developed and revised over the past two decades for recommended therapies for pneumonia [2] or other respiratory tract infections [3,4], urinary tract infections [5], bacterial meningitis [6] and many other clinical presentations [7] or for some specific pathogens such as methicillin-resistant *S. aureus* [8] or *Clostridium difficile* [9]. Interestingly, these guidelines do not all uniformly recommend specimen submission for culture and sensitivity. In some instances, culture is recommended only in more difficult clinical situations or where initial therapy has failed. Perhaps this indicates that antimicrobial therapy remains "distressingly empirical". In hindsight, perhaps the right approach was to submit specimens, isolate pathogens, determine susceptibility and then tailor therapy to be optimal for that pathogen and clinical presentation.

"...some 80% of patient management decisions are influenced by laboratory testing..."

Why has specimen submission to clinical microbiology for culture and sensitivity not been a priority in many clinical scenarios – especially in patients with community-acquired infections of mild-to-moderate severity? Some reasons could include:

- Not being necessary as clinical experience with a specific drug or a number of different drugs has been satisfactory with minimal clinical failure;
- Broad spectrum antimicrobial agents that can be safely administered orally;
- Administration of an antimicrobial agent was given empirically to alleviate patient symptoms and prevent clinical deterioration and/or complications from not being treated;
- It takes too long to get a result that is clinically useful.

Laboratory testing in chemistry and hematology has been automated for many years and current levels of automation with rapid reporting of results are impressive. Such has not been the case with clinical microbiology where semi-automation or automation has been slow to evolve. In fact, in many clinical microbiology laboratories, advanced automation or semi-automated technology is absent whereas in larger medical centers (including our own) more advanced technology exists. This in itself is problematic as some 80% of patient management decisions are influenced by laboratory testing and almost as large a percentage of testing is done outside of large medical or regional referral centers. As such, clinicians (general practitioners and specialists) have been rightfully frustrated with the time it often takes to provide a pathogen identification and susceptibility. **Figure 1** is a schematic diagram outlining approximate times for generation of results in clinical microbiology laboratories. Presumptive organism identification was and is by microscopy, colonial morphology and various biochemical reactions detected by colorimetric indicators. Latex agglutination or co-agglutination technologies also allow for rapid organism identification. Most of these methods can be performed in minutes but are dependent on the organism being present on an agar plate after 24 h (aerobic) to 48 h (anaerobic) of incubation. While rapid organism presumptive identification can occur in minutes, the necessity of culture has added 1–2 days to result generation from the time the specimen was received in the laboratory. Susceptibility testing, if done, would require an additional 18–24 h of incubation; however, a rapid screen for detection of β-lactamase enzyme could be performed in minutes. Such information could impact on clinical decision for initiation of antimicrobial therapy, for continuation of empirically initiated therapy or for a change in therapy; however, the β-lactamase screen is restricted to narrow spectrum β-lactam drugs. Such screening for extended spectrum β-lactamase enzymes is more complicated.

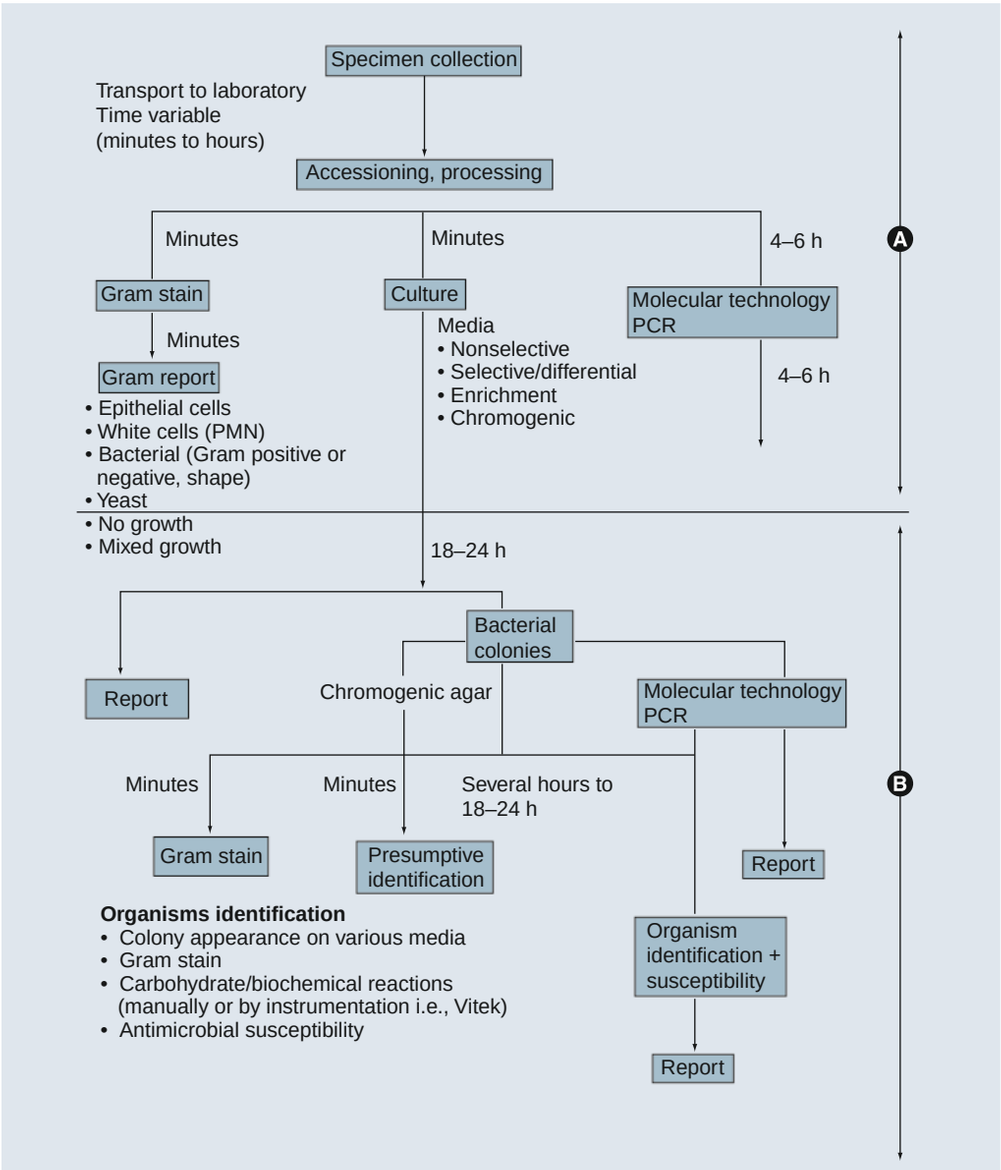

Figure 1. Bacteriology timeline. (A) <1 to approximately 6 h; **(B)** 16 to approximately 48 h.
PMN: Polymorphonuclear.
Data taken from [18].

Advances in blood culture technology improved detection of organisms present in blood; however, the time to organism identification and susceptibility testing is still problematic. Once a blood culture bottle flags as positive, a Gram stain provides initial information on organism morphology and Gram reaction; however, an additional 18–24 h of incubation to recover the organism on agar plates is necessary. Further identification and susceptibility can take up to another 18–24 h. As blood cultures are among the most important specimens analyzed in clinical microbiology laboratories, impacting on rapid identification and susceptibility testing or resistance detection is indeed a laudable and necessary goal.

Semi-automated technologies such as Vitek® (BioMerieux), Microscan® (Siemens) and Phoenix™ (Becton-Dickson) systems provide platforms for organism identification and antimicrobial susceptibility testing. Such systems have large data banks on various carbohydrate or biochemical characteristics of multiple strains of bacteria (e.g., *Escherichia coli*). When a test

organism is inoculated to the cards or plates (depending on instrument), reactions are determined (e.g., negative or positive or carbohydrate utilized) and the combination of reactions for that organism compared with the data bank and an organism identification given. Susceptibility testing follows a similar procedure except the cards or plates contain various antimicrobial agents over a range of different drug concentrations. Growth or inhibition of growth in the presence of the drug indicates susceptibility or resistance. This technology has transformed many clinical laboratories; however, a limitation on turnaround time (TAT) is the fact that the organism is required to be growing on an agar plate – hence, the time needs to be at least 18–24 h after the specimen was received in the laboratory. Organism identification and susceptibility testing on the above noted platforms can take approximately 4–18 h depending on the pathogen.

An advancement in technology that has impacted TAT is testing by PCR [10]. This technology allows for a unique sequence of nucleic acids to be recognized, amplified and detected, all within a number of hours, often same day. Such technology truly can influence same-day clinical decision-making. Three key pathogens (although there are others) detected directly from patient specimens are MRSA [11,12], *C. difficile* [13,14] and *Streptococcus agalactiae* (group B Streptococcus) [15]. Arguably, rapid detection of these organisms has consequences for the patient and institution including drug therapy and/or infection control intervention. Rapid detection of *S. agalactiae* from expectant females at or near the time of delivery could have a profound influence on prophylactic antimicrobial therapy if the expectant mother falls within any of the at-risk scenarios for postdelivery early-onset group B Streptococcus infection of the newborn. While rapid identification of an MRSA strain does not provide rapid identification of all the antibiotics the organism is susceptible or resistant to, it does immediately identify that β-lactam agents (e.g., penicillins, cephalosporins, carbapenems and monobactams) are not suitable drug choices. Other PCR advances influencing clinical decision making are for respiratory viruses. In some instances, PCR detection of viruses has reduced TAT from days (perhaps a week) to hours – a true advancement. Mycobacterial diseases would similarly benefit from rapid identification influencing TAT – especially in immunocompromised patients such as those with advanced HIV disease. Rapid differentiation of *Mycobacterium tuberculosis* from atypical *Mycobacterium* species could profoundly influence patient therapy.

"This technology impacts turnaround time for organism identification as it is accomplished in minutes versus hours..."

Recently introduced to North America but available earlier in Europe is the technology of matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry [16,17]. Our clinical microbiology laboratory has acquired this technology and the impact has been immediate and substantial. In a fashion similar to other technologies, a large data bank of organism profiles is built into the system. Spectrograms are compared against this data bank and organisms identified to the species level within 3–5 min or less. To use this technology, the organism from a colony on an agar plate is inoculated to a slide, overlaid with a drop of matrix solution, placed in the instrument and the spectrogram generated. For this technology, the well (on the card) containing the test organism is blasted multiple times by the laser technology and the profile generated. This technology impacts TAT for organism identification as it is accomplished in minutes versus hours, is cost effective and impacts on workload and workflow in the clinical laboratory. In some instances, organisms that are more difficult to identify and require multiple levels of reflex testing – often taking days to complete – can be identified in minutes. Ruling out a potential contaminant can be as important as identifying a true pathogen. Such information is clearly beneficial to clinical decision making. As an example, a recent case in our institution illustrates this point. A cerebral spinal fluid specimen from a child in the neonatal intensive care unit grew a single colony of bacteria on an agar plate. Our initial thinking was that the colony was insignificant but that we would require some 18–24 h to further resolve this. We tested the organisms by mass spectrometry and within minutes had an identification of an *Acinetobacter* species. In consultation with the neonatal intensive care physician, we learnt that the child was clinically stable; however, should her condition have deteriorated, the standard antimicrobial regimen used would have been potentially inadequate for this organism. The rapid identification

provided by mass spectrometry influenced the consideration of empiric therapy if necessary. Unfortunately, mass spectrometry technology does not yet truncate the TAT for susceptibility testing, which is arguably a more important variable for clinical decision making; however, such capabilities may not be far away.

The question remains: have technological advances in clinical microbiology laboratories impacted clinical decision making in patients with infectious diseases requiring antimicrobial therapy? In our opinion, we believe the answer to be clearly yes. Rapid organism identification clearly impacts therapy. For example, empiric antimicrobial therapy that does not include coverage for key pathogens such as *Pseudomonas aeruginosa* or *Enterococcus* species could be modified before susceptibility testing is completed as these pathogens have different antimicrobial susceptibility profiles with limited antimicrobial options. In addition, rapid identification of an unsuspected pathogen could impact numerous potential interventions including infection control.

If clinical microbiology laboratories wish to aspire to the levels of automation seen in other laboratory disciplines and have substantially reduced TATs, what will be necessary? Clearly, same-day reporting is desirable, which means that technology needs to advance for direct detection of a pathogen(s) from the patient specimen without the (albeit necessary) delays currently seen with overnight culturing. While detection of the pathogen is important, the name of the pathogen may be less important to clinicians than its susceptibility or resistance to antimicrobial agents, which is understandable. While such detection is currently available for, for example, MRSA, the full range of susceptibility results for different drug classes and specific agents require additional testing, adding 24–48 h to TAT. As such, although we are not quite there yet, we are closer to the goal of same-day reporting than we were a decade ago.

Tremendous advances have been made in clinical microbiology laboratories impacting TAT and such technologies have not compromised sensitivity or specificity. Rapid communication of organism identification clearly impacts some clinical scenarios but, in an ideal world, rapid identification of a pathogen and its susceptibility or resistance profile will have the greatest impact on influencing therapeutic discussions and optimizing therapy. Optimizing antimicrobial therapy may reduce the selective pressures for resistance selection. Organism identification by mass spectrometry technology directly from fluid specimens (i.e., positive blood cultures or urines with confirmed bacteria) will impact TAT, as will an expanded menu of pathogens detected directly from patient specimens by PCR. The next decade, and particularly the next few years, should prove intriguing as we strive to further impact clinical decision-making for infectious diseases by advances in clinical microbiology.

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