What can you expect from a microbiology diagnostic service?

A modern microbiology service offers a comprehensive diagnostic service to support patient management.

ELIZABETH WASSERMAN, ***MB ChB, MMed (Microbiol Path), DPhil (Social Science Methods)***
Professor and Head, Division of Medical Microbiology, Department of Pathology, NHLS, Tygerberg Hospital and Stellenbosch University
Professor Wasserman has an interest in innovative diagnostic techniques that can be effectively implemented in a limited resource environment, and also in medical education.

Although many microbiology practices are still rooted in the art of culturing pathogenic bacteria from patient specimens to identify them and test their response to antimicrobials *in vitro*, we have come a long way since bacteria were first visualised by Van Leeuwenhoek more than 300 years ago. The modern medical microbiology laboratory offers a comprehensive diagnostic service, designed to optimise specimen collection, to ensure quality processing and to assist with the interpretation of reports.

The role of a microbiology laboratory service is twofold: to diagnose the pathogens that cause disease in individual patients, and to keep track of the potentially pathogenic organisms that occur in a specific community.

The bacteria that colonise our patients form part of a larger, invisible community. We share our so-called 'normal flora' or colonising bacteria with the environment, and in particular with the people with whom we are in close contact. The epidemiology of infectious disease is therefore an integral part of society, which affects individual patients.

The role of a microbiology laboratory service is twofold: to diagnose the pathogens that cause disease in individual patients, and to keep track of the potentially pathogenic organisms that occur in a specific community. Such a community can be in a hospital (an intensive care unit, or a neonatal or surgical ward), but it can also comprise a specific geographical area, a crèche, or a nursing home. The microbiology laboratory should therefore not only inform the doctors submitting patient specimens regarding appropriate therapy for individual patients, but also compile data bases of current pathogen profiles occurring in specific communities. Such information can assist empirical therapy in cases where specimens are not usually submitted to the laboratory (e.g. uncomplicated urinary tract infections) or in cases of severe infection where therapy cannot await special microbiological results (e.g. septic meningitis).

Infections in specific patients

There are essentially three ways in which a microbiology laboratory can identify the bacterial agents of disease in a specific patient:

- direct detection of the pathogen in a patient specimen
- culture of bacteria for identification and sensitivity testing
- measurement of patient responses to the presence of a pathogen by means of antibody detection (serology).

Direct detection of a pathogen in a patient specimen

Microscopy

Microbiologists still rely on microscopy because it is cheap and relatively easy to perform. In certain specimens, it provides very useful information that can give an early indication of appropriate therapy. Direct microscopy on urine and stool specimens gives us information about the presence of pus cells, blood and parasites. Various staining methods help us to assess greater detail. The best example is the Gram stain, which enables us to characterise bacteria according to their morphology, spatial arrangement and characteristics of their cell walls. We use the Gram stain to distinguish between the so-called Gram positives and negatives, which form the basis of the classification system for medically important bacteria.

Within hours of receiving a specimen, a laboratory can report the results of microscopy. Although this will only be a preliminary report awaiting further results such as culture and sensitivity testing, a clinician may gain significant guidance from such a report towards tailoring of antimicrobial therapy. For example, the presence of Gram-negative diplococci in a joint aspirate should alert the clinician to the possibility of gonococcal arthritis, and therapy should be adjusted to cover for that pathogen. With good surveillance data at hand, the likelihood of resistance to certain antimicrobials in a community should also be known (e.g. high rates of ciprofloxacin resistance in gonococci in South Africa).[1] An accurate therapeutic choice can be made even before the sensitivity of a particular isolate to antimicrobials is tested and reported.

Antigen detection

Antigen detection is another example of direct detection of a pathogen from a clinical specimen, e.g. detection of the capsular antigen of *Cryptococcus neoformans* in the cerebrospinal fluid of patients suffering from chronic meningitis. The test is rapid and simple to perform: a reagent containing monoclonal antibodies

against the antigen is mixed with a drop of cerebrospinal fluid. The antibodies are fixed to small latex particles. When they are bound by antigen that may be present in the specimen, an agglutination reaction occurs that is visible with the naked eye. This test is both sensitive and specific for the diagnosis of cryptococcal meningitis.

Gene probes

The direct detection of genetic elements specific to pathogens in patient samples is becoming more commonplace. Hybridisation techniques and the polymerase chain reaction (PCR) are prime examples of this. Hybridisation involves fixing a patient specimen to a slide and probing it with gene sequences complementary to a specific part of the genome of a known pathogen. If the probe finds a matching sequence, it will bind to that site. Because the probe is marked with a fluorescent or an ensymatic marker that can be visualised, it flags the presence of the pathogen.

The PCR technique, developed in 1983 by Kary Mullis, not only targets specific genetic sequences, but also amplifies them logarithmically until the product of this amplification reaction is substantial enough to be visualised on a macroscopic level. New technologies, such as real-time PCR, enable us to quantify the original amount of DNA or RNA present in the patient specimen. It also makes it possible to automate molecular testing, which is faster, user friendly and cheaper. Multiplex PCR techniques allow us to look simultaneously for many different gene sequences in the same specimen. The gene fragments selected for molecular detection not only identify specific bacteria, but can also be used to identify their properties. For example, the presence of known genes that confer resistance to certain antimicrobials can easily be detected by PCR.

Molecular techniques are developing at a spectacular rate and will probably expand the range of services offered by the microbiology laboratory significantly in the future.

Culture of bacteria for further testing

The traditional phenotypic method (as opposed to the genotypic methods described above) of identifying bacteria and testing their sensitivity to antimicrobial drugs requires a pure culture of the organism, and further subculture on various media to visualise their biochemical characteristics and susceptibility to antimicrobial drugs. This is an art akin to gardening: a culture medium (or, in practice, a range of culture media) that will support the likely pathogens that may occur in a specific setting is selected, the specimen is seeded on this and then incubated for a time to allow for sufficient growth. Media are usually solidified by adding agar, as this helps to isolate a single colony from a mixed growth by simply picking it off with a sterile platinum loop. Organisms are then identified by exposing them to a range of biochemical substrates and documenting their reactions. This can be done manually, which is cheaper in consumables but expensive in time and labour. Currently automated systems for the identification of bacteria are utilised in many laboratories. Automation not only cuts down on hands-on labour, but also reduces the so-called 'turnaround time' of results. This implies that the final reports on specimen analysis can be produced faster. The higher cost can be offset by a positive impact on the total cost of care, as good-quality microbiology reports can have a significant impact on patient morbidity and mortality.[2]

We depend on good communication with clinicians to deliver an appropriate service.

The identification of pathogens helps us to formulate our clinical diagnoses and to establish the epidemiology of disease, and often it may also indicate the possible source of a systemic infection. This can be extremely valuable, as antimicrobial therapy is often insufficient without adequate source control.[3]

Despite the advances in molecular methods, phenotypic sensitivity testing is still the most common method currently used. The growing bacteria are exposed to specific concentrations of antimicrobials, and reactions to these are accurately measured. We can do this in several ways and a specific isolate is then reported to be either 'sensitive' or 'resistant' to a specific antimicrobial. Sensitivity can be more accurately established by the determination of the minimum inhibitory concentration (MIC) of a drug for a specific pathogen. This is useful in cases where the level of resistance relates to the site of the infection (e.g. *Streptococcus pneumoniae* meningitis).

Traditionally, all the processes listed above culminate in the final microbiology report: microscopy, culture and sensitivity (MC&S).

However, what about pathogens that cannot be visualised easily with ordinary microscopy, and those that do not grow well on conventional laboratory media? (Interestingly, these two properties are usually related.)

Serology

Pathogens such as *Rickettsiae, Mycoplasma* and *Treponema* cannot be cultured in cell-free media. Molecular testing is already providing alternative ways of detecting the presence of these organisms in clinical samples, but in most settings we still have to rely on the patient's reaction to the pathogen to make a specific diagnosis. Serology is the science of indicating the presence of specific antibodies in a patient's blood. It has several drawbacks: it takes time for the patient to develop such an antibody reaction; the reactions are often nonspecific; and it can be difficult to distinguish new infections from previous exposures.

There are a variety of techniques available for serology: haemagglutination, Coombs' testing, immunofluorescence and many permutations of the classic ELISA (enzyme-linked immunosorbent assay). Serology is never easy to interpret. Often a rise in titre is required to prove a current infection, which implies that two specimens have to be taken about 14 days apart. It is not a quick way to get an answer. The availability of various techniques complicates it further, e.g. results of tests done in different laboratories, using different methods, are often not comparable.

Serology may become less important in the future as other more reliable and reproducible technologies become available. Currently, it is still important in the diagnosis of many common and important diseases such as syphilis and tick-bite fever. The best advice with regard to the interpretation of troublesome serology results is to consult your clinical microbiologist.

Surveillance and pathogen profiles in specific communities

The traditional 'MC&S' usually has a turnaround time of ≥2 days. With automation, this can be reduced to just over a day. Molecular methods are currently restricted to specific pathogens in specific settings, but in future may produce results within hours. Nonetheless, life-threatening conditions such as septic shock and purulent meningitis often necessitate antimicrobial therapy before specific microbiological results become available. In a resource-constrained setting, it may not be feasible to send specimens to a laboratory for uncomplicated infections.

Surveillance data enable clinicians to make informed, empirical antimicrobial choices, which can be adjusted according to patient response or laboratory reports when they become available. Therefore, part of the service that a microbiology laboratory can offer is to compile data banks containing information on the organisms isolated from clinical specimens. These data can then be used to regularly supply updated information to clinicians regarding the most common pathogens isolated from specific sample types or from specific patient populations in specific areas (e.g. a certain community, hospital or ward), and the antimicrobial sensitivity of these organisms.

Data can be organised at a regional or national level, and over time certain trends become noticeable. We may become aware, for instance, that penicillin resistance is emerging in our *N. meningitidis* isolates[4] and that resistance to a range of different classes of antimicrobials is becoming a serious problem in Gram-negative bacteria.[5] Such epidemiological data enable us to pinpoint problem areas so that timely strategies can be employed to prevent further escalation of emerging health threats.

In South Africa, a national system of surveillance is orchestrated by the National Institute for Communicable Diseases (NICD), with particular emphasis on pathogens causing invasive infections, diarrhoeal pathogens and sexually transmitted diseases (http://www.nicd.ac.sa). In addition, local laboratories usually have their own regional system of surveillance, and if summaries of these are not disseminated routinely they can usually be specifically requested.

A different type of surveillance system can also be set up to flag specific pathogens that may present a public health hazard. These include notifiable diseases, such as *Salmonella typhi* and extremely drug-resistant (XDR) tuberculosis, that may cause outbreaks in a community that could be devastating if not recognised at a very early stage. Another group of pathogens that require surveillance are antimicrobial-resistant pathogens that may cause outbreaks in a hospital, e.g. methicillin-resistant *Staphylococcus aureus*, better known as MRSA. This type of surveillance system should be linked with infection control teams. Some hospital groups in South Africa now employ computerised antimicrobial surveillance technology which provides an early warning system of possible infection control issues.

Conclusion

Communication between the laboratory and clinical staff is perhaps the most important ingredient of a quality service from a microbiology laboratory. Good advice on specimen collection can be priceless (see Professor Forder's and Dr Orth's articles on this subject in this issue), while the relevance of microbiological results often only becomes clear in a discussion between a clinical microbiologist and a treating physician. Antimicrobial sensitivity testing may guide therapy, but optimal therapy cannot be advised without considering certain patient-related factors, such as the site of infection, which may have a profound effect on the ability of a specific agent to act effectively.

Where does all of the above leave the traditional culture of organisms, and phenotypic sensitivity testing?

For more than 50 years the standard MC&S ruled and practices changed very little in the average microbiology laboratory. But we are now approaching a frontier of irrevocable change. Amplifying and sequencing key areas of the bacterial genome enable us not only to identify pathogenic organisms, but also to determine their important characteristics, such as virulence properties and resistance genes. Sequence-based typing methods linked to the internet also enable us to compare strains to global data bases, tracking the spread of pathogens and providing us with the ability to instantly unravel the epidemiology and the evolution of infectious diseases. Technologies are becoming more user friendly and more affordable with time.

Nevertheless, our concept of culture and sensitivity may change in practice but not in implication. Although the way we derive our results may be different, faster, and even more accurate in the future, we will still be dependent on good communication with clinicians to deliver an appropriate service.

References

1. LewisD, Scott L, Slabbert M, *et al.* Escalation in the relative prevalence of ciprofloxacin resistant gonorrhea among men with urethral discharge in two South African cities – implications for HIV transmission. *Sex Trans Int* 2008; 84: 352-355.
2. Yuan S, Astion ML, Schapiro J, Ajit PL. Clinical impact associated with corrected results in clinical microbiology testing. *J Clin Microbiol* 2005; 43(5): 2188–2193.
3. Marshall JC, Maier RV, Jimenes M, Dellinger PE. Source control in the management of severe sepsis and septic shock: An evidence-based review. *Crit Care Med* 2004; 32(11) (Suppl.): S513-S526.
4. Du Plessis M, Von Gottberg A, Cohen C, De Gouveia L, Klugman KP. *Neisseria meningitidis* intermediately resistant to penicillin causing invasive disease in South Africa, 2001-2005. *J Clin Microbiol* (published online ahead of print on 23 July 2008).
5. Brink A, Feldman C, Richards G, Moolman J, Senekal M. Emergence of extensive drug resistance (XDR) among Gram-negative bacteria in South Africa looms nearer. *S Afr Med J* 2008; 98: 586-591.

In a nutshell

- The role of a microbiology service laboratory is twofold: to diagnose the pathogens that cause disease in individual patients, and to keep track of the potentially pathogenic organisms that occur in a specific community.
- There are essentially three ways in which a microbiology laboratory can identify the bacterial agents of disease in a specific patient:
  - direct detection of the pathogen in a patient specimen
  - culture of bacteria for identification and sensitivity testing
  - taking measurements of patient responses to the presence of a pathogen by means of antibody detection (serology).
- Direct detection of the pathogen relies on microscopy, antigen detection and molecular methods.
- Culture of bacteria for identification and sensitivity testing requires a pure culture of the organism, and further testing of its biochemical characteristics and susceptibility to antimicrobial drugs can be automated.
- Serology is mainly used for pathogens that cannot be cultured in cell-free media.
- Part of the service that a microbiology laboratory can offer is to compile data banks containing information on the organisms isolated from clinical specimens.
- In South Africa a national system of surveillance is orchestrated by the National Institute for Communicable Diseases (NICD), with particular emphasis on pathogens causing invasive infections, diarrhoeal pathogens and sexually transmitted diseases (http://www.nicd.ac.sa).
- Pathogens that are a public health hazard are also flagged by the microbiology laboratory. Pathogens that can cause hospital outbreaks should be reported to infection control teams.