A new tool for antimicrobial susceptibility testing

A new susceptibility testing system should help standardise and streamline the development of new antimicrobial agents.

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One of the most significant advances in healthcare during the twentieth century must surely have been the introduction of antimicrobial therapeutic agents - but the fight to control infectious disease is a continual process, with an ever greater need for new drugs. Whilst antibiotics have enabled major advances to be made in the treatment of bacterial infections, the fight against infectious disease is being undermined by the growing incidence of antibiotic-resistant strains of bacteria.

Although pharmaceutical companies have developed a wide range of antimicrobial agents, clinicians are now experiencing greater difficulty in selecting the most appropriate treatment for infections. The emergence of multi-resistant strains of bacteria is making the choice of antibiotic more limited. The more serious examples include outbreaks of methicillin-resistant Staphylococcus aureus (MRSA) infections in hospitals, inevitably resulting in ward closures to prevent the spread of infection, and resistant Mycobacterium tuberculosis, where antibiotic choice is more limited. Even with one of the most powerful antimicrobials, vancomycin, resistant forms of bacteria have started to emerge in the US and Japan.

Resistance to most antimicrobial agents has now been encountered, and the pharmaceutical industry is constantly fighting to stay ahead of the ever-changing resistance patterns of microbial populations. Based on current experience, even with a new antimicrobial, resistant forms of micro-organism can be anticipated to arise within two years of a product’s introduction. This has led to a pessimistic vision of healthcare in the future, in which no effective antimicrobial therapy is available to fight off infectious diseases.

In response to this challenge, the pharmaceutical industry is increasing its efforts to develop new antimicrobial compounds, which may be less likely to result in the production of resistant micro-organisms. In the race to beat emerging resistance, pharmaceutical companies are looking for new sources of potential antimicrobial agents, and techniques have been developed for increasing the number of compounds for initial screening. Areas such as combinatorial chemistry and genomics enable large databases of compounds to be designed, while high-throughput screening enables large numbers of compounds to be investigated for antimicrobial activity in a short time-period - it is now possible to assay thousands of samples per day. For drug development companies, this has increased the number of drug candidates that progress from the screening stage to further down the development route.

Such an increase in the number of experimental compounds for testing and candidate drugs for clinical research has the potential to overwhelm testing laboratories and create bottlenecks in the development process. This places a greater emphasis on streamlining testing, data capture and analysis,
At present, the majority of antimicrobial susceptibility testing in the UK is still based on the Stokes method without compromising the quality of data generated. With antimicrobial agents, the potency of a compound is determined through susceptibility testing, which determines the concentration of drug required to inhibit growth of a micro-organism, indicating the level of resistance to the antimicrobial.

**Antimicrobial susceptibility testing**

At present, the majority of antimicrobial susceptibility testing in the UK is still based on the Stokes method - with *ad hoc* modifications made according to the laboratory in-house method. With the Stokes method, the diameter of a zone of inhibition produced around a disc impregnated with an antimicrobial agent on an agar plate is used to determine the level of susceptibility of a micro-organism to an antibiotic (Figure 1). However, although a popular method, it does have a number of disadvantages with regard to performing the test and correctly reading the results. These include a lack of standardisation of the methodology, with individual laboratories applying minor variations to the technique, and operator error in terms of the difficulty in determining zone diameter where the zone edge tends to be ‘fuzzy’ and also in reading zones on some types of agar medium. This lack of standardisation was identified as a problem by the UK Select Committee on Science and Technology of the House of Lords in its report ‘Resistance to Antibiotics and Other Antimicrobial Agents’ (1998).

In 1999, the British Society for Antimicrobial Chemotherapy (BSAC), with support from the Public Health Laboratory Service (PHLS), launched a campaign to standardise disc susceptibility testing. This concentrated on all aspects of the susceptibility testing procedure, from the preparation of plates through to the measurement of zones of inhibition.

Although adoption of this method would indeed standardise testing, there would still be considerable scope for variation in the results generated at the zone reading stage. This is because if laboratories use rulers or electronic callipers to assess the diameter of the zone of inhibition, the results would still be subject to operator variation in terms of interpreting the zone boundary and size. This could compromise the value of research when it comes to producing pooled epidemiological data. To address this issue and speed up the throughput of samples, camera-based automated zone readers have been introduced. These can, however, present problems when reading opaque...
plates - such as blood or chocolate agar - in which case manual and automated reading may still often have to be run side-by-side.

Electronic callipers and automated zone readers offer a considerable advantage over completely manual measurement in that the information they generate can be fed directly into report forms - thereby avoiding transcription errors. The data can also be fed directly into epidemiology software, usually running separately - possibly on the pathology LIMS. Care has to be taken to ensure that the data is matched to the correct patient since, although both approaches cater for the use of bar-codes, they are not read at the same time as zones. This means that mis-matches can still occur between patient details and test results.

**A new testing system**

To address the need for standardised testing, and overcome some of the problems associated with manual determination of zone diameter, a new system for susceptibility testing has recently been launched by Oxoid. The system - Aura Image - combines fully automated zone reading with direct feeding of the data generated into powerful epidemiology software (Figure 2).

Designed to fit comfortably onto a laboratory benchtop, the Aura Image system has a sliding drawer at the front into which the plate to be analysed is inserted. Once loaded, the image is digitised via a high-performance scanner that can cope equally effectively with translucent and opaque plates. The enhanced, digitised image is then presented on the monitor screen, complete with defined zones of inhibition calculated using pre-set, best-fit algorithms. Should the need arise, users can ‘drag and drop’ using a mouse to enlarge or reduce the diameter of a zone if the image is deemed to need manual adjustment. There is also the facility to ‘zoom in’ on an image to better interpret the edge of a zone and to check for the presence of micro-colonies.

If Oxoid discs are used, then there is no need to place these in any particular order as the system uses optical character recognition to identify the antibiotics and link them to the correct zone. Results can be compared with a choice of four databases for classification: BSAC, NCCLS, DIN interpretive guidelines and the Oxoid reference database (based on SRGA-M), with a further database available for incorporation of the user’s own breakpoints.

Patient identity, together with tabulated numerical zone data and S, I, R classifications (susceptible, intermediate and resistant) are also displayed on the monitor alongside the plate. Again, the recommended classifications can be changed manually. The complete screen data set can be stored on the hard disk for later review or archiving.

A key feature of the Aura Image system is that the bar code, which is put onto each plate at the time of inoculation, is read at the same time as the zones, making it impossible to attribute the results to the wrong patient. Reading the bar code also allows the system to work in ‘host-query’ mode and to ‘fetch’ the patient information directly from the pathology LIMS, eliminating the need to enter any ID information or analyse the plates in any set order. It takes approximately 17 seconds to read...
the bar code, retrieve the patient ID, identify the antibiotic codes, digitise the image, interpret the result and designate the micro-organism as S, I or R. The system provides a major benefit to clinical laboratories in terms of epidemiology and infection control, in that the data generated can easily be used to monitor antibiotic use and trends in susceptibility, enabling resistance patterns to be detected at an early stage (Figure 3).

The system also provides a significant benefit to pharmaceutical companies during the development of new antimicrobial agents going through both the pre-clinical and clinical phases of development. As with all drug development programmes using multi-site trials, any procedure within the test protocol that has the potential for generating a variability in results needs to be minimised. This is especially true for antimicrobial susceptibility testing, where the test procedure is known to have a high level of variability between testing laboratories and is open to operator error in the reading of results.

These variabilities can be minimised using the Aura Image system, which provides both a standardised method (conforming to BSAC recommendations) and an automated zone reader to reduce any operator error. This standardisation can be followed throughout the development chain, from early phase development right through to completion of clinical trials, minimising the possibility of differences in susceptibility patterns occurring between sites, and also between pre-clinical and clinical phase data, providing greater confidence in the data generated.

The epidemiology software in the Aura Image system provides a powerful tool for handling the storage and manipulation of data. The system accumulates data to generate a database of susceptibility from single-site and multi-site generated testing. As the database builds, susceptibility profiles of new antimicrobials against different micro-organisms can be generated to give a fuller picture of the spectrum of activity of the drug. The database can then be used to provide early information on the level of resistance within a population, so that any trends in susceptibility profiles or geographic areas can be highlighted early on in development (Figure 4).

The software provides the trial manager with a useful tool for co-ordinating multi-centre trials and monitoring results. The database can generate reports on susceptibility patterns generated by site, locality and region for comparison of drug performance and identifying resistant samples; these can then be further analysed by MIC testing for clarification of breakpoint and to give a more accurate picture of susceptibility patterns. The database can also help to identify any ‘hot-spots’ of resistance, or any deviations in susceptibility profiles between sites, indicating possible differences in testing regimes. Longer term, the accumulated information in the database can be used to help monitor changes in susceptibility patterns, due to increasing levels of antimicrobial resistance arising within the population.

**Conclusion**

At a time when there is a greater urgency to develop new antimicrobial agents, the Aura Image system meets the need for standardising and streamlining the testing of new drug candidates, providing powerful software for co-ordinating clinical trials, processing data and monitoring trends in antimicrobial susceptibilities. Automating the zone-reading process helps to simplify disc diffusion testing for antimicrobial susceptibility determination, minimising any errors caused by manual reading; it also increases the throughput of samples in the laboratory and reduces the possibility of the occurrence of bottlenecks. Data can be easily captured and analysed for rapid determination of susceptibility patterns and trends, and report generation - reducing the need for time-consuming data manipulation.

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