

The CDS Antimicrobial Susceptibility Test Method: fulfilling a need



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The need for both the adoption of a uniform method of antimicrobial susceptibility testing by laboratories in Australia and the alliance of the laboratories with a reference laboratory was demonstrated clearly more than 40 years ago. This review outlines how the CDS Antimicrobial Test has fulfilled this need and demonstrates the value of the association of diagnostic laboratories with a readily accessible reference laboratory in reducing errors in antimicrobial susceptibility testing in practice.

In the years 1968 to 1970 the annual RCPA Microbiology Surveys (forerunners of the RCPA Microbiology QAP) revealed very serious errors in the antibiotic susceptibility testing of common bacterial pathogens in Australian and New Zealand laboratories. The errors arose in many cases because the methods used were unexplained, unvalidated and unauthorised modifications of published methods and in other cases the methods used were *ad hoc* and developed by laboratories without any scientific basis. In 1971, 1972 and 1973 the Microbiology Surveys compared the performance of laboratories when they used non-uniform methods with that when they used a uniform method of susceptibility testing. The uniform method was an agar disc diffusion test that was developed in the Microbiology Laboratory at the Prince of Wales Hospital, Sydney, Australia. It was clearly demonstrated that technical errors in antibiotic susceptibility testing could be eliminated almost entirely if laboratories adhered strictly to this method. The method was published as the Calibrated Dichotomous Sensitivity (CDS) test in Pathology in 1975¹. The

origin of the name of the test derived from the fact that the inhibitory zone sizes observed were calibrated to quantitative antibiotic susceptibilities determined by the WHO approved method published in 1971². Dichotomous appeared in the name because the method did not attempt to divide antibiotic susceptibilities any further than the two categories of sensitive or resistant. The experience gained in the conduct of the Microbiology Surveys also produced convincing evidence that a sustained improvement in the accuracy of susceptibility testing in Australia could be achieved only by the establishment of a reference laboratory dedicated to the interaction with and the provision of support to laboratories performing antibiotic susceptibility testing. As a consequence the CDS Reference Laboratory was set up within the Microbiology Laboratories at the Prince of Wales Hospital.

The role of the CDS Reference Laboratory

Initially the CDS Reference Laboratory's role was to assist those laboratories who were having difficulties in the application of a uniform method. As the diagnostic laboratories acquired greater skill in performing the CDS test the Reference Laboratory became more interactive with the CDS users. The diagnostic laboratories would draw attention to changes in the phenotypic expression of specific mechanisms of antibiotic resistance that then prompted modifications of the test method. Some notable examples of these changes were reduction of the disc potency of benzylpenicillin to 0.5 u, erythromycin from 15 µg to 5 µg and tetracycline 30 µg to

10 µg. Some of the initiatives and modifications remain unique to the CDS while others have been adopted by some overseas methods. The CDS Reference Laboratory also maintains and distributes, free of charge, the reference strains necessary for quality assurance in susceptibility testing. Every year for the past 30 years the staff of the CDS Reference Laboratory have conducted a workshop at the annual scientific meeting of the Australian Society for Microbiology. The workshops also are free of charge, open to all and are not restricted to CDS users. They provide an excellent interface between Reference Laboratory staff and those engaged in or interested in antibiotic susceptibility testing. In more recent times the Reference Laboratory has been called on to clarify or confirm the identity of an unusual organism or clarify atypical antibiotic susceptibility test results. The latter may also involve confirming the identification of organisms. Because of its ready access to molecular and a wide range of phenotypic techniques the Reference Laboratory is able to respond in a timely fashion to these requests. The laboratory averages at least 10 enquiries a week including telephone and email communication from CDS users.

Development of the CDS Test

When the method was introduced it was used to test only a restricted number of antibiotics against common bacterial pathogens. Major updates of the CDS method were published in 1984³ and 1988⁴, and in subsequent years the scope of the CDS test has been expanded to now include 63 antibiotics and 18 microbial genera including anaerobes and yeasts. Participation in what has become known as the CDS Users Group was broadened to include a number of overseas laboratories and Australian veterinary laboratories. In 1999 the first edition of Antibiotic Susceptibility Testing by the CDS Method – A Laboratory Manual was published and provided free to registered CDS users. The Manual is now in its 6th edition and the 7th edition is in preparation. In 1990 the CDS website (<http://web.med.unsw.edu.au/cdstest/>) was set up at the University of New South Wales. Developments in the CDS are reported regularly on the website and both web-based and downloadable pdf versions of the CDS manual are updated to include additions and modifications to the method. Access to the CDS website with all its resources and updates is available to all interested parties free of charge.

CDS innovations

Selection of disc potencies. The disc potencies in the CDS were selected on the basis of those that yielded an optimal separation of resistant and susceptible strains and followed the well-established principles of diffusion of antibiotics in agar enunciated by Humphrey and Lightbown⁵. Often the choice was at odds with that suggested by pharmaceutical firms promoting the antibiotic who

claimed (incorrectly) that their drug would be put at a marketing disadvantage if higher potency discs were not used in susceptibility testing. The pharmaceutical companies had a much greater influence on the developers of other methods of calibrated disc tests (such as NCCLS [now CLSI]) than they did on the CDS. The consequences of this difference were best illustrated by the calibration of cefotaxime, the first of the 3rd generation of cephalosporins, which was calibrated in the CDS using a 5 µg disc in preference to a 30 µg disc in 1984. As a result the CDS clearly demonstrated mechanisms of resistance such as elaboration of extended spectrum beta-lactamases (ESBL) well before the then NCCLS (now CLSI) method. It is worth noting that both EUCAST and CLSI have only recently adopted the breakpoints for these antibiotics first proposed by CDS some 30 years ago.

Inhibitory zone morphology. One unique feature developed by the CDS is the observation of inhibitory zone morphology either to confirm the identity of an isolate or to establish the mechanism of resistance. Previously the only use of zone morphology was the well-established practice of examination of the edge of the inhibitory zone with *Staphylococcus aureus* to detect resistance mediated by β-lactamase. The CDS method extended the close examination of zones to other Gram positives such as enterococci and to Gram negatives in identification and to detect a number of β-lactamases. These phenomena are shown in detail in photographs published on the CDS website and in the CDS manual. Feedback from CDS users indicates that the use of zone morphology is an essential tool in defining antibiotic susceptibility in many of the Gram-negative species in day-to-day testing.

Accuracy of the CDS Test in practice

The CDS team regularly reviews performance of laboratories that use the CDS method in the RCPA/QAP programs. Where susceptibility or resistance is readily demonstrated there is little difference in the results observed with those using the CDS method and other techniques including CLSI and automated systems such as Vitek. The errors with all methods are few and usually are clerical in nature. In the more difficult tests of antimicrobial susceptibility users of the CDS invariably outperform those who use other methods of susceptibility testing. Four recent examples of superiority of the CDS in the RCPA/QAP Surveys are shown here (the year, survey and question numbers, in italics, are included in the brackets showing the results): (i) detection of inducible clindamycin resistance in *Strep. pyogenes* (CDS 96% correct, other methods 78% correct, 2012, 8: 3); (ii) detection of vancomycin resistance in *Enterococcus faecium* (CDS 92% correct; other methods 73% correct, 2010, 1: 4); (iii) demonstrated resistance to cephalosporins mediated by an

ESBL (CDS 100% and others 82%, 2011, 6:1b); and (iv) detection of meropenem resistance in *Citrobacter freundii* mediated by a carbapenemase (CDS method 95%, other methods 71%, 2013, 4:1b).

The future of the CDS

Registrants as CDS users continue to grow and number over 200 at present. The CDS method is now being used by laboratories in South East Asia, India and South Africa. It is unfortunate that a number of Australian public laboratories have changed from using the CDS method to other methods as a result of executive decisions apparently based on reasons other than scientific merit. As long as antimicrobial susceptibility testing is performed in diagnostic laboratories the CDS will continue to provide a service to Australasian and a number of overseas laboratories. The original CDS team has been joined by younger scientific and medical staff who will carry on the tradition of supporting a high-performance national antibiotic susceptibility test method well into the future.

Acknowledgements

We acknowledge the Board of Education of the RCPA for its support of the development of a uniform method of susceptibility testing in Australia, the staff of SEALS Microbiology, many of whom have made significant contributions to the development of the CDS, The Australian Society for Microbiology (ASM) in supporting the Annual

CDS Workshops and finally the CDS users whose feedback has led to so many improvements and enhancements of the CDS.

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Vancomycin-resistant enterococci in hospitals



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Control measures for vancomycin-resistant enterococci (VRE) should be determined by the current epidemiology of infection and must be practical and effective. It is essential that emphasis is placed on consistent implementation of enhanced standard precautions (horizontal measures) in healthcare that reduce infections caused by all organisms,

not just VRE. Effective antimicrobial stewardship programs are paramount and should target reduction in the use of extended-spectrum cephalosporins, carbapenems and fluoroquinolones. VRE causes marked morbidity in a limited range of at-risk patient groups who require additional active measures to prevent their acquisition of virulent strains. The use of additional measures for patients at low risk from VRE morbidity is unlikely to be cost-effective and should be reserved for outbreak situations or for patients who are more likely to transmit VRE.

Epidemiology

Infectious agent and clinical significance

Enterococci are Gram-positive cocci that colonise the intestinal tract of humans and animals. They can persist on inanimate objects for weeks, have intrinsic resistance to many antibiotics and a capacity to develop multiresistance. Generally they have low virulence.