The evolution of in-vitro antimicrobial susceptibility techniques

Antimicrobial susceptibility testing (AST) is performed by laboratories every day on clinical isolates often with new antimicrobial agents. The techniques employed are often taken for granted and are frequently abused. This review traces the evolution of the generally used routine methods.

The first recorded observations of antibiosis in vitro were made by van Leeuwenhoek in 1676 (Balows, 1974). At a presentation to the Royal Society of London he described how his ‘living animalcules’ disappeared after he placed them in some ‘pepper water’. Agar diffusion methods were first utilized by Beijerinck in 1889 (Dufrenoy, 1947), for studying the effect of different auxins on bacterial growth. They have only been generally used for AST following the discovery of penicillin, and varying diffusion sources of the antibacterial agent have been used. These include the ‘ditch’ plate (Fleming, 1924); cutting wells into the agar (Reddish, 1929); and the cylinder-plate synonymous with ‘Oxford cup’, ‘fish spine’, or ‘Heatley cup’ (Abraham et al., 1941).

The use of absorbent paper as a means of carrying antibacterial solutions was first suggested by Pope in 1940 (Heatley, 1944). Filter paper discs incorporating penicillin were utilized by Vincent & Vincent (1944) for assaying this newly discovered compound. Mohs (1945) described a ‘radial streak’ technique with 15-mm diameter discs and interpretation of results for the first time was judged by comparison with a sensitive control. Further paper disc diffusion methods were then described (Copeland, 1945; Morley, 1945; Kokko, 1947; Kolmer, 1947). Hoyt & Levine (1947) described a technique using tablets incorporating penicillin instead of impregnated filter paper. The filter paper disc that is commonly utilized today has a diameter of 6.5 mm, and was first described by Bondi et al. (1947).

It was soon apparent to the early groups describing diffusion methods, that there was a need for standardization of methodology. This was achieved for single disc diffusion techniques by Bauer, Kirby and colleagues (Bauer et al., 1966) in the USA. The Bauer-Kirby method provided important advances in methodological standardization and zone diameter interpretation against a quantitative minimum inhibitory concentration (MIC) value. Earlier, workers in the UK had recognized the need for standardized susceptibility techniques (Gould & Bowie, 1952). Gould & Bowie devised a disc diffusion technique which was based on control organisms producing differing zone diameters with filter paper discs of varying concentrations of the antimicrobial agent. A single concentration disc was then tested against clinical isolates and the sensitivity determined by comparison to zone diameters produced by the control strains. Stokes (1955) described a disc diffusion technique whereby interpretation of zone sizes of test and control organisms was by comparison on the same plate. Variations of Stokes’ original technique are commonly used in British laboratories today.

The World Health Organization (WHO, 1961) and groups in other countries have also reported standardized AST methods, for example, in the UK (Stokes & Waterworth, 1972), in Sweden (Swedish Reference Group, 1981) and in Germany (Deutsches Institut für Normung, 1984). The results of a ten-year
International Collaborative Study (ICS) sponsored by WHO into AST were reported in 1971 (Ericsson & Sherris, 1971). The ICS disc diffusion method was largely based on that originally described by Ericsson and colleagues (Ericsson, Hogman & Wickman, 1954; Ericsson & Svartz-Malmberg, 1959). In the USA an approved standard for disc diffusion AST was published by the National Committee for Clinical Laboratory Standards (NCCLS, 1975), and this is updated every three years. Studies demonstrated no significant differences in error rates when a Stokes' disc diffusion technique was compared with a Bauer-Kirby method in national quality control assessments (Snell, Brown & Gardner, 1982; Snell, Brown & Gardner, 1984).

Dilution of antimicrobial agents in liquid medium was first suggested by Fleming (1929). The broth-dilution method usually relies on the antimicrobial agent being diluted in suitable medium in serial or two-fold dilutions. The test organism is then added and after incubation the end point assessed by visible inspection for growth or absence of growth. However, Fleming (1942) described a method where the end-point was assessed by a pH change. Descriptions of further broth-dilution techniques were later published (Rammelkamp & Maxon, 1942; Schmidt & Sesler, 1943; Spink & Ferris, 1945; Buggs et al., 1946). Determination of MIC values by the broth-dilution technique is time consuming, costly and technically demanding (Pykett, 1978). Recently dehydrated antimicrobial agents in microtitration trays have been produced (Philips, Warren & Waterworth, 1978). Standardized microdilution test procedures have been described (Thomsberry, Gavan & Gerlach, 1977). The microdilution technique has also been modified to incorporate fluorogenic growth substrates for detection of growth after 4-6 h incubation (Doern, 1987; Doern et al., 1987). Other rapid antimicrobial susceptibility techniques rely on optical density change for their end-point determination (Thomsberry, 1984). Other indicators of end-point have been described (Spencer & Wheat, 1986).

Determination of MIC values for sulphapyridine against gonococci was the first description of an agar dilution AST method (Schmith & Reymann, 1940). Frisk (1945) incorporated penicillin into agar when testing the susceptibility of strains of Streptococcus pneumoniae. Frank, Wilcox & Finland (1950) utilized agar dilution for numerous antibiotic/organism combinations. However, performing these quantitative MIC estimations by agar dilution was found to be time-consuming and cumbersome for routine use. The procedure was simplified by the introduction of multiple replica devices (Garrett, 1946; Steers, Foltz & Graves, 1959) and by increasing the size of the dilution steps, from serial or two-fold dilutions to one or several critical concentrations which separated organisms into resistant or sensitive categories (Steers et al., 1959; Tolhurst, Buckle & Williams, 1963; Haltalin, Markley & Woodman, 1973). This method is now commonly referred to as the breakpoint technique. Another factor that has facilitated the general introduction of agar dilution techniques in laboratory practice has been the availability of convenient sources of antimicrobial agents of known potency; firstly they appeared as filter paper strips (Rolinson & Russell, 1972); then as filter paper pads (Shafi, 1975) and finally as tablets (Snell, Danvers & Gardner, 1984). The choice of which breakpoint concentration to test is critical and is a very contentious issue; various regulative and advisory bodies have produced recommended breakpoint concentrations (NCCLS, 1985; European Committee for Clinical Laboratory Standards (ECCLS), 1985; Working Party of British Society for Antimicrobial Chemotherapy, 1988).

Variables affecting AST methods using disc diffusion or dilution methods have been comprehensively studied by many groups (Heatley, 1949; Erlanson, 1951; Waterworth, 1951; Gould & Bowie, 1952; Anderson & Troyanosky, 1960; Ericsson & Sherris, 1971; Stokes & Waterworth, 1972; NCCLS, 1975; WHO, 1977; ECCLS, 1985; NCCLS, 1985). There have also been studies comparing agar diffusion with dilution techniques (Jackson & Finland, 1951; Steers et al., 1960). These showed that if the two techniques are performed correctly, similar results are obtained.

Laboratories performing in-vitro antimicrobial susceptibility tests must utilize a standardized and reproducible technique. The in-vitro result should then enable the prescription of rational antibacterial therapy. However, an in-vitro susceptibility result can only be regarded as a useful indication to an in-vivo response and not as a definitive answer.

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