The problem of antibiotic resistance emerging from the use and misuse of antibiotics in veterinary practice was considered as long ago as the 1960s by the famous Swann Committee. What seems to be less well understood, and certainly less well documented, is the use of antimicrobials in the world of plant science. Antibiotics have been widely used in the control of contamination of micropropagation and plant tissue culture for 40 years or so and for a similar length of time in the topical treatment of bacterial diseases of fruit trees. It is probably time to bring this to the attention of medical microbiologists.

The first symposium to consider the problem of bacterial contamination and its control in plant tissue culture was held in Cork, Ireland, in 1987. The concept of propagation, on agar, of plant tissues from fast growing tissue such as apical meristems has been well established for many decades. In the same way as virologists incorporate antimicrobials into their tissue culture media to control bacterial and fungal growth, so plant propagators have included such agents in their agar media. What is relatively new, however, is the massive scale on which plant material is now propagated and the consequent financial importance of the industry in the supply of plants for house, garden and agriculture. When plants are propagated in their hundreds of thousands, the prospect of bacterial contamination of even a small proportion is clearly costly.

Plant tissue is normally prepared for propagation aseptically with sterile instruments in a clean air environment. The plant material is usually decontaminated with hypochlorite or another suitable ‘antiseptic’. The tissue is placed on a special medium (usually that developed by Murashige & Skoog, of which there are now many variations) and as it grows and develops it is transplanted on to agars of differing composition until the rooted plant is suitable for hardening off and planting in compost.

A II reports of the prophylactic use of antimicrobials by incorporating them in the culture media are prefaced by a statement to the effect that antibiotics should not replace sound aseptic technique. The problem is that the use of antibiotics in this way certainly conflicts with the normal principles of prophylactic use, in that the ‘pathogen’ is unknown and of uncertain susceptibility and the period of administration is prolonged. Plant tissue culture media are fairly hostile, at least to bacteria, particularly because of the high sugar concentration of these media. Under these circumstances the bacteria are reluctant to grow or metabolize and are, therefore, largely resistant to antibiotics; it is likely, therefore, that bacteria will remain as persisters for the duration of the phase of culture. It is well recognized that contamination can become evident during later growth phases in less hostile media, for instance where sugar concentrations are lower. Persistence is also likely to be an explanation for this covert contamination. The other unknown effect is that of the medium itself on the activity of the antibiotic. Plant tissue culture media are complex mixtures of a wide range of compounds including minerals, amino acids, plant hormones and sugars. It is easy to see how the antibiotic might not function under these conditions.

There are several possible sources of contaminating organisms. If the plant material was not adequately decontaminated it may carry plant-associated organisms from the field or organisms of animal or human origin from compost or manure. A nother possibility is the emergence of human-associated organisms derived from the staff who undertake the propagation: breakdown of asepsis is more likely to occur at certain times of the day, such as around break times. Coagulase-negative staphylococci, diphtheroids and other microorganisms from the skin or respiratory tract may appear as contaminants at a later stage.

The other consideration is the possibility of toxic effects...
of the antibiotics on the plant tissue. Reports of problems of phytotoxicity are numerous; it has been associated with most antimicrobials, including aminoglycosides and tetracyclines, but reports relate only to specific hosts. The antibiotics least likely to be phytotoxic are those acting at sites such as the bacterial cell wall rather than those which act on the ribosomes or DNA. Mycoplasmas and possibly L-forms may cause significant contamination, so β-lactams are not the whole answer.

At the first symposium in Cork, the opening paper was a thoughtful review of the problems; it was designed to encourage the delegates to consider the possible role of commensal organisms and the relationship between plant and pathogen, commensal or contaminant. There was a discussion of the difficulties of using antibiotics in this way and reviews of technology available for the identification of bacterial contaminants. Many papers described the use of antibiotics in tissue culture and in discussion there were anecdotal reports of very large but unspecified quantities of third-generation cephalosporins being used commercially.

Since that meeting, many of the problems have been addressed so it was interesting to attend the second symposium, again held in Cork, in September 1996. It was notable how, in the intervening years, a more rational approach to the use of antibiotics had been adopted and how there had been a move away from ‘prophylaxis’ towards treatment of tissue before propagation. This ‘treatment’ involves immersing the tissue in an ‘appropriate’ antibiotic following confirmation of the presence of a likely pathogen.

There was also a greater awareness that contaminants might not be harmful: two delegates reported beneficial effects of Bacillus subtilis in protecting against further bacterial or fungal contamination. Also of interest were reports from Eastern Europe and North Africa. Many papers described the use of antibiotics in tissue culture and in discussion there were anecdotal reports of very large but unspecified quantities of third-generation cephalosporins being used commercially.

Administration by injection of oxytetracycline to fruiting and ornamental palm trees has been used successfully for some time in Florida. One feels that this is perhaps an encouraging development if the fruit are not loaded with oxytetracycline as a consequence. It is fair to point out, though, that monitoring fruit for streptomycin residues since the 1950s has not yet revealed detectable levels. No antibiotics are permitted for these purposes in the UK or, as far as I can tell, in Europe.

While a more logical approach seems to have been adopted in micropropagation and plant tissue culture, the use of antibiotics in the plant world should remain under scrutiny. It must be a matter for concern that antibiotics such as oxytetracycline are in apparently carefree and widespread use because, while the amounts used in this way are small compared with the total amount of antibiotics used in clinical and veterinary practice in the USA (30 million pounds weight), the mode of application is likely to have consequences out of proportion to the quantities used. While tetracyclines may not be in widespread clinical use, resistance to tetracyclines is often closely tied in with resistance to other antibiotics. Streptomycin selects resistant bacteria which are resistant to other antibiotics and there is some measure of cross-resistance to other aminoglycosides. The relative ease with which certain important human pathogens may become dependent on streptomycin is also a matter for concern. It should not go unnoticed that a decrease in the clinical use of streptomycin and tetracycline in Denmark has corresponded with the decline of one strain of methicillin-resistant Staphylococcus aureus which was also resistant to these two drugs.

Plants and humans do not generally share pathogens and there seems to be no term analogous to ‘zoonosis’ for an infection acquired from a plant. However, organisms which are of clinical importance, such as Burkholderia cepacia (a pathogen of onions), are deemed ‘environmental’ and many more are closely related to our hospital pathogens, e.g. other pseudomonads, Xanthomonas and Erwinia spp., so transfer of resistance can be readily envisaged. Perhaps a renewed interest might be shown in pot plants brought in to patients, as a potential source of human pathogens or a source of transmissible resistance, since many of these will be from micropropagated stock.

As a final thought, one can only hope that tractor drivers on fruit farms in the USA are adequately protected and that those who like to talk to their trees are not unduly disappointed if, through the auditory damage of streptomycin, they fail to hear any response!

Acknowledgements

I would like to thank my various colleagues who read the manuscript at various stages and Professor Alan Cassells.
of University College, Cork who stimulated my interest in the subject.

References


Clinical trials of antibacterial agents—a commentary

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Clinical trials are difficult to perform but are an essential prerequisite to establishing the efficacy and safety of a treatment intervention. They have an important role in protecting the public against ineffective or harmful therapies and are extensively used by the pharmaceutical industry in support of licensing applications of new drugs.

It is 8 years since the BSAC Working Party on Clinical Trials of Antibacterial Agents published its recommendations in this journal. This document provided an acknowledged stimulus for the Infectious Diseases Society of America (IDSA) to revise earlier outdated general guidance on trial design and to produce disease-specific guidelines for use in the development of anti-infective agents. This initiative was sponsored by the USA Food and Drug Administration (FDA). Contemporaneously, the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) established a working party with a similar brief which agreed to harmonize its recommendations in line with the IDSA documents. This working party added other disease-specific guidelines such as those concerned with trials of drugs for treating human immunodeficiency virus infection. In both the USA and Europe these guidelines have no official status, although they are used extensively by regulators, the pharmaceutical industry, trialists and even ethics committees. Thus, within the space of a few years there has been much debate and published guidance on the design and conduct of clinical trials of anti-infective drugs.

It is important to stress that these guidelines were never intended to be the final statement. In particular it is interesting to note that the statistical sections of the IDSA document generated considerable controversy during their development and hence it is no surprise that the report of the Statisticians in the Pharmaceutical Industry (PSI) Working Party, published in this edition of the Journal, has been produced. The report is a welcome addition to existing guidelines and extends, in a more pragmatic and complete manner, the statistical advice provided in earlier guidelines. However, in our view this document is unlikely to be the final word, in an area of therapeutics that is undergoing rapid change.

Clinical trials of anti-infective agents present a number of particular challenges, as a result of the intrinsic dynamics between the host, infecting organism and any therapeutic intervention. Studies of the treatment of site- or disease-associated infections usually include a heterogeneous population. For example, a common therapeutic target, such as community-acquired pneumonia (CAP), includes illnesses caused by many different pathogens which vary seasonally, by underlying disease, and by virulence so that disease outcome is highly variable. Furthermore, although the management of CAP may take place within the community or in hospital, the criteria for admission to hospital are poorly standardized and are likely to differ between the centres where trials are conducted and, in turn, from the broader community where the antibiotic will be prescribed, once licensed.

Endpoints for clinical trials rightly focus on clinical efficacy and microbial eradication. These can be measured at various time points, e.g. after 7, 10 or 14 days’ therapy for many common infections, but up to 28–42 days post-therapy in the case of urinary tract infections. Outcomes such as the risk of relapse or superinfection and the rate of return to baseline health are rarely incorporated as meaningful endpoints in trial design.

It is also worth noting that placebo recipients show a high rate of spontaneous clinical cure for some infections. For example, in some patients pathogenic bacteria are eliminated from the middle ear by host defences alone, others with acute bacterial otitis media have persistent symptoms despite effective antibacterial therapy, while some improve despite the inability of a drug to eliminate the offending pathogen. Thus an antibiotic with modest antibacterial activity will appear to be almost as clinically effective as a more microbiologically active agent—the so-called ‘Pollyanna phenomenon’.

The advice captured in the PSI report is largely helpful and pragmatic for those undertaking clinical trials, particularly with regard to the way in which data might be...
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The maintenance of a log of patients eligible for trial entry is stressed. However, we have some concern that the ‘per protocol’ analysis is emphasized as the primary analysis rather than the more conventional intention-to-treat analysis. Other analyses may be appropriate; these should be defined in the protocol in advance of the trial which should be sufficiently powered to accommodate additional analyses. Furthermore, any subset analysis should show consistency with the primary analysis. It is of interest to note that the report contains no discussion of the Bayesian approach to statistical analysis, despite its current popularity.

One concern is the emphasis the report places on the impact of ‘resistant’ pathogens on disease outcome. It is unfortunately too common that breakpoints indicating susceptibility and resistance are set in advance of clinical evidence of efficacy. It should be remembered that breakpoints are a synthesis of information based on in-vitro susceptibility and pharmacokinetic information, with appropriate adjustment where concentration-dependent safety concerns exist. Ideally, clinically relevant breakpoints should be decided once there is sufficient experience in treating defined infections with organisms requiring known inhibitory concentrations of drug, including those that might be considered of ‘marginal’ susceptibility. It would, therefore, appear inappropriate to exclude all patients with resistant pathogens from any evaluation, as suggested in the report. Regulators, microbiologists, prescribers and pharmaceutical companies all have interests in the results of treatment of less sensitive pathogens.

A further issue is the increasing recognition of the importance of pharmacodynamic studies in supporting proposed dosing regimens. These complement conventional pharmacokinetic information. Likewise it may be shown that site-specific data derived from studies of tissue penetration and intracellular disposition can also predict drug performance. Some of this information may not emerge until well beyond licensing. It is important, therefore, that statistical robustness should not negate any requirement for further evaluation of new agents once licensing has been achieved.

The Working Party supports the recruitment of patients who have failed other therapies, providing there is clear clinical and microbiological evidence of the infecting organism. This is welcome and endorses guidance provided in the IDSA and ESCMID documents.2,3 Another area in which the Working Party’s statements are to be applauded is the issue of confounding events. Within clinical medicine and, inevitably, within clinical trials confounding events arise which may prevent the full therapeutic potential of an intervention, be it favourable or otherwise, from being assessed in relation to outcome. This includes issues such as inappropriate antimicrobial therapy, inadequate medical or surgical management, underlying or unsuspected complicating disease, and the use of ‘do not resuscitate’ decisions at a time when the outcome remains unclear. These issues cannot be anticipated at the time of patient recruitment. The matter of confounding events has been emphasized recently in trials of therapeutic interventions in sepsis syndrome which have highlighted the importance of such events on trial analysis.7 This issue deserves wider discussion and a decision about the extent to which confounding events might be anticipated within a particular trial setting. Provided the sample size is adequate to accommodate them, the robustness of a particular trial might then be preserved.

The recommendation that patients with infections at more than one site be permitted entry into studies of one particular type of infection is pragmatic and particularly relevant to studies of intra-abdominal sepsis and of infections in intensive care unit patients. In contrast, the PSI Working Party appears to have avoided one other particular challenge, namely clinical trials in patients with neutropenic sepsis. This is unfortunate since it is one indication in which there are a large number of additional variables such as the nature of the underlying disease, the impact and recovery from cytotoxic therapy, infrequent microbiological confirmation of infection and the frequent use of systemically active agents for chemoprophylaxis. Efficacy in neutropenic sepsis is important for any new anti-infective drug since it often endorses the use of a new compound in other life-threatening infections.

One final area that deserves comment relates to the evaluation of drug safety. This is rarely the primary focus of any clinical trial and while information may be gathered both actively and passively on the spectrum of adverse events, trials are rarely designed to capture them in a statistically robust manner. The data gathered are often presented by body system and contrasted with data collected from patients receiving the comparator agent(s). However, information on the latter is not sufficiently standardized and may vary according to the target infection, its severity and host factors. Here again, no amount of statistical purity can compensate for poor quality information.

In conclusion, the science and practice of clinical trials of anti-infective drugs has made considerable progress in the past decade as a result of a number of thoughtful documents and guidelines. These have clearly influenced trial design and data submission in support of licensing applications of new drugs for the better. This process of evolution is still far from complete but, none the less, the PSI Working Party report is a welcome addition to this process.

References


