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LETTER TO THE EDITOR

Challenge tests for antimicrobial agents

In a communication in this issue Richards (1975) has reported on the antipseudomonal activity of contact lens solutions. His data on the efficiency of certain contact lens solutions in killing *Pseudomonas aeruginosa* are in marked contrast to those reported by our colleagues and ourselves in a recent report (Norton, Davies & others, 1974). Richards attributes this difference to the fact that we filtered off and washed the cell suspensions, used as sources of inocula, with a minimal salts medium, while he used an inoculum of a broth culture. This difference in antipseudomonal activity existed not only with commercially available solutions but also in solutions containing benzalkonium chloride 0·004% and EDTA 0·1% prepared in the different laboratories. The reported difference is large in four of the six preparations tested, Richards finding growth after 4 h contact while we found no growth after 15 min contact.

To test whether this difference was due to the harvesting procedure as Richards suggests, we have repeated an evaluation of two solutions of benzalkonium chloride 0·004%, using the growth and preparation techniques of Richards & McBride (1971) and of Norton & others (1974). One solution, containing benzalkonium chloride 0·004%, unbuffered, had a pH of 4·6, the other was buffered to pH 7·2.

In each case the inoculum was about 5×10^6 organisms ml^{-1} of *Pseudomonas aeruginosa* NCTC 6750 and recovery and growth conditions were as described by Norton & others (1974). The results are given in Table 1 and it can be clearly seen that as tested in our laboratory the differences in efficiency are not greatly altered by the growth conditions and harvesting procedure. It appears therefore that factors other than those claimed by Richards are responsible for the reported differences in antipseudomonal activity.

The communication however, does raise an important issue that is relevant to a wider range of preparations, in that clear guidelines need to be given on the method of assessment of antimicrobial efficiency so that different laboratories may produce comparable results when testing the same product. Kelsey & Maurer (1974) have framed similar requirements for a detailed protocol in devising their improved test for the evaluation of disinfectants. Furthermore the severity of the challenge test to be carried out should be related to the harmful consequence to patients of utilizing a product that has failed to kill microbial contamination.

For the experimental technique of carrying out official challenge tests, the following parameters, in our opinion, must be stated:—

1. the organisms to be used, their Type Culture reference number and the conditions and time of growth;
2. the methods of harvesting the cells and obtaining populations of known viability;
3. the volume of test solution to be used and the size of the bacterial inoculum to be added;
4. the temperature of holding the experimental solutions and the frequency of sampling;
5. the recovery medium to be used and the temperature and period of incubation;
6. the criterion of antimicrobial activity to be considered acceptable.

Variation in any of these conditions will markedly affect the results obtained, yet at present the B.P. stipulates none of these points, stating simply that bactericides for Injections "should be capable of sterilising the injection within three hours of inoculation with one million vegetative bacterial cells per ml". The B.P.C. has no test for

Table 1. *Antipseudomonal activity of 0·004% benzalkonium chloride.*

Treatment	pH of solution	Contact time (h) 0	¼	½	1	
Overnight culture + Dilutions in TSB	4·6	++	++	++	−−	all—ve at 2, 6 and 24 h
Overnight culture + Dilutions in M9	4·6	++	+−	−−	−−	
Overnight culture + Dilutions in TSB	7·2	++	++	++	−−	
Overnight culture + Dilutions in M9	7·2	++	++	−−	−−	

Initial viability level in test solution = $5{\cdot}4 \times 10^6$ organisms $ml.^{-1}$
TSB = Trypton Soya Broth. M9 = Minimal salts medium.
+ = Growth. — = No growth.

bactericides used in Injections or Eye Drops but it does state those that are acceptable for use and gives the recommended concentrations.

For the different dosage forms containing antimicrobial agents then the acceptable performance to be expected when coping with a fully described challenge test should be stated for the guidance of manufacturers and the safety of the public. The B.P. does state the expected efficiency of bactericides in Injections but no guidance for other preserved preparations exist. It would seem to be unreasonable to expect the same antimicrobial efficiency for the different types of preparations as the danger from using a contaminated injection will be much greater than from using, for example, a contaminated wetting solution for contact lenses. It would seem more sensible for the challenge test to be based on a knowledge of the danger to the patient consequent on the use of a contaminated solution. A knowledge of the contamination levels that might reasonably be expected in normal use would also appear to be necessary before designing challenge tests. It could be argued that sufficient information is already available for ophthalmic preparations, but if it is intended to bring contact lens solutions under the Medicines Act then more information is needed before relevant challenge tests can be designed. To this end we would recommend that a survey into the incidence of eye infections amongst contact lens wearers should be carried out.

Official compendia in different parts of the world are, quite rightly, autonomous. It would seem sensible however to see if a measure of agreement exists, in different countries, on the level of antimicrobial activity to be expected from different medicinal forms and how this might best be tested. The present situation is unsatisfactory both from the manufacturers and health authorities points of view and decisions regarding these issues should be made. A conference attended by experts from different countries would seem to be the most logical way to achieve a measure of conformity, so that differences of the order reported by Richards and ourselves might no longer appear.

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