TESTING OF PRESERVATIVES FOR PHARMACEUTICAL PREPARATIONS

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A number of pharmacopoeias suggest preservative testing procedures for pharmaceuticals involving inoculation of organisms into preserved product and measurement of subsequent loss of viability. In our investigations we have studied aspects of preservative testing known to affect results of such tests and their reliability in predicting adequate preservation of manufactured products. These include methods for maintenance, cultivation and preparation of test organisms and variations in resistance of strains from different origins.

The BP 1980 test states that 'several subcultures may be needed after revival before the organism is in its optimal state'. This has been demonstrated for some laboratory strains by Hobbs et al (1979). In this paper effects of successive subculture on resistance of an Enterobacter cloacae strain isolated from a nutritional pharmaceutical product compared with that of a laboratory strain NCTC 5920 to parabens, chlorhexidine acetate and bronopol are described.

Cells taken directly from contaminated product and from a broth culture of laboratory strain were subcultured successively on tryptone-soy agar (TSA), incubating at 30°C for 20-24 hours. The sensitivity of the first four subcultures was measured by collecting cells on 0.45 μm membranes, washing, resuspending in 0.1% peptone water, and inoculating 0.1 ml of suspension (diluted to 10^8 cells ml^{-1}) into 9.9 ml of antibacterial solution at 23°C. Samples (1 ml) were withdrawn at intervals, inactivated in medium containing tween 80 and lecithin for chlorhexidine and parabens with added thioglycollate for bronopol and surface-viable counts performed on TSA using peptone water as diluent. Plates were incubated at 30°C for 24 hours, counted and survivor curves drawn.

Cells isolated from the contaminated product showed equivalent resistance from the first to the fourth subculture. For the laboratory strain primary subcultures showed greater resistance to parabens and bronopol than second and subsequent subcultures which were of equivalent sensitivity. From these results it seems reasonable to suggest that, where TSA is used as culture media (as in the BP 1980 test), two subcultures are required to ensure a consistent response in preservative tests. This conclusion is supported by similar experiments using a waterborne strain of Pseudomonas aeruginosa from a pharmaceutical manufacturing area and a laboratory strain NCTC 6750.

It is recognised that, of necessity, preservative tests are artificial and even where isolates rather than laboratory strains are used, these are cultivated on laboratory media and may be subcultured many times before use. In further experiments samples of contaminated pharmaceutical product and the laboratory strain of Ent. cloacae were inoculated and grown in fresh unpreserved product at 25°C for 17½ hours to give 10^8 cells ml^{-1}. For preservative testing, 0.2 ml of freshly contaminated product was added to 19.8 ml chlorhexidine solution at 23°C. The product strain (primary subculture) and laboratory strain (secondary subculture) grown on TSA were tested in an equivalent system.

Using product strain grown in a fresh sample of product, chlorhexidine (0.002% solution) produced 10^3 reduction in cell numbers in 6 hours but cells regrew within 24 hours and produced cell numbers greater than the original inoculum size within 5 days. Attempts to reproduce this preservative failure by cultivation of laboratory strain in unpreserved product or the product or laboratory strain grown on TSA were unsuccessful; although there was some difference in resistance over the initial period, chlorhexidine at the some concentration produced no detectable survivors within 6 hours and no regrowth thereafter in all three systems. Results suggest that product isolates cultivated under conditions relating to the product may be used to obtain a more reliable indication of preservative efficiency.

Hobbs, R.J. et al (1979) J.Pharm.Pharmac. 31:58P

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