A NEW APPROACH TO BACTERIAL MUTAGENICITY TESTS OF β-LACTAM ANTIBIOTICS

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Bacterial mutagenicity screening of antibiotics is often of limited value as test concentrations are usually restricted to very low levels. However, marketing authorities in the UK and Europe are proposing that all new medicinal products should be screened for bacterial mutagenicity.

The test strains used in mutagenicity screening e.g. Ames Salmonella strains (McCann et al 1975), are sensitive to most β-lactam antibiotics (mic's ~5mg per ml). However, these compounds will only kill growing cells laying down new cell walls. Therefore it is possible to test high β-lactam concentrations for DNA mutation by exposing the test strains in buffer or unsupplemented minimal media followed by mutant selection (Paes and Tweats 1980).

Although these conditions can be achieved in liquid, the cells of the test strains have to be removed from contact with the test β-lactam by filtration followed by resuspension in selective media. As an alternative more convenient method, a test was devised in which the test strains, held on Millipore membrane filters, were exposed to plates containing high concentrations of antibiotic in agar.

The test strains were grown overnight in fully supplemented Davis and Mingioli (DM) minimal media. The cells were washed twice with and diluted to 10^{-2} in unsupplemented DM salts media. One ml of each suspension was filtered through a black Millipore membrane (pore size 0.45μm, diameter 25mm). Three replicate filters were placed on unsupplemented DM minimal agar plates and incubated for 1.5 hours at 37°C to starve the cells. The filters were transferred to plates of the same media containing various concentrations of test agent. These were then incubated for two hours at 37°C. The filters were then transferred to selective DM minimal agar plates containing glucose, relevant supplements and for the Ames strains, a trace of histidine. These plates were incubated for three days at 37°C before counting revertant colonies on each membrane filter. Exposure to known mutagens was carried out using the same procedure.

Using this method β-lactams have been screened at concentrations up to 1000 ug per ml witnout any evidence of lethality. Furthermore, concentrations of potent mutagens such as N-methyl-N-nitro-N-nitrosoguanidine have been successfully detected at 1 ug per ml with this modified technique.

This method, although not ideal, as some mutagens will only exhibit optimum mutagenesis when acting on growing cells, does provide a quick, economical mutagenicity screen of β-lactam antibiotics at realistic test concentrations.

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