THE USE OF IMAGE ANALYSIS TO EXAMINE THE EFFECTS OF ANTIBIOTICS ON THE ADHERENCE *STAPHYLOCOCCUS EPIDERMIDIS* TO HEP2 CELLS

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Antibiotics at sub-MIC concentrations can interfere with bacterial attachment to host tissue by disturbing the metabolism and processing of bacterial surface components, or by disorganising surface architecture (Schifferli & Beachey 1988). Interference of initial bacterial adherence to host cells may aid in the prevention of the early stages of bacterial pathogenesis. The importance of studying the effects of antibiotics on bacterial adhesion as a guide to the treatment of peritonitis complicating continuous ambulatory peritoneal dialysis (CAPD) is therefore necessary. This communication reports on the use of image analysis to examine the adherence of isolates of Staph. epidermidis, cultured in the presence or absence of sub-MIC concentrations of vancomycin to HEp2 cell monolayers.

Three clinical isolates of Staph. epidermidis (900,901 and 904) were cultured statically in the presence or absence of sub-MIC concentrations of vancomycin in nutrient broth at 37°C in a 5% CO2 atmosphere. Cells were harvested at 30h in late log/early stationary phase, washed with phosphate buffer and resuspended in Minimum Essential Medium with Earle's Salts. Confluent monolayers of HEp2 cells (Flow Labs,Rickmansworth), cultured on glass coverslips (Old et al 1986), were incubated with 1mL aliquots of bacterial suspension at cell densities of 10^9 cells/mL (OD 1.6 at 620 nm) at 1/2 h - intervals over a 2h period at 37°C in a 5% CO2 environment. Monolayers were washed with phosphate buffered saline, fixed with 3.5% gluteraldehyde, and differentially stained using a modification of Gram's staining method. Bacterial adherence to HEp2 cells was examined by using a computerised image analysis programme based around a Semper 6+ (Synoptics Camb. UK) kernel. Counting of bacteria utilised the difference between in and out of focus images produced by transmitted light. Bacteria could be highlighted despite the uneven background caused by the monolayer. The programme differentiated between HEp2 cell surface area and interstitial cell spaces. Bacterial cells adhering per HEp2 cell were calculated.

Figure 1 Attachment of Staphylococci to HEp2 cells in the absence (a) and presence (b) of 1/2 MIC vancomycin

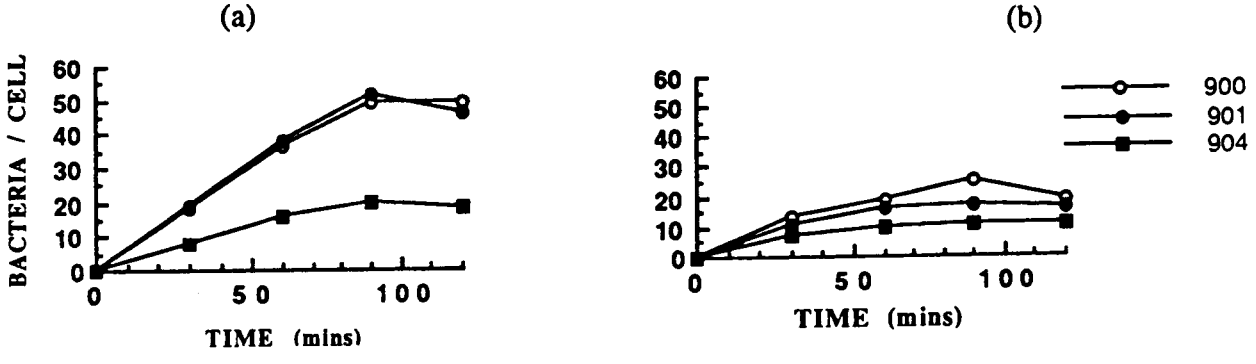

Image analysis, in conjunction with differential staining methods, proved to be a valuable rapid and objective method for the assessment of bacterial adherence to HEp2 monolayers. All three strains had the capacity to adhere to HEp2 cells, with maximum adherence achieved after 1 1/2h contact ; strain 904 demonstrated lowest levels of attachment (Figure 1a). When cultured in 1/2 MIC vancomycin the adherence of all strains was significantly reduced at all contact times (Figure 1b).
Clearly vancomycin can have significant effects upon adherence in vitro. Similar effects have been reported for vancomycin and other antibiotics (Chugh et al 1989). If these results reflect the in vivo situation they will have important implications in the selection of antibiotics for the treatment of staphylococcal peritonitis.

Schifferli,D.M & Beachey,E.H (1988) Antimicrobiol. Agents & Chemother. 32: 1603-1613
Old,D.C. et al (1986) J.Appl. Bacteriol. 61: 563-568
Chugh,T.D. et al (1989) Chemother. 35: 113-118

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