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ABSTRACTS OF MICROBIOLOGICAL INTEREST

SYMPOSIUM: THE MOLECULAR BASIS OF INTRACELLULAR SURVIVAL

Chairman: P. J. Watt

NEISSERIA MENINGITIDIS INTERACTIONS WITH HUMAN ENDOTHELIAL CELLS—IDENTIFICATION OF MULTIPLE ADHESIVE LIGANDS AND MOLECULAR MIMICRY IN MENINGOCOCCAL PATHOGENESIS

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Phase and antigenic variations are common characteristics of *Neisseria meningitidis* surface expressed ligands such as pili and outer membrane opacity proteins Opa and Opc. Since blood and CSF isolates are pilate and pili are lost rapidly on non-selective subculture, pili are selected for *in vivo* and may play an important role during dissemination. The roles of pili, the significance of phase and antigenic variation and the interplay between distinct surface structures have been investigated with a library of defined variants and mutants derived from meningococcal isolates. These studies have shown that pili increase meningococcal adhesion to human endothelial and epithelial cells. They also modulate cytotoxicity mediated by LPS and cellular invasion mediated by outer membrane proteins. Pili confer both host and tissue tropism and are essential adhesins in sialylated phenotypes. Analysis of the structural features of pili (that may modulate function) have led to the discovery of post-translational modifications. Covalently linked constituents such as O-linked trisaccharide Gal β 1-4Gal α 1-3 diacetamidotrihexose and α -glycerophosphate have been identified by mass spectrometry. These are unusual substitutions perhaps with unique functional roles. Meningococcal opacity proteins are another family of adhesins and Opc is an effective invasion for human endothelial cells. Studies on the mechanisms of cellular invasion have shown that Opc requires the presence of Arginine-Glycine-Aspartic acid (RGD)-bearing serum factors, such as vitronectin, to interact with apically expressed receptors of polarised endothelial cells. The major receptor for Opc appears to be the integrin α v β 3 (vitronectin receptor). Opc therefore helps in the invasion of host cells by mimicking as an integrin ligand.

THE INTRACELLULAR FATE OF MYCOBACTERIA IN MACROPHAGES: EXCLUSION OF BCG FROM THE ENDOSOMAL-LYSOSOMAL PATHWAY

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The interaction of mycobacteria with host phagocytic cells is central to the pathogenesis of tuberculosis and to the protection afforded by baccille Calmette-Guerin (BCG) vaccination. Macrophages act both in antigen presentation and as effector cells in the anti-mycobacterial response, while on the other hand also providing an environment for mycobacterial replication during survival. To enable study of these processes at the subcellular level, we have used density gradient electrophoresis to isolate intracellular compartments from macrophages infected with BCG. Viable BCG were found in a novel phagosome completely separate from the endosomal-lysosomal pathway. In contrast, phagosomes containing nonviable BCG co-migrated with endosomes and lysosomes. These findings may help to explain differences in the immune response induced by dead and viable BCG, and indicate that density gradient electrophoresis provides a powerful new approach to study interactions between macrophages and intracellular pathogens at the subcellular level.

THE IMMUNOBIOLOGY OF CHLAMYDIAL INFECTIONS

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Chlamydiae are obligately intracellular bacterial pathogens of eukaryotic cells responsible for a wide variety of important human and animal infections. In man, chlamydial infections are generally localised to superficial epithelial or mucosal surfaces, are frequently asymptomatic and may persist for long periods if untreated, inducing little protective immunity. Nevertheless, neutralising antibodies of limited efficacy are produced against the main chlamydial outer envelope protein, while IFN γ is chlamydiostatic and, paradoxically, may play a role both in chlamydial persistence and in protective immunity. Delayed hypersensitivity responses to chlamydiae caused by repeated or persistent infection are thought to be important in the development of the severe scarring sequelae characteristic of trachoma or of chronic salpingitis. Chlamydial heat shock proteins bearing close homology with their human equivalents may be major targets for immunopathological responses and their expression is upregulated in IFN γ induced persistent infection. *C. pneumoniae*, a common cause of acute respiratory infection in man, may persist in coronary arteries and is strongly implicated as a risk factor in atherosclerosis and in acute myocardial infection. The presentation reviewed current ideas of the immunopathology of chlamydial infection.

THE MOLECULAR BASIS OF ENTEROVIRUS DISEASE

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Members of the enterovirus genus of the Picornaviridae family are associated with a wide range of clinical syndromes including poliomyelitis, aseptic meningitis, pancreatitis, Bornholm disease, myocarditis, pericarditis, exanthema and common cold-like symptoms. This range of clinical conditions is due, in part, to differences in tissue tropisms of the various members of the group. A principal determinant of tropism is the ability to recognise a given cellular receptor, which may be differentially expressed on certain cell types. In recent years, considerable effort has been devoted to identifying the cellular receptors for different members of the enterovirus genus. A number of

these have been characterised in detail. Our work has focused on identifying receptors and secondary accessory molecules used by certain echoviruses. We have identified the decay accelerating factor (DAF), a regulator of complement activation as the receptor for echovirus 7 and related viruses. DAF is expressed on a range of cell types, including erythrocytes. We have gained evidence that these viruses and others also utilise $\beta 2$ microglobulin at a post-binding stage in the penetration process. Our work has also focused on trying to identify intracellular molecules that specifically interact with picornaviruses during their replication and to determine whether these are differentially expressed such that they influence efficiency of replication in certain cell types. Our approach to this has been to construct chimeric viruses and to look for differences in growth properties in different cell types. Results to date were described.

ORAL PRESENTATIONS

THE SURVIVAL OF ACINETOBACTER IN THE ENVIRONMENT AS A FACTOR IN HOSPITAL INFECTION

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Acinetobacter baumannii is of increasing importance as a hospital pathogen, especially in the intensive care unit (ICU). During a recent outbreak in Nottingham in the adult ICU that was associated with considerable morbidity and mortality, significant environmental contamination was found. The ability of *Acinetobacter* compared with other bacteria to survive on clinical surfaces was investigated by semi-quantitative techniques. Inocula ranging from 10^4 – 10^8 cfu/ml of *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), the outbreak organism, *A. baumannii* (AB) and *A. lwoffii* (AL) were used to contaminate formica shelving. This was then sampled with contact plates containing CLED agar at 0, 2, 4 and 18 h to initially identify the most appropriate inoculum. Subsequently, the formica shelving and a treatment trolley were contaminated over areas 8–9 cm² with 0.25 ml of a chosen inoculum (10^6 cfu/ml). A drip stand was also contaminated. Sampling of this was carried out in triplicate for one week. Sampling of the formica shelving and treatment trolley was carried out for up to 3 weeks on three or more occasions. The median duration of survival of PA on the formica shelving, treatment trolley and drip stand was 3, 7 and 2 days, respectively. SA was recovered 14 days after contamination of the formica shelving and treatment trolley and 7 days after contamination of the drip stand. AL was recovered at 7 days on the formica shelving, but AB persisted for 21 days. AL persisted for 8 days on the treatment trolley and 4 days on the drip stand. However, AB survived for 21 days or longer on the formica shelving and treatment trolley, and was recovered after 7 days on the drip stand. In conclusion, the strain of *A. baumannii* tested was capable of surviving in a viable state on clinical surfaces for long periods. This may partly explain

the increasing importance of these bacteria in hospital, especially in ICUs.

THE ROLE OF MICROBES IN INTERSTITIAL CYSTITIS

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Interstitial cystitis (IC) is a chronic disease with no known aetiology. It has been suggested that fastidious or non-culturable bacteria may be implicated. The aim of the present study was to investigate, by molecular methods, the possible role of bacteria in the pathogenesis of IC. 2–10 years old bladder biopsy specimens, preserved in paraffin wax, originally obtained from 10 IC patients, were examined. Their DNA was extracted by proteinase-K incubation followed by phenol-chloroform extraction. Samples were subjected to the polymerase chain reaction (PCR) and the presence of human DNA was confirmed by successful amplification of the mitochondrial genome. Further amplifications with primers directed against the universal regions of the 16S rRNA gene failed to indicate the presence of bacterial DNA, while appropriate controls were included. The identification of eubacteria by targets within the 16S rRNA gene region is a highly sensitive and reproducible method for the detection of most commonly encountered bacteria. In this study, despite the recovery of human DNA from all paraffin wax sections, we consistently failed to detect the presence of bacteria. Bacterial DNA, however, may not be preserved in paraffin wax over a significant time and, consequently, we are now examining fresh specimens by PCR methodology.

THE PREVALENCE OF *CHLAMYDIA TRACHOMATIS* IN WOMEN WITH A HISTORY OF MISCARRIAGE OR ECTOPIC PREGNANCY

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Chlamydia trachomatis is a leading cause of sexually transmitted disease both in the UK and world-wide. Genital tract infection may be sub clinical or silent, and in females, if untreated, can ascend *via* the endometrium to the Fallopian tubes resulting in tubal dysfunction, which can lead to ectopic pregnancy and infertility. By use of a polymerase chain reaction (PCR) specific for *C. trachomatis*, and an enzyme linked immunoassay (ELISA) to measure anti-*C. trachomatis* IgG, the prevalence of *C. trachomatis* was investigated in three study groups; women with a history of miscarriage; women with a history of ectopic pregnancy; and fertile female controls. Endometrium, Fallopian tube, ovary, and serum samples were obtained from each patient. *C. trachomatis* DNA was detected from tissue samples from all study groups, as was serum anti-chlamydial IgG. More specifically *C. trachomatis* DNA was detected in endometrial, Fallopian tube, and ovarian biopsies. Evidence of *C. trachomatis* infection was found most frequently in the ectopic pregnancy group, followed by the miscarriage group, and finally controls. We conclude that there may be an association between prior exposure to *C. trachomatis* and the incidence of ectopic pregnancy, and miscarriage.

INVASIVENESS OF *CAMPYLOBACTER JEJUNI* AND INCREASE OF VIRULENCE ON PASSAGING THROUGH CACO2 CELLS

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Campylobacter jejuni is a major cause of gastroenteritis though the disease mechanism is poorly understood. This pathogen has been shown to colonise and adhere to intestinal surfaces prior to damaging epithelial cells by translocation or invasion. To model aspects of the disease, cell lines have been used to investigate the mechanism of invasion, particularly Caco-2. This cell line differentiates to give typical enterocyte apical and basolateral surfaces, representative of those for which *C. jejuni* has a natural affinity. Fresh isolates of *C. jejuni* were utilised in the adherence and invasion assay to assess the extent of strain variation. Two statistically different groups emerged, one able to invade and multiply more rapidly over 6 h (5–10 fold) than the other. Four strains were selected for repeated passaging through the cell line. Invasiveness increased in all of these strains but to varying extents (10–100 fold). The protein profiles of these passaged strains altered with novel expression at 83–84, 71–73 and 65-kDa. 2-D SDS PAGE also showed similar changes with up-regulation of several proteins. Invasiveness of *C. jejuni* varied greatly between strains indicating two mechanisms of invasion or intracellular replication with implications for virulence. The up-regulated proteins which correlated

with increased invasiveness on Caco-2 passage may represent important virulence determinants.

A SEVERE COMBINED IMMUNODEFICIENT (SCID) MOUSE MODEL FOR *CAMPYLOBACTER JEJUNI* INFECTION

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The lack of a suitable small animal model is hampering studies of campylobacter pathogenicity. Immunodeficient mice have been reported to show increased susceptibility to enteric infections. Three strains of adult immunodeficient mice (SCID-Beige, CB17-SCID-Beige and RAG-2) were inoculated intragastrically with *Campylobacter jejuni* NCTC 11168. All mice became colonised but none developed signs of disease. The co-administration of iron dextran had no effect on colonisation levels or the development of clinical symptoms. Out of 40 CB17-SCID-Beige mice inoculated with one of a series of fresh clinical isolates of *C. jejuni*, all were heavily colonised for up to 5 months. Four mice became ill with diarrhoea over this period. One recovered, but the remaining three displayed histopathological lesions of the lower gastrointestinal tract typical of the human disease. Such pathology was evidenced by severe inflammation, crypt destruction and mucosal erosion. Blood was detected in the stools but no evidence of tissue invasion was found by immunohistology. Two of the ill mice had been given the same isolate, 228584. *C. jejuni* consistently colonises CB17-SCID-Beige mice, with levels of 10^7 – 10^9 cfu/g detectable in the faeces for at least 5 months post inoculation. Clinical symptoms including diarrhoea have been detected in a small number of mice.

INFLUENCE OF ENCAPSULATION ON SUSCEPTIBILITY OF *STREPTOCOCCUS MILLERI* TO PHAGOCYTOSIS

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Members of the *Streptococcus milleri* group (SMG) are responsible for a variety of oral and extra-oral infections of man. As such the three species constituting the group (*S. constellatus*, *S. intermedius* and *S. anginosus*) display some degree of variation in terms of their expression of extracellular capsule composed of hyaluronic acid provides some protection from the host's phagocytic cells. Encapsulation was not correlated with species. Isolates of SMG varying in their degree of encapsulation were tested for their susceptibility to ingestion by and stimulation of a respiratory burst in isolated human polymorphonuclear leucocytes (PMNL). In all instances the bacteria were preopsonised with human serum lacking specific antibodies. Those strains which possessed a capsule were more resistant to phagocytosis (mean percentage ingestion was 12.2 SD 4.8 versus 36.5 SD 8.1). Similar differences were seen in the peak level of respiratory burst induced by opsonised bacteria. Removal of the capsule by hyaluronidase enzyme treatment increased the susceptibility of capsule SMG to phagocytosis.

SYMPOSIUM: PATHOGENS IN THE FOOD-CHAIN

Chairman: I. Clarke

GROWTH, SURVIVAL AND PERSISTENCE OF
CAMPYLOBACTER

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Campylobacter spp., principally *C. jejuni/coli*, are important, human gastro-intestinal pathogens with increasing prevalence. *C. jejuni* is a predominantly food-borne, "thermophilic" (42°C) microaerophile (4% DOT), its growth being restricted largely to the warm and anaerobic GI tract. Environmental exposure is ordinarily deleterious and mechanisms prolonging survival on foodstuffs and in water would heighten its disease-causing potential. Indeed, campylobacters have already been shown to carry genetic and protein elements homologous to chaperonins and related molecules. Resistance to environmental extremes is poor when measured by conventional culture. Although sub-lethal injury is involved, *C. jejuni* may also adopt more persistent, viable but non-culturable (VNC) forms, which may retain disease potential. However, metabolic and infectivity studies have provided equivocal results regarding VNC status ("dormant" vs. "degenerate"). Experiments of our own have utilised the high level of control possible in chemostat-based systems to examine the responses of *C. jejuni* to environmental extremes. For example, in model water distribution systems. The culturability of *C. jejuni* rapidly declined, dependent on temperature and oxygenation. However, labelling (antibody, rRNA) experiments demonstrated that extensive persistence may be encouraged by incorporation within the indigenous biofilm. In separate experiments, high DOT elicited changes to a putative coccal (VNC) state which was associated with large increases ($\times 100$) in invasiveness (Caco2 cells) and correlated with the upregulation of particular cellular proteins (in contrast iron restriction reduced invasiveness). The molecular characterisation of these physiological responses coupled with the application of models relevant to the human disease will help identify the mechanisms involved in maintaining the persistence, viability and virulence of *C. jejuni*.

YERSINIA ENTEROCOLITICA – AN UNDER-RECOGNISED
FOODBORNE PATHOGEN?

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Yersinia enterocolitica is a recognised foodborne pathogen which commonly causes enteritis but may also cause a wide variety of other symptoms. Pathogenicity depends upon the presence of a plasmid, which is found in only a few serotypes. Pigs are the main reservoir of these strains. Plasmidless strains of *Y. enterocolitica* and other closely related strains are found much more widely in the environment and in food products. The pathogenic significance of these strains is debatable but they may be capable of causing mild illness. A number of food poisoning outbreaks attributed to contamination with pathogenic *Y. enterocolitica* have been described, some of which have caused thousands of cases of yersiniosis. Few of these outbreaks were associated with pork consumption; despite

this the link with sporadic cases and consumption of pork is well established. Changes in butchery practices and improved consumer education on handling and eating pork are significant routes to minimising foodborne illness. The presence of this organism in food is particularly significant because of its ability to grow at refrigeration temperatures. The infectious dose is not known, but some evidence suggests that it may be low. It is, therefore, essential to exclude its presence from food that is ready to eat, especially if it is to be stored under refrigeration. It does not survive heat processes such as pasteurisation and so presence of any *Yersinia* strain indicates post-processing contamination or inadequate heating. Because of cultural isolation difficulties few laboratories examine routinely for the presence of *Y. enterocolitica* in food or clinical samples. Its true role as an enteric pathogen is therefore underestimated.

THE ROLE OF WATER IN THE TRANSMISSION OF
INFECTIOUS DISEASE

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Classically, consumption of contaminated water is associated with the transmission of diseases such as cholera and typhoid and these diseases are still common in developing countries where drinking water and poor methods of sewage disposal continue to contribute significantly to the spread of these diseases. In developed countries, however, there has been a considerable change in emphasis in the role of water as a potential vehicle of disease. In the late 1970s, Legionnaires Disease became recognised as a significant cause of mortality and morbidity associated with the inhalation of bacteria found growing in hot water systems and cooling towers. The spread of this disease was shown to be associated with inappropriate maintenance procedures within buildings, and more recently similar issues have arisen with the *Mycobacterium avium* complex. In recent years, the pathogenic protozoa have come to the fore. In particular, *Cryptosporidium parvum* and to a lesser extent *Giardia intestinalis* have become household names in many parts of the developed world due to large and well publicised waterborne outbreaks. Waterborne disease continues to be a potential threat to human health although in the developed world, the organisms involved pose new problems.

VIRUSES IN THE FOOD-CHAIN

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There are two main foodborne viral infections in the UK – hepatitis A and viral gastroenteritis. Foodborne transmission of both these infections is believed to be grossly under-recognised. Several different viruses can cause gastroenteritis, but in foodborne outbreaks it is the small round structured viruses (SRSV), otherwise known as Norwalk-like viruses,

that are most frequently reported. Both hepatitis A and the gastroenteritis viruses are normally transmitted directly from person to person, but on occasions may be food or waterborne. Viruses do not multiply or produce toxins in foods and transfer is merely passive. However, these viruses are infectious in very small doses. They survive well in the environment. Foods may be contaminated in two ways:

1. Contamination in the growing and harvesting area by sewage polluted water; molluscan shellfish have been particularly implicated.
2. Contamination by infected food handlers.

Until recently it has not been technically feasible to detect virus in foodstuffs, and investigation of outbreaks has depended on identifying virus in patients and using epidemiological findings to link illness to a possible food source. Recent advances in PCR techniques has opened up the opportunity to look for viruses directly in foods, particularly shellfish, although this is not yet practical on a routine basis. Prevention and control depends on good basic food handling practices and control of food production. Current cleansing procedures for some shellfish, although adequate to remove bacterial contamination, cannot guarantee the absence of viruses.

MYCOTOXINS IN FOOD

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Mycotoxins are toxic secondary metabolites produced by fungi infecting agricultural crops, particularly cereals and oilseeds, both during crop growth and in storage, as well as in processed foods and feeds. When ingested, mycotoxins can produce various toxic syndromes (mycotoxicoses) in man and animals. People can be exposed directly by consumption of contaminated plant-based foods or indirectly by ingestion of mycotoxin residues in animal-derived foods. At least 300 different mycotoxins have been identified but only about 20 are of real concern. Some like aflatoxin (a human carcinogen) primarily contaminate crops from warm countries and enter the EU by importation whereas others such as the trichothecenes, the fumonisins and ochratoxin A occur in European crops. The effects of mycotoxins on human health are generally complex and mostly little understood. Most information has been derived from epidemiological data or extrapolation from animal studies. While substantial exposure is unlikely to occur in most EU countries because of strict food regulations, concern remains on possible adverse effects of long-term exposure to low levels of mycotoxins in the food chain. It is recognised that better information is required on actual exposure levels. Concern about potential health hazards has led to the development of various strategies to minimise mycotoxin contamination of foods.

POSTERS AND DEMONSTRATIONS

IS SEROLOGICAL EVIDENCE OF *CHLAMYDIA PNEUMONIAE* INFECTION A RISK FACTOR FOR ACUTE MYOCARDIAL INFARCTION?

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An association between coronary heart disease and *Chlamydia pneumoniae* (CP) infection has been suggested serologically and by demonstrating the organisms in atheroma. Twenty coronary care patients (average age 65 years, range 45–82 years) with clinical symptoms of acute myocardial infarction (AMI) were tested for antibodies to the three species of *Chlamydia* by the micro-immunofluorescent test (MIF) with immunoglobulin classes A, G and M. Patients were tested for creatinine phosphokinase (CKMB) isoenzyme activity and raised CKMB isoforms (Helena Lbs UK). 20 randomly selected sera from adults (average age 55 years range 47–74 years) post “needle-stick” injury were examined by MIF as above and used as comparable group. Sixteen of the CCU patients had biochemical evidence of AMI. Fifteen of these were IgG antibody positive for CP. One of the four negative patients had IgG antibody to CP, $p = 0.0013$. No IgA or IgM class antibody were demonstrated in the CCU patients. The absence of IgM response would not rule out acute infection as no second specimens were examined. Twelve of 20 needle-stick subjects had IgG antibody specific for CP. CP antibody prevalence was not statistically significant between AMI and needle-stick subjects. The latter group might have been the subject of coronary heart disease or even suffered from earlier MI. These interesting results suggest a direct or an indirect cause and effect of CP and AMI, further investigations are warranted.

THE DETECTION OF *CAMPYLOBACTER JEJUNI* IN A WATER BIOFILM BY USE OF rRNA PROBES

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Water and aquatic biofilms are potentially of great significance as vehicles for the transmission of *Campylobacter jejuni* within the food chain. Fluorescently-labelled *Campylobacter* specific rRNA probes were used to detect the presence of *C. jejuni* in aquatic and biofilm models. The persistence of *C. jejuni* was investigated in a water model which consisted of a continuous culture of heterotrophic aquatic organisms with its surface area optimised for the formation of biofilm. Different combinations of temperature and oxygenation were used in the water model (4°C, 30°C; anaerobic, 4% dissolved oxygen tension, aerobic), with a constant dilution rate of 0.025 h⁻¹. Viable cells of *C. jejuni* in the biofilm and planktonic phase in the water model became rapidly undetectable (by culture) after introduction into the system, particularly at higher temperature and dissolved oxygen tension. Analysis of the biofilm (on removable glass coupons) after this time with *Campylobacter* specific rRNA probes demonstrated the continuing presence of a range of cell morphologies of *C. jejuni* at least 6 weeks post introduction. The detection of *C. jejuni* in the water model with rRNA probes after an extended period suggests that the organism becomes integrated and persists within the heterotroph consortium of the biofilm and thus may survive.

USE OF PCR AS AN ALTERNATIVE TO TISSUE CULTURE TO DETECT ENTEROTOXIGENIC *BACTEROIDES FRAGILIS*

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Although the association between enterotoxigenic *Bacteroides fragilis* (ETBF) and diarrhoea in sheep, cows and other domestic animals is established, their isolation from man has been little studied and is of uncertain significance. One reason for this is the lack of a simple laboratory test to detect the presence of the enterotoxin. The only method current available, apart from the ligated lamb ileal loop test, depends upon the direct detection of enterotoxigenic activity by demonstration of a cytotoxic effect on a human colon-derived cell line, HT-29. In this study, a PCR assay was developed based upon the N terminal sequence of the toxin gene to amplify a 294-bp product and the results were compared with the tissue culture assay. One or two colonies of *B. fragilis* were scraped off plates into 500 µl of distilled water and boiled for 5 min. PCR was performed directly on the supernate. Thirty two strains of *B. fragilis* which had previously been shown to produce enterotoxin by their cytotoxic action on HT29 cells were studied. All were found to be positive by PCR. A further 27 uncharacterised strains of *B. fragilis* isolated from wound infections (14), blood (9) and faeces (4) were then tested. Two of these strains were positive by PCR, only one of which was positive by tissue culture, suggesting that in the other strain at least part of the enterotoxin gene was present but not expressed. These results show that PCR provides a rapid and accurate alternative to tissue culture and should be useful in further epidemiological studies.

A COMPARISON OF THREE COMMERCIALY AVAILABLE ELISAS WITH CYTOTOXIN ASSAY FOR THE DETECTION OF *CLOSTRIDIUM DIFFICILE* TOXIN IN FAECES

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Clostridium difficile infection is an increasing problem as the cause both of sporadic cases and nosocomial outbreaks of antibiotic associated diarrhoea in hospital patients, especially in the elderly and the immunocompromised. Increasingly, a diagnosis of *C. difficile* infection is made by using a commercially produced ELISA kit to detect faecal toxin A or toxin B, or both, rather than by isolation of the organism or by detection of the cytotoxic activity of toxin B on a mammalian cell line. In this study, three commercially available toxin A ELISA kits were compared with the results of tissue culture cytotoxin assay and stool culture on CCFA medium. One hundred and sixty two faecal specimens were studied; these consisted of 80 specimens from consecutive unselected patients and 82 specimens from patients in whom *C. difficile* infection was suspected. On culture, 65 specimens yielded *C. difficile* of which 51 were toxigenic; all these 51 specimens were positive in the tissue culture assay; in addition one specimen was positive on the tissue culture assay but negative on culture and in all three ELISA tests. The sensitivities and specificities of the ELISA kits compared to the tissue culture were as follows: Meridian Premier – sensitivity 86.3%, specificity 88.3%; Porton

Cambridge – sensitivity 91.7%, specificity 90.0%; BioWhittaker – sensitivity 92.2%, specificity 92.5%. None of the kits was as sensitive as the tissue culture assay, but they are considerably quicker and easier to perform. The relative values of ELISAs, tissue culture and culture in the diagnosis of *C. difficile* associated disease were discussed.

COMPARATIVE PHENOTYPIC CHARACTERISTICS OF *STAPHYLOCOCCUS AUREUS* ISOLATES FROM LINE AND NON-LINE ASSOCIATED SEPTICAEMIAS, CAPD PERITONITIS AND HEALTHY NASAL CARRIERS

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The aim of this study was to demonstrate whether there were any phenotypic differences between *Staphylococcus aureus* invasive infections; 291 isolates – 153 from blood cultures (line associated 68, non-line 85), 78 from CAPD peritonitis and 60 from healthy nasal carriers – were studied. Lipolytic, proteolytic, fibrinolytic and haemolytic activities were tested. Phage typing, crystal violet reaction and biochemical reactions were studied. Production of enterotoxins A, B, C, E and TSST-1 and protein A were tested by ELISA. Almost all the isolates were lipolytic with more intensity among nasal isolates; 70–80% were fibrinolytic. Proteolytic activity of nasal isolates was more frequent (62%) compared to septicaemic isolates (35–37%). All showed higher frequency of haemolysis on rabbit blood agar. Production of enterotoxin A ranged from 14% to 27% among the four groups. Enterotoxin B, 57–78%, production was significantly higher among P.D. isolates compared to nasal carriers. Enterotoxin C 11–25% and enterotoxin E 7–14% did not differ. Production of TSST-1 ranged from 13% to 62%, significantly higher among nasal isolates (62%) compared to the infections (29%), particularly the non-line septicaemias (13%). No differences were noted regarding the other tested characteristics. TSST-1 does not seem to be an important factor for invasive infections. Enterotoxin B seems to be more important in infections, particularly CAPD peritonitis. Nasal colonisers tend to be more frequently proteolytic and more intensely lipolytic.

FREQUENCY-DYSURIA SYNDROME: A SURVEY TO DETERMINE ITS PREVALENCE AND RELATED FACTORS AND TO IDENTIFY ANY ROLE OF THE FASTIDIOUS MICRO-ORGANISM *GARDNERELLA VAGINALIS*

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Frequency-dysuria syndrome (recurrent cystitis) affects young women who present with symptoms of urinary frequency and dysuria, which are temporarily relieved by a course of antibiotics. It is thought that fastidious micro-organisms have a role. A survey was conducted amongst female university students. Their mid-stream specimens of urine (MSU) were collected and cultured in conditions selective for the growth of fastidious micro-organisms. DNA was extracted from the resulting colonies and subjected to PCR with *Gardnerella vaginalis*-specific primers.

Of the 55 questionnaires handed out, 32 (58%) were

returned along with MSU samples. Age range was 17–40 years, 66% in the age group 21–30 years. Nine (28%) of 32 gave history of recurrent cystitis without any other abnormality in the urinary tract. Seven (78%) of nine with recurrent cystitis grew fastidious micro-organisms on selective culture, as opposed to six (33%) of 18 who never had any urinary infection. However, PCR to amplify *G. vaginalis* DNA failed to detect the presence of this species in the DNA extracted from these colonies, positive and negative controls were included. We conclude that fastidious micro-organisms have a significant role in the aetiology of frequency-dysuria syndrome, but bacteria other than *G. vaginalis* are the most likely cause.

DEVELOPMENT AND ASSESSMENT OF HUMAN RECOMBINANT ANTIBODIES TO CYTOMEGALOVIRUS

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Cytomegalovirus (CMV) is the single most important infectious complication of renal and bone marrow transplant recipients. Treatment with antiviral drugs alone is poor, but survival rates are increased when drugs used in combination with i.v. human serum to CMV. Immune immunoglobulin would also be valuable in prophylaxis against CMV infection in CMV seronegative recipients receiving seropositive transplants. Unfortunately, pooled human immunoglobulin is prohibitively expensive, difficult to standardise and not without risks. Therefore, our overall objective is to produce one or more human recombinant antibodies which could be used as an alternative to pooled immunoglobulin. Two gene libraries encoding variable light (VL) and heavy (VH) immunoglobulin domains were constructed. Both scFv and Fab forms of the libraries were designed, using the phage display vectors pEXmide 3 and 4. The libraries were produced with cDNA, synthesised from mRNA obtained from the peripheral blood lymphocytes of patients exhibiting both primary and secondary CMV infections. Both libraries were assessed for diversity, by *Bst* NI DNA fingerprinting. They were then panned against a selection of peptides taken from epitopes of human CMV antigens, antibodies against which show neutralising activity. The resulting clones were then reassessed and sequenced.