PROCEEDINGS OF THE PATHOLOGICAL SOCIETY
OF GREAT BRITAIN AND IRELAND

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SYNOPSIS OF PAPERS OF MICROBIOLOGICAL INTEREST

SYMPOSIUM: STREPTOCOCCI: ELUCIDATION OF THEIR PATHOGENICITY
Chairman: M. W. Casewell

VIRIDANS STREPTOCOCCI AND EXTRA-ORAL DISEASES

D. Beighton
Joint Microbiology Research Unit, KCSMD, Denmark Hill, London SE5 9RW

Viridans streptococci are members of the normal oral flora and it has recently been demonstrated that this group consists of at least 15 species. The individual species may be differentiated by phenotypic tests to determine the ability of strains to ferment carbohydrates, hydrolyse arginine, aesculin and urea, produce glycosidic activities, including a-fucosidase, a-arabinosidase, sialidase, and ß-N-acetylgalactosaminidase and to produce IgA protease. The changes in the taxonomic status of the viridans streptococci have enabled the disease associations of individual species to be determined. The major extra-oral diseases with which viridans streptococci are associated are infective endocarditis, septicaemia in neutropenic patients, respiratory infections and deep-seated abscesses particularly in the liver and brain. It is now apparent that infective endocarditis is primarily associated with Streptococcus oralis, S. gordonii and S. sanguis whereas septicaemia is associated with S. oralis and S. mitis, respiratory infections are associated with S. oralis and S. mitis, and S. intermedius is isolated from deep-seated abscesses. The development of reliable identification tests for these previously difficult organisms has permitted these disease associations to be demonstrated. Now that such close associations between particular species and specific diseases have been demonstrated, these data may provide a basis on which to investigate potential virulence factors of individual species.

ENTEROCOCCUS FAECIUM – INFECTIONS FOR THE POST-ANTIBIOTIC ERA

J. J. Wade
Dulwich Public Health Laboratory and Medical Microbiology, King’s College School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ

Enterococcus faecium is an important cause of nosocomial infection and hospital outbreaks. Acquired resistance mechanisms can render this species resistant to all clinically useful antimicrobial agents. It is known that E. faecium bacteremia carries a poor prognosis with an attributable mortality. At this hospital the incidence of E. faecium bacteremia is increasing, especially amongst liver patients (p < 0.01). In a study of 284 liver transplant (LT) patients, E. faecium was the second commonest pathogen. E. faecium infections affected those patients with the most severe underlying conditions or complicated post-operative courses. Patients who subsequently developed E. faecium infections had higher aspartate transaminase levels (AST: p = 0.037), international normalised ratios (p = 0.0308), and longer ITU admissions (p = 0.016) pre-LT; they more often required emergency transplant (p = 0.0004), and post-LT more often developed fungal sepsis (p < 0.00001), required tracheostomy (p = 0.0019), or had prolonged admissions (p = 0.0001). Three LT patients developed serious intra-abdominal sepsis due to E. faecium with MICs of ampicillin, vancomycin, teicoplanin, and streptomycin of 16–256, 128–1024, 16–256, and >1000 mg/L, respectively; two patients had isolates that were also highly resistant (MIC > 1000 mg/L) to gentamicin. All isolates were sensitive to pristinamycin (MICs 0.25–0.5 mg/L) but this oral agent proved ineffective possibly due to poor absorption. For these patients it proved impossible to identify antimicrobial regimens to control recurrent bacteremias, and surgery was required in each case. Untreatable enterococcal infections are likely to occur increasingly, particularly in immunocompromised patients.

EPIDEMIOLOGICAL TYPING OF ENTEROCOCCI

D. Morrison
Epidemiological Typing Unit, Laboratory of Hospital Infection, Central Public Health Laboratory, 61 Colindale Avenue London NW9 5HT

In the last two decades enterococci have emerged as an increasingly important cause of nosocomial infection. As a result of the threat posed by these organisms, several phenotypic and genotypic typing methods have been applied to investigate their epidemiology. These include serotyping, phage typing, enterococcine typing, biotyping, antibiograms, analysis of whole or digested plasmid DNA, restriction endonuclease analysis (REA) of chromosomal DNA, ribotyping and various pulsed-field gel electrophoresis (PFGE) and PCR-based techniques. Comparison of PFGE, ribotyping, PCR-ribotyping, whole plasmid profile, antibiogram and biotyping suggest that PFGE is the most discriminatory method. Amongst a collection of geographically distinct isolates, ribotyping distinguished a number of types, although, one or two types predominated. PCR-ribotyping has not proved a satisfactory typing method even when combined with digestion of the products. Whole plasmid profiles and antibiograms may vary considerably with time, even within a PFGE type, which potentially limits their usefulness. A number of unusual biochemical reactions, such
as raffinose, sorbitol and mannitol fermentation in *Enterococcus faecium*, may provide a simple method for indicating possible clonality.

**CANDIDATE EXTRACELLULAR PROTEINS FOR EXPRESSING STREPTOCOCCAL PATHOGENICITY**

K. A. Homer

Joint Microbiology Research Unit, KCSMD, Denmark Hill, London, SE5 9RW

Streptococci are associated with a number of life-threatening diseases including endocarditis, septicaemia, deep-seated internal abscesses, respiratory tract infections and meningitis. Of the viridans streptococci, the major pathogens appear to be *Streptococcus intermedius*, *S. oralis*, *S. gordoni* and *S. sanguis* while of the pyogenic streptococci, the major pathogens include *S. pyogenes* and *S. agalactiae*. The mechanisms by which these individual species are responsible for the onset of disease are varied and no single factor explains their virulence. For these to cause disease they must gain access to the potential sites of infection and resist host defences. Binding to host tissues localises bacteria and degradation of tissue components, including glycoproteins and glycosaminoglycans (GAGs), leads to tissue damage, facilitating spreading and providing nutrients for cell proliferation. The mechanisms by which streptococci bind to host tissues include the presence of specific lectin-like receptors on the bacterial surface and these may be responsible for the interactions between streptococci and platelets in endocarditis. The growth of bacteria within tissues may involve the production of specific glycosidases such as sialidase and β-N-acetylglucosaminidase which have been demonstrated, by 1H-NMR spectroscopy, to remove N-acetylated sugars from serum glycoproteins. The degradation of GAGs by streptococci involves the production of hyaluronidase and chondroitin sulphate depolymerase activities. The pathogenicity of individual species (or strains) of streptococci may involve the presence of different sets of virulence determinants.


J. F. P. Schellekens

National Institute of Public Health (RIVM), P.O. Box 1, 3720 BA Bilthoven, the Netherlands

The possible increase of severe invasive infections and toxic shock syndrome (TSS) with β-haemolytic streptococci and Lancefield's Group A (GAS) which has occurred in the USA, the UK and Scandinavia, was noted in the Netherlands at the beginning of 1992. A nation-wide laboratory-based surveillance was started, including submission of invasive GAS strains and acquisition of clinical and demographic data of involved patients (response rate 57%). Submitted GAS-strains were characterised with T-serotyping, M-genotyping and assessment of the presence of *speA1, speA2, speA3, speA4, speB* and *spec*. From January 1993 to July 1995, this surveillance yielded 449 patients with clinically and bacteriologically documented invasive GAS infection, of whom 93 (21%) fulfilled the criteria of TSS (incidence of 1.2/105 and 0.25/105/year, respectively). Lethality was over 14% and in TSS patients 58%. In individuals >60 years old, the incidence of invasive GAS-infection was highest (1.6/105) and strongly associated with underlying disease (61%). In individuals 20–50 years old with TSS (n = 38), early signs and symptoms of systemic toxicity (rash, vomiting, diarrhoea; 66%) and the occurrence of necrotising fasciitis (42%) was most prominent. Among the many different T/M-types found, T1/M1 was the single dominant type, causing 20% of invasive infections and 36% of TSS-cases. In the first half of 1995, T3/M3 emerged in association with TSS (7 of 22 cases). All isolates of T1/M1 and T3/M3 contained the exotoxin A gene with the variants *speA2* in T1/M1 and *speA3* in T2/M3.

**ORAL PRESENTATIONS**

**CHARACTERISATION OF A NOVEL TOXIGENIC STRAIN OF CLOSTRIDIUM DIFFICILE**

S. Hyde, P. Dodson and S. P. Borriello

Microbial Pathogenicity Research Group, Institute of Infections and Immunity and Division of Microbiology, Queen's Medical Centre, Nottingham NG7 2UH

*Clostridium difficile* is the cause of pseudomembranous colitis and many cases of antibiotic-associated diarrhea. Not all isolates are toxigenic, these being considered avirulent. One such strain, an infant isolate, failed to produce detectable levels of toxins conventional growth conditions. However, disease was occasionally produced in clindamycin-pre-heated hamsters. Culture in a dialysis bag system to produce disease in the hamster animal model and produces low levels of a cytotoxin. The extent to which this is also true of other non-toxigenic isolates needs to be determined.

**MORTALITY ASSOCIATED WITH HOSPITAL ACQUIRED CLOSTRIDIUM DIFFICILE COLITIS**

M. Lesna, D. Parham and S. Hill

Department of Histopathology, Royal Bournemouth Hospital, Bournemouth, Dorset and Public Health Laboratory Service, Poole General Hospital, Poole, Dorset

Pseudomembranous colitis due to *Clostridium difficile* in patients on antibiotics is regarded as usually a self limiting disease after the antibiotic therapy is withdrawn. In the elderly the outcome may be fatal but the mortality, in our experience, has been underestimated. Having previously
reported two fatal cases of *C. difficile* colitis with toxic megacolon. We have analysed the data from 47 cases of *C. difficile* diarrhoea (diagnosed by culture and toxin detection) occurring in December 1993 in our hospital (average age of patients 84 years) and compared the outcome with 59 age-matched controls who received antibiotic therapy in our hospital at approximately the same time but did not suffer from diarrhoea. In the *C. difficile* group 30/47 patients died: 24/47 within 18 days of the diagnosis, 10/47 within a week and 7 before the result of the stool culture became available. It appears that in all these cases the infection was acquired in the hospital and in none of these cases was *C. difficile* colitis mentioned on the death certificate. In the control group, 10 out of 57 patients died 2–30 days after admission of various natural causes (3/10 as a result of disseminated neoplasm). During the hospital treatment they received a similar selection of antibiotics including cephalosporins. We conclude that, in the elderly, *C. difficile* colitis is a very serious and potentially fatal complication and the speed of the diagnosis is most important if death in that stage is to be prevented.

**PCR RIBOTYPING AND PYROLYSIS MASS SPECTROMETRY OF CLOSTRIDIUM DIFFICILE: EXPERIENCES OF THE PHLS ANAEROBE REFERENCE UNIT**

J. S. Brazier  
*PHLS Anaerobe Reference Unit, University Hospital of Wales, Cardiff*

A typing service for strains of *Clostridium difficile* has been in operation in Cardiff for 2 years. The service was initially based on pyrolysis mass spectrometry (PMS) of whole cells which enables the rapid fingerprinting of strains from hospitals in a putative outbreak but does not assign a permanent type. A PCR ribotyping method based on analysis of the intergenic spacer region of the 16S-23S ribosomal RNA gene complex has recently been developed in our laboratory. This method was applied to strains typed by the serotyping method of Delmee and to wild strains submitted for typing. Serogroups A-X and sub-serogroups A2-AlO all gave different banding patterns. Initial results comparing this method with previous analysis by PMS has given good correlation for typing. Serogroups A and B of *C. difficile* have been typed and this method is currently being applied to large collections of *C. difficile* strains held at the Anaerobe Reference Unit.

**THE ECOLOGY AND EPIDEMIOLOGY OF CLOSTRIDIUM DIFFICILE**

Nabeel M. Al-saif, J. S. Brazier and B. I. Duerden  
*PHLS Anaerobe Reference Unit, UWCW, Heath Park, Cardiff CF4 4XN, UK*

*Clostridium difficile* is known as the main causative agent of antibiotic associated diarrhoea. Whilst much is known about nosocomial acquisition of this organism, little is known about how the organism may be acquired by healthy people in the community. The study examined the role of the environment as a potential source of human infection. It included development of optimal methods for detection of *C. difficile* in different habitats. Stool samples were taken from animals that included cats, dogs, sheep, cattle, pigs, horses, pigeons, chickens and ducks. Samples of human food of plant origin were also examined. Various environmental sites including soil, different sources of water, hospital wards, family houses, nursing homes for the elderly and student hostels were screened for the presence of *C. difficile*, *C. difficile*-positive samples were obtained from cats, dogs, horses, pigeons, sheep and from environmental sites including soil, different sources of water, hospital wards, family houses, nursing homes for the elderly and student hostels. The isolates were confirmed as *C. difficile* by standard laboratory techniques and tested for toxin production. The isolates were examined for their sensitivity to different antibiotics. Isolates were typed by molecular methods (PCR) and compared to the PCR typing of the clinical isolates of *C. difficile* collected from different areas of the UK to establish the relationship between environmental isolates and the isolates from cases of human disease. Typing studies will also be used to clarify the uncertain situation regarding transitional versus long term carriage of *C. difficile* by healthy people and animals.

**A MOLECULAR COMPARISON OF VANCOMYCIN-RESISTANT ENTEROCOCCUS FAECIUM ISOLATES FROM PATIENTS AND FROM CHICKENS**

*King's College School of Medicine and Dentistry and the Central Public Health Laboratory, London, UK*

The origin of the vanA gene that confers resistance to glycopeptides in enterococci is unknown, although vancomycin-resistant *Enterococcus faecium* (VREF) has been isolated from poultry and farm animals. We examined, by standard methods, the plasmids, the presence of the vanA gene and the macro-restrictive analysis of total DNA in gentamicin-resistant VREF (VGREF) isolated from patients and from chickens obtained from a local market. Four isolates of VREF cultured from the guts of 38 chicken carcasses were compared with 40 patient isolates of VGREF. All chicken isolates contained a non-transferable 35.5-kb plasmid which reacted on Southern blotting with digoxigenin-labelled vanA probe. Similarly, the vanA probe reacted with a 42-kb transferable plasmid which was present in clinical strains. Digestion of total DNA with *Sma* I followed by pulsed field gel electrophoresis (CHEF) yielded approximately 25 DNA fragments of 27–295 kb. Using the gel Gelcompar and MVSP programmes to examine the phylogenetic relationship, we showed that at the 80% similarity level there were four groups of strains and there was only 50–80% similarity between some clinical and poultry strains. These findings suggest the need to examine more poultry VGREF but, thus far, there seems to be two distinct populations and no evidence for direct transfer of poultry derived DNA to the human population.

**THE EFFECTS OF THE CAPSULE OF STREPTOCOCCUS UBERIS ON BOVINE NEUTROPHILS**

J. A. Leigh, T. R. Field, P. Norton and A. P. Bland  
*Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berks RG20 7NN*

*Streptococcus uberis* is capable of infecting the lactating bovine mammary gland and is responsible for around 30% of all clinical episodes of mastitis in the UK. The influx of neutrophils following challenge does not control infection. The bacterium can also be induced to become highly resistant to phagocytosis and killing *in vitro* and this correlates with the production of a hyaluronic acid capsule. However, this layer does not deter opsonisation. Extracts of the capsular layer, generated by treatment with hyaluron-
dase, were shown to inhibit the bactericidal action of neutrophils directly following incubation at 37°C but not at 0°C, 4°C or 25°C. Inhibition of neutrophil function coincided with the appearance of membrane blebs at the neutrophil surface and the failure to produce pseudopodia, as determined by electron microscopy. Further investigation also revealed that changes to the distribution of actin had occurred within neutrophils following incubation with the capsule extract. The inhibitory action of components within the capsule may play a role in the ability of S. uberis to resist phagocytosis by neutrophils.

STUDIES OF VEROCTOTOXIN PRODUCING ESCHERICHIA COLI IN CATTLE
C. Wray, I. M. McLaren and L. P. Randall
Central Veterinary Laboratory, New Haw, Addlestone, Surrey KT15 3NB

Verocytotoxin producing Escherichia coli (VTEC) O157 is an important cause of human disease and farm animals have been shown to be a reservoir for the organism.

Although investigations have been carried out on farms, experimental infections allow more detailed studies and this paper describes such investigations in calves and adult cattle. Six calves between two and four-weeks-old were infected orally with c. 10^9 VTEC O157:H7. The calves excreted the challenge strain in their faeces for periods ranging from one to 58 days (mean 22 days). The excretion was often intermittent and the challenge strain was not isolated from any calf at necropsy two weeks after the last isolation of VTEC O157. Three adult cows were similarly infected and intermittent excretion ranged from 13 to 44 days. None of the animals showed signs of illness. Of the different VTEC O157 detection techniques used, immunomagnetic separation was the most sensitive.

STUDIES INTO THE ROLE OF THE SEF14 FIMBRIAL ANTIGEN IN THE PATHOGENESIS OF SALMONELLA ENTERITIDIS
C. J. Thorns1, C. Turcotte2, C. G. Gemmell3 and M. J. Woodward4
1Central Veterinary Laboratory, New Haw, Addlestone, Surrey, KT15 3NB, United Kingdom, 2Animal Diseases Research Institute, Agriculture Canada, Nepean, Ontario K2H 8PG, Canada, 3Department of Bacteriology, University of Glasgow, Glasgow G4 0SF

To investigate the role of the SEF14 fimbrial antigen in pathogenesis, a single defined sefA (SEF14-) inactivated mutant of Salmonella enteritidis strain LA5 was constructed and tested in a number of biological assay systems. There was no significant difference between the wild-type strain and the isogenic SEF14- mutant in their abilities to adhere to and invade HEp-2 epithelial cells or their survival in mouse peritoneal macrophages, whereas the SEF14- mutant was ingested more rapidly by isolated human PMN. Both strains colonised the intestine, invaded and spread systematically in one-day-old chicks, laying hens and BALB/c mice equally well. A significantly greater number of chicks excreted the wild-type SEF14+ strain during the first week following infection as compared to those infected with the SEF14- mutant. However, similar numbers of chicks excreted the two strains between two and seven weeks after infection. These results indicate that possession of SEF14 fimbriae alone does not appear to play a significant role in the pathogenesis of S. enteritidis although its contribution to virulence may depend on the host species infected.

ADHERENCE OF AEROMONAS CAVIAE TO MONOLAYER CELLS AND CHARACTERISATION OF A POSSIBLE NEW ADHESIN
Jonathan P. Thornley, Jonathan G. Shaw and Adrian Eley
Department of Medical Microbiology, University of Sheffield Medical School, Beech Hill Road, Sheffield, S10 2RX

Adherence of Aeromonas caviae to HEp-2 and Caco-2 cell monolayers was investigated with 24 clinical isolates. Factors affecting adherence included growth phase, multiplicity of infection length of incubation, growth temperature and pH. Light microscopy, together with scanning and transmission electron microscopy were employed in the investigation of bacteria-bacteria and bacterial-monolayer interactions, and indicated similarities with the enterogaegregative adherence patterns of Escherichia coli. The presence of extracellular bacterial appendages and their correlation with an increase in adhesive capacity suggested a possible role in the adherence process. Extracellular filamentous structures, from a strain previously isolated from a patient presenting with gastro-intestinal symptoms, were purified and characterised. Two morphologically distinct flagella types were seen with molecular weights of 31.5 and 33 kDa. N-terminal amino acid sequence homology was noted with the polar flagella of Pseudomonas aeruginosa and the lateral flagella of Vibrio parahaemolyticus, respectively. Further studies have indicated that the lateral flagella may be responsible for the adhesive potential; genetic characterisation is being studied further.

GENE CLONING OF A FLAGELLAR SHEATH PROTEIN OF HELICOBACTER PYLORI
A. C. Jones1, C. J. Luke1, A. Cockayne2 and C. W. Penn1
1School of Biological Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT and 2Department of Microbiology, University Hospital, Queen’s Medical Centre, Nottingham, NG7 2UH

Helicobacter pylori possesses sheathed flagella which are essential for colonisation of the gastric mucosa. A component of the sheath is a 29-kDa protein which is reactive by immunoblot with a monoclonal antibody derived from whole H. pylori cells. The antibody decorates the flagellar sheath of H. pylori. A genomic library of strain NCTC 11637 in the vector lambda Zap Express (Stratagene) was screened with the anti-sheath monoclonal antibody, and antigen-expressing clones were identified. Plasmids were excised from these clones and Escherichia coli strains bearing the plasmids were analysed by SDS-PAGE and immunoblotting. The antigen was expressed strongly in recombinant E. coli both with and without IPTG induction of the vector promoter. The recombinant protein was prominent on Coomassie blue-stained SDS-PAGE gels and was strongly reactive with the monoclonal antibody, showing an identical mobility on SDS-PAGE with the protein expressed by H. pylori. The nucleotide sequence of the smallest clone, designated pACJ193, was found to be highly similar to the gene hpaA which is reported to encode a fibrillar haemagglutinin. The hpaA sequence was revised and the transcriptional start mapped by primer extension from H. pylori mRNA. The hydrophobic nature of the 29-kDa protein was demonstrated by partitioning in a Triton-X114 two-
Microbial Pathogenicity Research Group, Institute of GREAT BRITAIN AND IRELAND

TRANSDUCTION

restricted serotypes, which are epidemiologically associated with severe persistent diarrhoea disease in infants and young children. Recent research into the pathogenic mechanism of these organisms has revealed that initial attachment to the microvilli of small intestinal enterocytes leads to the development of a characteristic attaching and effacing (AE) ultrastructural lesion. Infected cells eventually die and are lost from the villus surface, significantly reducing absorption in the gut. Effacement of microvilli and pedestal development is an early event in EPEC mediated signalling. Tyrosine kinase activity and phosphorylation of signalling proteins associated protein caveolin, suggests that EPEC pathogenesis involves binding to host receptors and requires recruitment of numerous host proteins that have functions in signal transduction, endocytosis and cytoskeletal architecture.

Young children. Recent research into the pathogenic mechanism of these organisms has revealed that initial attachment to the microvilli of small intestinal enterocytes leads to the development of a characteristic attaching and effacing (AE) ultrastructural lesion. Infected cells eventually die and are lost from the villus surface, significantly reducing absorption in the gut. Effacement of microvilli and pedestal development is an early event in EPEC mediated signalling. Tyrosine kinase activity and phosphorylation of signalling proteins associated protein caveolin, suggests that EPEC pathogenesis involves binding to host receptors and requires recruitment of numerous host proteins that have functions in signal transduction, endocytosis and cytoskeletal architecture.

Immune Response to Chronic Disseminated Candidosis: Evidence for Defective Monocyte Function

R. A. Barnes, C. H. Poynton and M. Williams

Emerging immunological evidence supports the hypothesis that several intracellular pathogens may survive within the host by modulating the T-helper cell cytokine response from Th 1 to Th 2. A number of mechanisms have been postulated including down-regulation of co-stimulatory molecules on antigen presenting cells such as those of the B7 family. Lack of B7 expression in the context of antigen presentation is thought to result in a down-regulatory Th 2 response. Chronic disseminated candidosis (CDC, also known as hepatosplenic candidosis) is a specific syndrome that usually occurs as white cells are regenerating following myelosuppressive therapy. It is characterised by persistent fever and fungal infection of the liver and spleen, associated abdominal pain, elevated alkaline phosphatase and characteristic CT scan defects in the liver and spleen with survival of the fungus within these lesions. Down-regulation of the immune response by fungal mannoproteins is thought to be involved in the pathogenesis. We measured macrophage function in patients with chronic systemic fungal infection and attempted to correct the defect with some of the up-regulatory cytokines of the Th 1 family (GM-CSF, IFN-gamma, TNF, IL1, IL2). Four patients with chronic fungal infection (3 CDC, one invasive aspergillosis) who had failed therapy with conventional and liposomal amphotericin B received GM-CSF +/- IFN-gamma. Two patients had a complete microbiological and clinical response, one had a partial response and one patient responded but died of their underlying disease. Continued response in the absence of antifungal treatment was seen in one patient. Pre-cytokine treatment, macrophage function and respiratory burst activity was markedly reduced in vitro. This could be reversed by exogenous cytokines and following treatment, supranormal respiratory burst activity was noted. We are attempting to correlate the extent of these defects with quantitative B7 expression on circulating monocytes from infected patients.

MECHANISM OF ENTEROPATHOGENIC ESCHERICHIA COLI PATHOGENICITY; HIJACKING OF HOST CELL SIGNAL TRANSDUCTION

T. J. Baldwin

Microbial Pathogenicity Research Group, Institute of Infections and Immunity, University Hospital, Nottingham NG7 2UH

Enteropathogenic Escherichia coli (EPEC) are frequently defined as non-invasive, non-toxigenic isolates belonging to restricted serotypes, which are epidemiologically associated with severe persistent diarrhoeal disease in infants and young children. Recent research into the pathogenic mechanism of these organisms has revealed that initial attachment to the microvilli of small intestinal enterocytes leads to the development of a characteristic attaching and effacing (AE) ultrastructural lesion. Infected cells eventually die and are lost from the villus surface, significantly reducing absorption in the gut. Effacement of microvilli precedes intimate attachment of bacteria to the plasma membrane of the enterocyte and the formation of pedestal like structures beneath the sites of bacterial attachment. The major component of pedestal structures is filamentous actin in association with myosin and other actin binding proteins. Effacement of microvilli and pedestal development is dependent on elevations in intracellular calcium in the vicinity of attached bacteria. This calcium originates from host cell intracellular stores, indicating bacterial activation of host signal transduction pathways. A view further strengthened by observations that EPEC infection results in protein kinase C (PKC) activation and myosin light chain phosphorylation. Calcium mobilisation and protein kinase C activation are regulated by second messengers generated from phosphatidylinositol lipids in the membrane by phospholipase C (PLC). These have been identified in EPEC infected cells. Analysis of EPEC infected cells has recently revealed increase in tyrosine kinase activity and phosphorylation of proteins of 85-90 kDa. These events are dependant on the product of the EPEC eaeB gene. Inhibitor studies show that tyrosine phosphorylation is essential for pedestal formation and is probably an early event in EPEC mediated signalling. These and other findings such as the involvement of the signalling proteins phosphatidylinositol-3-kinase and vesicle associated protein caveolin, suggests that EPEC pathogenesis involves binding to host receptors and requires recruitment of numerous host proteins that have functions in signal transduction, endocytosis and cytoskeletal architecture.
SYMPOSIUM: HELICOBACTER PYLORI – EVOLUTION AND REVOLUTION
Chairman: J. Philpott-Howard

CLINICAL RELEVANCE OF HELICOBACTER PYLORI INFECTION
Ian Forgacs
Department of Gastroenterology, King’s College Hospital, Denmark Hill, London SE5 9RS

Helicobacter pylori is the cause of chronic antral gastritis yet the relevance of this infection to an individual is obscure. The clinical role of H. pylori is contentious but this review will separate fact and mainstream opinion from conjecture and the maverick view. Technical advances have made easy identification of the organism possible by serology, 13C- or 14C-urea breath test or by endoscopic gastric biopsy (rapid urease test or histology). Peptic ulcer: H. pylori gastritis is associated with duodenal (95%) and gastric (75%) ulcers. The mechanism of association is unclear but lengthy remissions from ulcer follow eradication and relapse of infection is unusual. Non-ulcer dyspepsia: There is no evidence that H. pylori gastritis causes symptoms and eradication is clinically effective. Real controversy exists over the management of symptomatic patients with positive serology or breath tests in the community. Gastric cancer: Large-scale epidemiological studies indicate an increased risk of gastric carcinoma with H. pylori infection, but some populations with very high H. pylori carriage rates have low gastric cancer incidence – suggesting that infection may be a confounder. Gastric lymphoma: Eradication of H. pylori has been consistently associated with regression of MALT lymphoma of the stomach. H. pylori treatment has not proved simple but two- and (particularly) three-drug regimens for 1–2 weeks give clearance rates of 80–95%.

DETECTION OF HELICOBACTER PYLORI IN CLINICAL SPECIMENS
C. T. Keane
Department of Clinical Microbiology, Trinity College Dublin, St James’s Hospital, Dublin 8, Ireland

Evidence of Helicobacter pylori infection may be obtained by non-invasive and invasive methods. Of the former, two urea breath tests (U.B.T) are used. The 13C-UBT employs the non-radioactive 13C isotope of urea. It is thus preferred in pregnant women, children and when multiple tests are required rather than the 14C urea test which is a radioactive isotope measured with a beta scintillation counter. H. pylori infection can also be detected by serology. The enzyme linked immunosorbent assay (ELISA) is widely used. There are drawbacks to its use such as the inability to distinguish active from prior infection. Moreover, after effective treatment titres may take months to fall. It is ideal for epidemiological studies. Laboratory methods of detection and antigens used vary widely. Invasive microbiological tests on biopsy specimens include the Gram stain, urease test, culture and molecular techniques to detect the presence of H. pylori. Urease tests exploit the large quantity of urease produced by H. pylori. A gastric biopsy specimen is placed in a carrier containing urea and a pH indicator. A positive test is indicated by a colour change when urea is hydrolysed. Two new rapid urease tests have been introduced, one membrane based and the other a gel test. Culture is valuable for clinical and research purposes. Numerous solid media have been used. Many supplements have been added which probably act in protecting the bacteria by neutralising or binding toxic factors. Molecular techniques including amplification of the target DNA by the polymerase chain reaction (PCR) have been applied to H. pylori. We have used randomly amplified polymorphic DNA (RAPD) fingerprinting to distinguish between re-infection and recrudescence of infection.

HELICOBACTER DETECTION BY HISTOLOGY – A GOLD STANDARD?
J. I. Wyatt
St James’s University Hospital, Leeds

The observation by Warren, a histopathologist, that the gastric spiral organisms have the morphology of campylobacters, led to the first successful culture of ‘Gastric campylobacter-like organisms’ in 1982. Some authors still prefer the terminology ‘Helicobacter-like organisms’ where Helicobacter pylori is seen in histological sections, if there is no bacterial culture. However, by virtue of their generally high population density, characteristic morphology and position in gastric biopsies, and (usually) diffuse distribution in the stomach, histological detection of H. pylori in endoscopic biopsies is one of the most accurate diagnostic tests. Sensitivity can be improved by increasing the number of biopsies, or making multiple sections, but this is not usually required. In situations of low bacterial load (post treatment, use of proton pump inhibitors, gastric atrophy/ intestinal metaplasia), histology, like other biopsy-based tests, is less sensitive. There will also be inter-observer variation amongst histopathologists depending on experience and interest. A mixed bacterial flora may be present in hypochlorhydria, and must be distinguished from H. pylori. Occasionally H. heilmannii (Gastrospirillum) gastritis will be discovered. Recognition of H. pylori allows histopathologists to classify gastritis by aetiology; if both antrum and corpus (body) mucosa are biopsied, the distribution and severity of gastritis is determined which relates to the risk of complications of ulceration and malignancy.

ANTIMICROBIAL RESISTANCE IN HELICOBACTER
A. J. Howard
PHLS Wales, Department of Medical and Public Health Microbiology, University Hospital of Wales, Heath Park, Cardiff CF4 4XW

Helicobacter pylori shows good in-vitro susceptibility to most antimicrobial groups with the exception of glycopeptides, polymyxins, sulphonamides and trimethoprin. It is also inhibited by bismuth salts and benzimidazole proton pump inhibitors. This high level of in-vitro sensitivity does not correlate with in-vivo response and the use of any of the drugs active against helicobacter as single agent therapy for gastritis or peptic ulceration is associated with a high rate of
relapse. Factors responsible for this discrepancy may include: the large pH gradient between the gastric lumen and the mucosa; a lack of bactericidal activity; the influence of gastric mucus; the presence of non-dividing or slowly growing cells; and the development of acquired resistance. The latter is a particular problem in relation to nitroimidazoles. The emergence of nitroimidazole resistance is reduced but not eliminated when they are administered in combination with other antimicrobial agents and is a major determinant of the outcome of dual or triple therapy. The prevalence of primary resistance to nitroimidazoles varies in different populations and is probably mainly related to previous exposure to the drug. Acquired resistance to macrolides and quinolones has also been reported. Proposed resistance mechanisms were discussed.

**HELICOBACTER AND GASTRIC NEOPLASIA**

E. de Koster, J. Otero, M. Buset and M. Deltenre

Brugmann University Hospital, Van Gehuchtenplein 4, B1020 Brussels, Belgium

Gastric cancer is the world's overall second most common cancer. The incidence of *Helicobacter pylori* decreases with progressing preneoplastic lesions. The prevalence of *H. pylori* was elevated in patients with gastric cancer. Family members of patients with gastric adenocarcinoma have a higher *H. pylori* prevalence than controls; patients infected with *H. pylori* have more family members with gastric cancer. There is a higher *H. pylori* prevalence in regions or populations with high gastric cancer risk vs low-risk populations. In China and Europe a correlation was shown between *H. pylori* prevalence and gastric cancer incidence and mortality. Three nested case-control studies showed that infection with *H. pylori* increased the risk of further development of gastric adenocarcinoma, showing that *H. pylori* infection precedes the development of gastric cancer. *H. pylori* has been recognised by the International Agency for Research on Cancer as a type one carcinogen. Gastric cell proliferation is increased in parallel with inflammation. The ascorbic acid concentrating mechanism is abolished in gastritis. Ammonia, generated by *H. pylori* urease, gives rise to gastric mucosal atrophy. Salt increases the gastric cell proliferation. The organism's toxin may play a role in gastric cancer. Gastric lymphoma is rare (about 5% of all gastric tumours), but its incidence is steadily increasing. It was shown that *H. pylori* also increases the risk for low-grade as well as high-grade gastric lymphoma. Eradication of *H. pylori* has been shown to cure several cases of unequivocally proven gastric low-grade lymphoma.

**IDENTIFICATION OF SALIVARY RECEPTORS FOR HELICOBACTER PYLORI**

F. Namavar and E. C. I. Veerman

Departments of Medical Microbiology and Oral Biochemistry (ACTA), Vrije Universiteit, van der Boechorststraat 7, 1081 BT Amsterdam

*H. pylori* is a causative agent in chronic active gastritis, duodenal ulcer and presumably gastric malignancies. The mode of transmission of *H. pylori* in man is still unknown. It is suspected that this micro-organism can be transmitted orally, since *H. pylori* has been detected in various sites of the oral cavity by culture and by PCR. Oral tissues are covered by a mucus layer containing salivary mucin and attachment of bacteria to these compounds may facilitate bacterial adherence to different sites of the oral cavity. We have studied, therefore, the binding of *H. pylori* to salivary mucins. Under physiological conditions of saliva, *H. pylori* was able to bind to the high molecular weight salivary mucins and the binding was pH dependent. Sulphated-rich high molecular weight mucins from palate lingual glands were bound with the highest avidity, compared to the mucin species in sublingual and submandibular secretions. *H. pylori* bound in vitro to sulphated-Lewisa blood group antigens which is abundantly present on the sulphated salivary mucin species. Other salivary proteins such as α-amylase, lysozyme and proline-rich proteins did not bind to *H. pylori*. In conclusion, *H. pylori* possesses adhesins recognizing SO3-3-Lewisa residues which are common antigens of salivary mucins.

**POSTER PRESENTATIONS**

**PREVALENCE OF THE CAGA GENE IN HELICOBACTER PYLORI STRAINS FROM GASTRIC BIOPSY AND DENTAL PLAQUE SAMPLES**


1Department of Oral Microbiology, 2Dental Auxiliary School and 3Department of Gastroenterology, London Hospital Medical College, London, E1 2AD

The cytotoxin-associated (cagA) gene of *Helicobacter pylori* has been proposed as a virulence marker because of its strong association with the vacuolating cytotoxin of this organism. Infection with cagA+ strains results in increased gastric mucosal erosion compared to infection with cagA− strains and increases the risk for development of gastric cancer. In developed countries the oral-oral route of transmission is supported by reports of *H. pylori* in oral samples. The prevalence of cagA+ strains in matched gastric biopsy and dental plaque samples from patients undergoing gastroscopy was determined to establish whether such potentially virulent strains might be available for transmission via the oral-oral route. One gastric biopsy and three dental plaque samples were taken from each of 34 patients. The presence of *H. pylori* was determined by PCR of the urease A gene. Those positive samples were tested for the cagA gene by PCR of a 348-bp fragment of this gene. *H. pylori* was detected in 22 (64.7%) of the 34 gastric biopsies, and 9 (40.9%) of these 22 were cagA+. Ten patients had *H. pylori* in their dental plaque, of whom 7 (70%) carried cagA+ strains; four of these patients were found to harbour cagA− strains in their gastric biopsies. Of the 102 dental plaque samples examined, 20 (19.6%) were *H. pylori* positive and 14 (70%) of these were cagA+. The prevalence of the cagA gene in strains from gastric biopsy samples found in this series is lower than reported in recent studies,
but the proportion of \textit{cagA}\textsuperscript{+} strains in dental plaque is relatively high. It appears that potentially virulent strains of \textit{H. pylori} are carried in the mouths of both gastric biopsy positive and negative individuals, and such strains may be transmitted via the oral-oral route.

**EVALUATION OF PCR FOR THE DIAGNOSIS OF HELICOBACTER PYLORI INFECTION**

D. Hyde\textsuperscript{1}, M. Buckley\textsuperscript{2}, C. T. Keane\textsuperscript{1} and C. A. O’Morain\textsuperscript{2}

\textsuperscript{1}Department of Clinical Microbiology, Trinity College Dublin, St. James’s Hospital and \textsuperscript{2}Department of Gastroenterology, Meath/Adelaide Hospitals, Dublin 8, Ireland

PCR was performed on 76 paraffin embedded antral biopsies from 73 patients under investigation for dyspepsia or under review at 4 weeks after cessation of \textit{Helicobacter pylori} eradication treatment. Southern blot was performed on a representative sample of amplified products to confirm specificity. Patients were classified as being \textit{H. pylori} positive if two or more conventional tests (histology, culture, Gram’s stain and rapid urease test) were positive. Twenty-three (31\%) patients were positive and 51 (69\%) patients were negative. PCR was compared to the conventional criteria. Twenty-one patients, negative by conventional tests, were positive by PCR and eleven patients were followed up with the \textsuperscript{13}C urea breath test or endoscopy. Two patients (14\%) were subsequently positive for the bacterium. Preliminary results indicate that PCR has a high sensitivity but a low specificity. Contaminated endoscopes are the most likely source of the false positive results. We conclude that PCR has limited application for the diagnosis of \textit{H. pylori} unless the issue of endoscope decontamination is resolved.

**EFFECT OF VISCOSITY ON MOTILITY OF HELICOBACTER PYLORI**

Q. N. Karim\textsuperscript{1}, M. L. Worku\textsuperscript{2}, T. Keshavarz\textsuperscript{2}, R. P. H. Logan\textsuperscript{1}, J. H. Baron\textsuperscript{1}, R. L. Sidebotham\textsuperscript{1} and J. J. Misiewicz\textsuperscript{1}

\textsuperscript{1}Parkside Helicobacter study group, St. Mary’s Hospital London and Central Middlesex Hospital, London and \textsuperscript{2}University of Westminster, London

Helicobacter pylori (HP) has to travel through viscid gastric mucus to colonise the surface of the gastric epithelial cells. There is inadequate information on the effect that viscosity or the composition of viscous material has on the motility of HP. Dextran (grade A from BDH), sodium alginate (from BDH) and xanthan gum (from Sigma) were used as source of viscous materials of different compositions. They were dissolved in nutrient broth (Brain Heart Infusion, Oxoid) at room temperature (23\°C) to produce viscosity values of 3 and 13 mPa.s as measured by Physica ViscoLab LM. Seven strains of HP, including the reference strain 11637, were grown in conventional broth cultures. Exponential phase cultures were exposed to these three viscous substances at 3 and 13 mPa.s viscosity values, and motility was measured by the Hobson Bac Tracker, a real-time image processing computer for tracking and measuring motile bacteria. The results showed that the motility of all the strains was reduced as the viscosity increased from 3 and 13 mPa.s. Different strains were differently affected by exposure to these three substances; however, motility was most affected by xanthan gum for all the strains studied.

**MEASUREMENT OF HELICOBACTER PYLORI MOTILITY IN DIFFERENT GROWTH PHASES IN CONVENTIONAL BROTH CULTURES BY REAL TIME COMPUTER TRACKING**


Parkside Helicobacter study group, St. Mary’s Hospital London and Central Middlesex Hospital, London

Motility is an important colonisation factor in \textit{Helicobacter pylori} (HP), yet remains largely unexplored. We have applied an image processing technology to study HP motility. The microscopic images of the bacteria are recorded by a video camera and tracked by the computer. The movements of 120 bacteria can be simultaneously and continually tracked in real time, and measures of motility seen as histograms or trail draws on the screen. The motility of HP in 27 broth cultures obtained from 14 patients with duodenal ulcer and 13 with non-ulcer dyspepsia were examined for motility daily with the Hobson Bac Tracker for 23 days. They showed a similar pattern of motility being slow in the lag phase, median curvilinear velocity (CVL) 6.9 \mu m/sec (range 1–6); were actively motile with a variety of movement, in the mid log phase, median CVL 26.8 \mu m/sec (range 1–113); with slowing in the stationary phase, median CVL 7.3 \mu m/sec (range 2–40). There was no significant difference in motility between the two disease groups. Motility was greater in spiral or rod shaped HP that stained intensely red on Gram’s stain. HP are actively motile for a short phase in their growth cycle, and the same bacteria may appear as motile or non motile depending on their growth phase.

**EPSTEIN-BARR VIRUS (EBV) INFECTION IN THE COMMON MARMOSET: EFFECT OF IMMUNISATION WITH EBV ENVELOPE GLYCOPROTEIN (gp)340**

C. Cox\textsuperscript{1,3}, J. R. Arrand\textsuperscript{2}, M. Mackett\textsuperscript{2}, B. Griffin\textsuperscript{1} and N. Wedderburn\textsuperscript{1,3}

\textsuperscript{1}The Royal College of Surgeons of England, London, \textsuperscript{2}Patterson Institute of Cancer Research and Christie Hospital, Manchester, and \textsuperscript{3}Royal Postgraduate Medical School, London

EBV causes long term infection in the common marmoset. Prolonged seropositively accompanies viral shedding into buccal fluids and the ability to transfer infection to naive animals. 139 samples of ‘whole mouth fluid’ (WMF) from 21 EBV infected animals were analysed by PCR, after removal of inhibition by Chelex\textsuperscript{8} 100; 79\% were positive. Five marmosets received three doses of 30\mu g of gp340 from a bovine papillomavirus expression vector, plus Alhydrogel, and six received Alhydrogel only. EBV was injected into Waldeyer’s ring 4 and 16 weeks after the last vaccine dose. PCR of WMF, with primers specific for the viral repeat sequence, \textit{Bam}HI W, was positive in all animals 4 weeks after the first EBV infection and in none of five immunised and three of six non-immunised, at 12 weeks. Over 11 months, 43\% of WMF samples from immunised, and 74\% from non-immunised animals were positive for EBV DNA. Twelve marmosets received EBV, with oral cyclosporin A (60 mg/kg p.d.) for 30 days; 10 of 12 WMF were positive for EBV by PCR, before subsequent immunisation. Over 9 months, 44\% of WMF samples from immunised, and 73\% from non-immunised, animals were PCR positive. These results indicate that immunisation with
gp340, either before or after infection, can reduce viral shedding in buccal fluids of EBV-infected marmosets.

SMOKING AS A RISK FACTOR IN DISEASE: A POSSIBLE NEW MECHANISM
W. G. F. Ditcham, J. A. Leigh, A. S. Blinkhorn
Oral Health and Development Unit, Turner Dental School, University of Manchester, Manchester M15 6FH

Smoking is a risk factor in a number of diseases. For example, the relative risk from Sudden Infant Death Syndrome (SIDS) is greatly elevated with increased exposure to nicotine. Our recent work has shown that nicotine can act synergically with the extracellular products of both gram-positive and gram-negative bacteria isolated from SIDS victims. The aim of this study was to assess whether synergy between nicotine and bacteria could be a general phenomenon in other bacterial diseases. To achieve this, extracellular and lysed-cell products of the putative periodontopathogens Porphyromonas gingivalis and Prevotella intermedia were tested with and without nicotine in the chick embryo assay. Nicotine significantly enhanced toxicity of extracellular toxins (Pr intermedia, p = 0.00005; P gingivalis, p = 0.00002). There was also synergy with cell lysates of P gingivalis (p = 0.024). We conclude that diseases in which the pathological condition involves bacteria might be exacerbated by nicotine. This would be achieved via the potentiation of bacterial toxins by nicotine.

AMINO ACID REQUIREMENTS OF STREPTOCOCCUS UBERIS AND THE UTILIZATION OF PLASMIN DERIVED CASEIN PEPTIDES
A. J. Kitt and J. A. Leigh
Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berks RG20 7NN, UK

Streptococcus uberis is capable of infecting the lactating bovine mammary gland and is responsible for around 30% of all clinical episodes of mastitis in the UK. This organism grows in an environment in which the availability of free amino acids and peptides is growth limiting. Despite this S. uberis does not produce protease activity but does synthesise a protein capable of activating bovine plasminogen to the serine protease, plasmin.

The amino acids required for growth of four strains of S. uberis were determined by the single omission of each amino acid from a chemically defined medium. All strains had a requirement for eight amino acids: L-glu, L-arg, L-trp, L-met, L-ile, L-leu, L-val and L-his. In addition, there were strain specific requirements for: L-phe, L-tyr, L-ser, gly and L-cys. The common requirement for eight amino acids was shared by a further eight strains. The ability of S. uberis to acquire amino acids from plasmin-derived casein peptides was demonstrated. Further investigation of this route of nutrient acquisition may identify key processes and bacterial molecules involved in the interaction between bacterium and host which enable growth of S. uberis in vivo.

ADHERENCE OF STREPTOCOCCUS UBERIS TO EPITHELIAL CELL CULTURES DERIVED FROM THE BOVINE MAMMARY GLAND
J. A. Leigh, A. P. Bland and A. W. Hill
Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berks RG20 7NN

Streptococcus uberis is capable of infecting the lactating bovine mammary gland and is responsible for around 30% of all clinical episodes of mastitis in the UK. The role of adherence in the pathogenesis of bovine mastitis caused by S. uberis has not been established. The ability of two bacterial strains to adhere to epithelial cells derived by primary culture of isolated secretory alveoli was examined by scanning electron microscope. The cultured monolayers consisted of two types of epithelial cell, one of which possessed many microvilli (MV+) and another which exhibited only sparse or no microvilli (MV−). Both strains adhered equally to MV+ cells and more readily to the MV− cells. This was most apparent for strain EF20 (low virulence) and was reflected by both greater numbers of bacteria adherent per MV− cell and interaction with a greater proportion of MV− cells than MV+ cells. The slightly increased adherence to MV− cells by strain 0140J (high virulence) was reflected only by the interaction with a greater proportion of the cells of this type and not adherence of a greater number of bacteria per cell. Unlike resistance to phagocytic killing and growth in milk, an increase in ability to adhere to host tissues does not correlate with the differing virulence of these two strains.

AN EPIDEMIOLOGICAL STUDY OF CLOSTRIDIUM DIFFICILE ISOLATES IN THE GREATER MANCHESTER AREA TYPED BY RANDOM AMPLIFICATION OF POLYMPHORIC DNA (RAPD)
Helen C. Duckworth, Karen W. Loudon and James P. Burnie
Department of Medical Microbiology, 2nd Floor, Clinical Sciences Building, Manchester Healthcare Trust, Oxford Road, Manchester M13 9WL

Clostridium difficile is well established as a major enteric pathogen in hospitals causing pseudomembranous colitis (PMC), antibiotic-associated colitis (AAC), and diarrhoea. It produces significant morbidity and mortality in hospitalised patients. Recently there have been several outbreaks due to C. difficile in the Greater Manchester area. The investigation and management of such outbreaks depends on the availability of typing methods for C. difficile to establish if a single epidemic strain is responsible and if cross-infection has occurred. So far the epidemiological study of these outbreaks has been hindered by the lack of a universally accepted typing method. We used random amplification of polymorphic DNA (RAPD) to type 100 strains of C. difficile from the Greater Manchester area. Common strains were found in Manchester Royal Infirmary and North Manchester Hospital. RAPD was found to be a rapid and simple typing method. It is easy to implement as there are now PCR facilities available in most laboratories. Our results suggest that RAPD could be a valuable tool for epidemiological studies of this organism.

THE ROLE OF HSP 90 AS A VIRULENCE ENHANCING FACTOR IN DISSEMINATED CANDIDOSIS
Samantha J. Hodgetts and Ruth Matthews
Department of Medical Microbiology, 2nd Floor, Clinical Sciences Building, Manchester Healthcare Trust, Oxford Road, Manchester M13 9WL

Candidosis is now the fourth most common hospital acquired infection in the USA, accounting for almost 80% of nosocomial fungal infections. Currently the mainstay for therapy is amphotericin B (often liposomal, to reduce toxicity) or fluconazole (but fluconazole resistance is
It has been demonstrated previously that mice infected with a lethal dose of *Candida albicans* were protected when given serum or immunoglobulin from patients recovered from systemic candidosis containing antibodies to hsp 90, but not when given normal human serum. Protection was also observed with a human recombinant antibody or a murine monoclonal antibody to LKVIRK – an immunodominant conserved epitope of hsp 90 recognised by patients recovering from systemic candidosis. Injection (i.v) of (1) parental *Saccharomyces cerevisiae*, or (2) an *S. cerevisiae* clone which over-expresses hsp 90 tenfold, into mice demonstrated that clearance of the latter from multiple organs was delayed, as shown by the number of mice culture-positive and cell counts taken from specific organs. The possibility that hsp 90 enhances the virulence of *C. albicans* infections was investigated further and these results were discussed.

**LOCALISATION OF THE ANTIGENIC DETERMINANTS ON THE FIMBRIAL PROTEINS OF *BORDETELLA PERTUSSIS* WITH PATIENTS' SERA**

Peter Williamson and Ruth Matthews

*Department of Medical Microbiology, 2nd Floor, Clinical Sciences Building, Manchester Healthcare Trust, Oxford Road, Manchester M13 9WL*

Control of whooping cough depends primarily on prevention with whole-cell vaccine. The putative link between whole-cell vaccination and encephalopathy and problems with local and sometimes systemic reactivity, has led to pressure to produce acellular vaccines. At least six components of *Bordetella pertussis* have been identified as important in providing immunity and acellular vaccines containing different combinations of these components have been produced. This study was focused on the two major fimbrial proteins (Fim2 and Fim3) of *B. pertussis*. The sequences of the proteins were synthesised on solid supports as nonapeptides, overlapping by eight amino acids. These peptides were screened, using the Pepscan technique, with 12 infected and 4 vaccinated patients' sera to identify epitopes reactive with human antibody. Eight major epitopes were identified on Fim2 and ten on Fim3. The epitopes most commonly reactive with IgG were not the same as those which were immunodominant with serum IgA. Vaccinated patients produced no detectable IgA. Use of paired sera confirmed that reactivity was associated with seroconversion to pertussis and not merely due to cross-reactive existing antibodies. These epitopes have been resynthesised as unbound peptides and are being used in an ELISA test to screen more patients' sera to assess their use in diagnosis.