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IMPACT OF HIB VACCINE

Mary P.E. Slack1, P. Heath2, R. Booy1, N. Begg3, E.R. Moxon1

1 Haemophilus Reference Laboratory, Public Health Laboratory, John Radcliffe Hospital, Headington, Oxford, OX3 9DU.
2 Department of Paediatrics, John Radcliffe Hospital, Headington, Oxford, OX3 9DU.
3 PHLS, Communicable Diseases Surveillance Centre, 61 Collindale Avenue, London, NW9 5EQ.

Routine immunisation of children against Haemophilus influenzae type b (Hib) began in the UK and Republic of Ireland in October 1992. Hib vaccine has been well received and vaccine coverage is high (90%) in children aged 12 months. Annual laboratory reports of invasive Hib disease in children < 4 years have declined by 90 - 95%.

A prospective study to monitor Hib vaccine failure was initiated in October 1992 by the P.H.L.S., the Oxford Vaccine Group and the British Paediatric Surveillance Unit. Paediatricians and Microbiologists are asked to report invasive H. influenzae disease in any child < 10 y who has received any Hib vaccine. H. influenzae isolates are verified by the Haemophilus Reference Laboratory. Acute and convalescent sera are also collected. A case of invasive Hib disease is designated a True Vaccine Failure (TVF) if it occurred after at least 2 doses of Hib in the first year of life or after the single dose given to children > 12 months of age. In the first 2 years of the study there have been 23 TVF. Thirteen were cases of meningitis and 6 were epiglottitis. Five were born prematurely (median age 32 weeks) and 3 had Down's syndrome. There have been 50 apparent vaccine failures where the infection occurred before protection could reasonably be expected to have developed. In addition there have been 18 cases of non-encapsulated H. influenzae invasive disease and 6 infections caused by other serotypes of H. influenzae in immunised children. Active surveillance for vaccine failure is a vital part of any immunisation programme. Hib immunisation has resulted in a dramatic decline in disease and vaccine failures are rare.

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THE INFLUENCE OF CONJUGATE VACCINATION ON THE CARRIAGE OF HAEMOPHILUS INFLUENZAE TYPE B (HIB).

ML Barbour
Department of Paediatrics, John Radcliffe Hospital, Oxford.

Conjugate vaccines against Haemophilus influenzae type b (Hib) may modify Hib pharyngeal colonisation. We performed two controlled studies to assess this in Oxfordshire.

The first study compared the prevalence of Hib carriage and the concentrations of serum IgG antibody to the type b capsular in 120 four year old children who had been involved in an immunogenicity study of the HM)C vaccine. Sixty children (vaccinees) had received the HM)C vaccine at the ages of three, five, and seven months of age. Additionally, six unvaccinated Hib carriers were vaccinated, then swabbed weekly for six weeks. The Hib acquisition rate was lower in vaccinees than controls (p < 0.01). During surveillance 1.5% vaccinees and 6.3% controls carried Hib (p = 0.04), and among those with family Hib exposure the carriage rates were 8.7% and 38.5% (p = 0.07) respectively. Hib carriage rates were lower among vaccinees' unvaccinated siblings. Giving conjugate vaccine to an unvaccinated Hib carrier did not rapidly terminate carriage.

We conclude that: i) conjugate vaccination may reduce or delay the initial acquisition of Hib, ii) children who have received vaccine do acquire the organism, but immunisation primes for a booster response on exposure to Hib, iii) there may be a relationship between previous conjugate immunisation and the density of Hib colonisation in children. A delay in acquisition of Hib in infancy, a reduction in organisms carried or shortening of the duration of carriage may all contribute to the potential for herd immunity to Hib in a vaccinated population.

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THE CHALLENGE OF MENINGOCOCCAL INFECTIONS

E R Moxon
Oxford University Department of Paediatrics, John Radcliffe Hospital, Oxford OX3 9DU

Neisseria meningitidis is a major cause of meningitis and septicaemia and results in substantial morbidity and mortality, especially in children.

Invasive N. meningitidis infections occur on a worldwide basis and may be epidemic (e.g. African "meningitis belt", China, Norway) or sporadic.

There are three major serogroups (defined by the capsular polysaccharides), designated A, B and C. Prevention of N. meningitidis diseases through immunisation is already a possibility for serogroups A and C, although improvements in these vaccines are needed. The major problem is in developing successful vaccines against serogroup B strains. This presentation will review the status and prospects for the several vaccines which are currently available, under investigation or in the process of development.

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WHERE VIRAL VACCINATION MEETS GENE THERAPY

G.W.G. Wilkinson
Department of Medicine, University of Wales College of Medicine, Cardiff CF4 4XX, UK.

Live viral vaccines have had a major impact world-wide on a limited number of viruses diseases. Certain features of live vaccines make them particularly effective: (i) they can generate humoral, cell mediated and mucosal immunity, (ii) they are inexpensive to produce and (iii) elicit long-lived protection. However, not all potential vaccine targets are amenable to this approach. Replication-competent viral vector systems have been heavily exploited over the last decade to study the immunobiology of virus disease and recently both vaccinia virus and adenovirus recombinants have been used in human clinical trials. A technology aimed at in vivo gene delivery is being developed for gene therapy. The demands made by gene therapy on its vectors are extreme: the transgene has to be efficiently delivered to a target cell population, be non-toxic and expressed for an appropriate time and level to correct a disease phenotype. For vaccination it is usually sufficient for a protein to be expressed long enough to induce an immune response. Replication-deficient viral vectors and novel systems for the direct transfer of naked DNA are being exploited both in gene therapy and vaccine strategies. We have concentrated on using replication-deficient adenovirus recombinants both to elicit and monitor immune responses. In vivo gene delivery systems are proving to be extremely efficient vehicles for priming both humoral and cell-mediated immunity. The induction of such immune responses while being the object of a vaccination represent a potential hazard for gene therapy.
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CYTOTOXIC T LYMPHOCYTE INDUCING VACCINES AGAINST MALARIA

Adrian V.S. Hill
Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU.

No effective vaccine is available for any parasitic disease of humans. The design of such a vaccine presents particular challenges but one new approach is based on the analysis of peptides bound to HLA molecules. I shall outline how we have applied this approach to identify components of a new cytotoxic T lymphocyte (CTL) inducing vaccine against malaria. However, a similar approach may be useful in designing vaccines against other complex pathogens.

A central challenge in the design of effective subunit vaccines is the identification of protective antigens and epitopes in infectious microorganisms. Our approach to this issue, in the case of Plasmodium falciparum malaria, has been based on the analysis of peptides eluted from HLA molecules. By analogy with 'reverse genetics', a term used to describe the identification of a disease gene product by localisation of its gene, we have termed this process 'reverse immunogenetics'.

After identifying an association between the HLA class I antigen, HLA-B53, and resistance to severe malaria, we predicted that HLA class I restricted cytotoxic T lymphocytes (CTL) were likely to play a protective role in this disease by acting against the liver-stage of the parasite's life-cycle. To test this prediction, we set out to identify and characterise CTL in Gambians naturally exposed to malaria. We focused initially on CTL restricted by HLA-B53. We eluted self-peptides from this molecule and sequencing of these indicated a peptide binding motif consisting of proline at position 2 and a hydrophobic amino acid at position 9 of bound peptides. We then synthesized peptides corresponding to this motif from the four sequenced pre-erythrocytic antigens of P. falciparum and tested them for binding to HLA-B53. Although peptides from all four antigens were found to bind with high affinity, only one peptide, from liver-stage-specific antigen-1 (LSA-1), was the target of CTL in Gambians exposed to malaria.

We have recently extended this work to identify malaria CTL epitopes for several common HLA class I antigens that are found in the great majority of both Gambian and caucasian individuals. The present challenge is to find a suitable delivery system for inducing high levels of CTL in humans safely and some exciting new approaches to this question will be discussed.

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A CAPSULE-LIKE STRUCTURE OF A NON-TYPABLE HAEMOPHILUS INFLUENZAE REPRESENTS A LAYER OF GLYCOPROTEIN.

D.M. MacLaren1, F. Namavar1, B.J. Appelmelk1, D.M. Jones2, and A. Curry2
1 Dept. of Medical Microbiology, Vrije Universiteit, Amsterdam. 2 Public Health Laboratory, Withington Hospital, Manchester M20 8LR.

O-lactamase positive Haemophilus influenzae strains isolated from a nosocomial outbreak of respiratory infections belonged to three clones on the basis of DNA restriction enzyme analysis. One clone (A) appeared to be non-capsulate by India-ink staining, whereas the other two clones (B and C) seemed capsule. All three were non-typable in Pittman antisera, and were non-iridescent on Fildes agar. Under electron-microscopy B and C possessed a capsule-like structure that stained with Alcian blue/glutaraldehyde, but not with Ruthenium red. In immuno-electrophoresis B and C showed precipitation lines towards the positive pole as did type A. The "capsular" antigen of B seemed heat-labile and was sensitive to periodate and proteinase K. We conclude it is a layer of glycoprotein. Its role in adherence will be studied.

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COMPARISON OF DIFFERENT RIBOTYPING METHODS FOR HAEMOPHILUS INFLUENZAE

J. Zoe Jordens and N. I. Leaves
Haemophillus Reference Laboratory, Oxford Public Health Laboratory, Level 7, John Radcliffe Hospital, Headington, Oxford OX3 9DU.

Ribosomal RNA genes (rDNA) are used as target sequences in a variety of bacterial typing techniques. Ribotyping is a widely used technique and probes for this have been generated by several different methods. A more rapid technique based on rDNA involves amplification of 16S rDNA followed by digestion of the product with restriction enzymes. The relationship between the techniques has not been studied.

The present study characterised 39 isolates of Haemophilus influenzae by ribotyping with two different probes, a cDNA probe generated by reverse transcription of 16S + 23S rRNA and a PCR-generated 16S rDNA probe. The cDNA probe and 16S probe produced 24 and 22 different patterns respectively. The cDNA probe differentiated between a clone with reduced susceptibility to beta-lactam antibiotics and a susceptible isolate whereas the 16S probe did not. Four strains which produced distinguishable ribotyping patterns with both probes were also characterised by digestion of PCR amplified 16S rDNA. The four strains were indistinguishable by this technique using five enzymes.

The present study showed that ribotyping with a 16S + 23S cDNA probe is more discriminatory for H. influenzae than a probe for 16S rDNA alone and also that the heterogeneity identified with these techniques concerns flanking restriction sites and not sites within rDNA. This study also highlights the need for standardisation of ribotyping protocols.

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CHARACTERISATION OF NON-CAPSULATE HAEMOPHILUS INFLUENZAE PRIOR TO THE NATIONAL INTRODUCTION OF HIB VACCINE

N.I. Leaves, J. Zoe Jordens
Haemophillus Reference Laboratory, Oxford PHL, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK.

Recently, Haemophilus influenzae serotype b (Hib) vaccination of infants was implemented in the UK and this has led to a dramatic decline in the incidence of Hib disease. However, non-capsulate Hib influenzae (NCHi) is a well recognised cause of severe disease in both children and adults and will probably remain unaffected by the introduction of the vaccine.

For a six month period prior to the implementation of vaccine, all isolates of NCHi (n=128) received by the Haemophilus Reference Laboratory were characterised by biotyping, antibiogram, outer-membrane protein (OMP) profiling and ribotyping. The purpose of this study was to describe the pre-vaccine population of NCHi thus permitting a comparison of post-vaccine isolates using similar methods.

The study showed that the NCHi population is heterogeneous, consisting of multiple clones. A statistical measure of discrimination (Simpson's index of diversity) revealed that biotyping combined with either antibiogram, OMPs or ribotyping was highly discriminatory for this collection of NCHi with indices of 0.901, 0.992 and 0.990 respectively. When all four techniques were combined, the results were even more discriminatory with an index of 0.999. These methods are therefore highly descriptive of the pre-vaccine population and so may prove suitable markers of any changes which may occur following the introduction of the Hib vaccine.
NEW LIPOPOLYSACCHARIDE SEROTYPES OF SERRATIA MARCESCENS

H.M. Aucklen and T.L. Pitt
Laboratory of Hospital Infection, Central Public Health Laboratory, 61 Colindale Ave. London, NW9 5HT, UK

A new O serotyping scheme has been developed for S. marcescens because it has been shown that the current scheme reflects capsular antigens as well as true O or LPS types. The scheme is based on the chemical structures of the LPS neutral polysaccharides (as determined by Professor Wilkinson's team), as well as the ELISA reactions of appropriately absorbed antisera, and comprises 17 serotypes. It was used to type a collection of 423 clinical (patient and hospital environment), 57 insect and 32 environmental (animal, plant and soil) strains. This scheme has improve typability from 72% to 87%, and increased discrimination from 0.639 to 0.866 (Simpson's index of discrimination). When combined with the new K typing scheme and a secondary typing method such as phage-typing, reliable epidemiological data can readily be obtained.

The results showed a radically different distribution of LPS types among the two groups of strains:

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Insect</th>
<th>Environmental</th>
</tr>
</thead>
<tbody>
<tr>
<td>O6</td>
<td>4%</td>
<td>29%</td>
</tr>
<tr>
<td>O8</td>
<td>4%</td>
<td>22%</td>
</tr>
<tr>
<td>O14</td>
<td>33%</td>
<td>0%</td>
</tr>
<tr>
<td>O28</td>
<td>6%</td>
<td>13%</td>
</tr>
</tbody>
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Thus the distribution of serotypes in hospitals is not a simple reflection of the serotypes in the natural environment, suggesting that O14 strains are better able to respond to the selective pressures imposed by the hospital environment.

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PCR DROP AMPLIFICATION AND ANALYSIS OF A 320 bp FRAGMENT OF OmpR FROM Neisseria meningitidis

D B Wells, B W Wren, S P Borriello.

Microbial Pathogenicity Research Group, Department of Microbiology and the Institute of Infections and Immunity, Queen’s Medical Centre, Nottingham NG7 2UH, and Department of Medical Microbiology, St. Bartholomew’s Hospital, London EC1A 7BE.

N. meningitidis is a major cause of bacterial meningitis with approximately 80% of cases being observed in children under five years of age. Bacteria spread rapidly throughout the body resulting in the colonisation of three main sites, the nasopharyngeal mucosa, the blood and the CSF. N. meningitidis is able to sense and adapt to environmental stimuli such as nutrient availability which may increase survival potential in specific microenvironments encountered. Many of the identified mechanisms for sensing environmental changes involve two component regulator systems which may also be involved in virulence regulation. Deletion mutants in one such response regulatory ompR has been shown to severely impair virulence in Salmonella typhi and Salmonella typhimurium.

Comparisons of published amino acid sequence data from several global regulatory proteins reveals conserved regions between the different families. By constructing degenerate oligonucleotide primers to these regions of patch homology we have been able to amplify a 320 base pair fragment from N. meningitidis chromosomal DNA. This potential OmpR homologue was cloned into the vector pUC19 and sequenced. Comparison of the sequence obtained, with known sequences from E. coli and S. typhimurium showed a 80% and 75% homology respectively. Southern hybridisation analysis of restricted Neisseria meningitidis chromosomal DNA revealed a single band for each enzyme used. This information will be useful for cloning the whole gene and subsequently constructing isogenic deletion mutants.

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COMPARATIVE STUDY OF FUSOBACTERIUM NECROPHORUM STRAINS FROM ANIMAL AND HUMAN SOURCES

V Hall, B I Duerden, J T Magee, H C Ryley, J S Brazier
PHLS Anaerobe Reference Unit and Department of Medical Microbiology, University Hospital of Wales, Cardiff CF4 4XW.

Fusobacterium necrophorum causes necrobacillosis in man and animals. Although indistinguishable by conventional tests, there is evidence that distinct host-specific sub-species may occur. Biovar A strains are pathogenic for mice, macropods and other animals while biovar B strains show little pathogenicity for these species. A collaborative study was co-ordinated by the PHLS Anaerobe Reference Unit (ARU), Cardiff to investigate the phenotypic and genotypic properties of F. necrophorum strains originating from human and animal sources. Fifty strains comprising 21 human clinical isolates, 24 animal isolates, 1 NCTC strain and 4 duplicates were blind-coded and distributed to each of six participating laboratories for examination by various methods.

The ARU tested morphological characteristics, gas-liquid chromatography of metabolic end-products, carbohydrate fermentation, pre-formed enzymes, pyrolysis mass-spectrometry and SDS-PAGE of whole cell proteins.

In conventional tests all strains identified as F. necrophorum but differences in colonial and cellular morphology were observed. For each isolate results of conventional tests were converted to a 23 digit numerical code and subjected to cluster analysis. Human strains (biovars unknown) formed a cluster with biovar B animal strains. Biovar A animal strains clustered separately. Cluster analysis of pyrolysis mass-spectrometry data and SDS-PAGE data revealed similar groupings.

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ADHERENCE CHARACTERISTICS OF COAGULASE-NEGATIVE STAPHYLOCOCCI FROM ENDOCARDITIS

R G A Cree, I Philips, W C Noble
Division of Microbiology, United Medical and Dental Schools, St Thomas’ Hospital, London, SE1 7EH, UK

A collection of 95 coagulase-negative staphylococci, chiefly S. epidermidis, isolated from patients with endocarditis was examined using tests believed to relate to the ability to adhere to plastics and tissue. These strains could be divided into native valve or prosthetic valve endocarditis or into hospital acquired or community acquired strains, though most native valve isolates were community acquired and the reverse was true for prosthetic valve strains. A collection of similar strains from line associated bacteraemia was used as controls.

Bacteraemia strains differed from endocarditis strains in that they were more likely to produce slime, were more hydrophilic but less likely to adhere to Hep2 tissue culture cells. Amongst the endocarditis strains there was little difference in slime production but hospital acquired or prosthetic valve strains were more hydrophobic and more likely to adhere to catheter silicone than were native valve or community acquired strains. Adherence to Hep2 cells, fibronecin or laminin were more frequently found in strains from community acquired or native valve strains. This may reflect the exposure of extracellular matrix proteins prior to clinically apparent infective endocarditis selecting strains or variants capable of adhering.
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**PSEUDOMONAS AERUGINOSA INFECTIONS ASSOCIATED WITH ERCP DISINFECTOR WASHERS**

G F S Edwards, D J Platt, E T Curran, R Wilson, J Hood

Department of Microbiology, Glasgow Royal Infirmary, G4 0SF

Between November 1991 and July 1994 a strain of *Pseudomonas aeruginosa* identifiable by its pyocin type and unusual antibiogram was isolated from the blood of 6 patients and from other sites of a further 11. All 17 had undergone endoscopic retrograde cholangiopancreatography (ERCP). In the ERCP suite the endoscopes were disinfected with gluteraldehyde and later, 1,000 ppm hypochlorite were used to disinfect all the machine's channels before lists. The outbreak was only controlled when other aspects of endoscope use were changed. Disposable or autoclavable equipment is now used in the manual cleaning of the endoscopes before disinfection, accessories such as the bottles that hold irrigation fluid are autoclaved rather than disinfected and the nurse who cleans the endoscope before disinfection does not handle it afterwards.

Initial, unsuccessful, attempts to control the outbreak were directed at the disinfection process. Alcohol drying of endoscope channels was carried out. Gluteraldehyde and, later, 1,000 ppm hypochlorite were used to disinfect all the machine's channels before lists. The outbreak was only controlled when other aspects of endoscope use were changed. Disposable or autoclavable equipment is now used in the manual cleaning of the endoscopes before disinfection, accessories such as the bottles that hold irrigation fluid are autoclaved rather than disinfected and the nurse who cleans the endoscope before disinfection does not handle it afterwards.

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**MENINGOCOCCI BIND MORE IRON-RICH (HOLO) THAN IRON-FREE (APO) TRANSFERRIN AND EXPRESS MORE TRANSFERRIN BINDING PROTEIN 2 (TBP-2) THAN TBP-1.**

N.B.L. Powell, D.A. Alataloon, A.B. Schryvers, S.P. Borriello

1 Microbial Pathogenicity Research Group, Department of Microbiology, and Institute of Infections and Immunity Queen's Medical Centre, Nottingham, NG7 2UH, U.K.

2 Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada.

It is generally considered that meningococci cannot differentiate between iron-rich (holo) and iron-poor (apo) transferrin. We are of the opinion that it would be advantageous for meningococci to be able to do so, and re-examined this question. Apo-transferrin and holo-transferrin were separately conjugated to [55S]sodium chloride gold binding SD (B15P1.16) bound 74% more holo-HTF than apo-HTF (P=0.0014).

In addition we used holo and apo-gold labelled transferrin (Au-HTF) to investigate the relative distribution and ratio of TBP-1 and TBP-2 on the surface of isogenic mutants of N16, B2aP1.2. Strain N97 (TBP-2 mutant) bound as much gold-labelled transferrin as the parent strain, whereas strains N91 (TBP-2 mutant) and N96 (TBP-1 & TBP-2 mutant) were less effective at binding gold-labelled transferrin (P>0.001). The TBP-2 expressing mutant bound twice as much Au-HTF as the TBP-1 expressing mutant. Also, inhibition studies of Au-HTF binding to strain SD using polyecylonal anti-TBP1/2, suggest that the ratio of TBP-2:TBP-1 is approximately 2:1.

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**HEPATITIS C ANTIBODIES IN INJECTING DRUG USERS IN MERSEYSIDE 1971 - 1991.**


* Public Health Laboratory Service, Fazakerley Hospital, Liverpool, L9 7AL

**Infectious Diseases Unit, Fazakerley Hospital, Liverpool, L9 7AL

++Department of Medical Microbiology, Royal Liverpool Hospital, Liverpool, L7 8XP.

++Liverpool John Moores University, School of Biomolecular Sciences, Liverpool L3 3AF

HBV and latterly HCV have been recognised as a major risk factor for intravenous drug users (idus). Merseyside has a large group of idus's with one of the highest rates of HBV transmission currently reported. HBV-positive sera, stored at -20°C since 1970, was recovered and tested for the presence of HCV antibodies by Elisa testing. Sera giving equivocal results were tested by a second Elisa test. Sera which proved to be repeatedly positive were tested by PCR to confirm the result, and also tested for the HCV strain.

The sera recovered from 1971-72 consisted of 23 HBV-positive idus (15 male and 8 female) and a control group of 24 HBV-positive BTS sera sent for marker testing at that time. Age and sex-matched stored sera for both idus and BTS patients from 1981-82 and 1991-92 were recovered and tested in parallel.

Results showed that 54% of HBV-positive idus were HCV positive in 1971-72, 36% in 1981-81 and 40% in 1991-92 compared with none for BTS sera in 1971-72, none in 1981-82 and only 4% in 1991-92. HCV infection has been present idu’s on Merseyside for over 2 decades, and has remained apparently constant.

**SYMPOSIUM: THE MOLECULAR BASIS OF DRUG RESISTANCE**

Chairman: Mary Slack

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**PENICILLIN BINDING PROTEIN MEDIATED RESISTANCE IN KENYAN PNEUMOCOCCI**

J. Paul

Public Health Laboratory, Level 7, John Radcliffe Hospital, Oxford, OX3 9DU

Until recently, penicillin has been an effective therapeutic agent against *Streptococcus pneumoniae*. The growing population at special risk of pneumococcal disease (asplenic and HIV-positive patients) and realisation of increasing penicillin resistance have promoted an interest in vaccination. 53 of 206 (26%) isolates collected during the course of the Wellcome Trust/KEMRI HIV Project in Nairobi were resistant to penicillin (MIC>0.1u/ml). Penicillin MICs ranged from <0.008 to 0.25 µg/ml. Penicillin resistance is pneumococci is conferred by alterations in at least three of the five high molecular weight penicillin binding proteins (PBP's), (1A, 1B, 2A, 2X, 2B). It is important to know whether resistance occurs due to the spread of a single clone or because multiple resistant clones have evolved independantly. 23 penicillin-resistant strains were studied in detail at the University of Sussex and Oxford to determine overall genetic relatedness of the strains (by serotyping, ribotyping and multilocus enzyme electrophoresis) and the relatedness of PBP's (by sequencing and DNA fingerprinting of PBP genes). The different typing methods showed close concordance, suggesting that at least eleven resistant clones have developed independantly. Two serotype 7 and two serotype 14 isolates were otherwise indistinguishable, suggesting a serotype switching event and indicating that pneumococci may be able to evade vaccines aimed at particular serotypes.
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EVOLUTION & MOLECULAR PROPERTIES OF EXTENDED-SPECTRUM \( \beta \)-LACTAMASES

David M. Livermore
Department of Medical Microbiology, The London Hospital Medical College, Turner Street, London E1 2AD.

Newer generation cephalosporins are stable to the TEM-1 and SHV-1 plasmid-mediated \( \beta \)-lactamases, which are common causes of amino- and carboxy-penicillin resistance in Gram-negative bacteria. Their use has, however, selected extended-spectrum \( \beta \)-lactamase and extended-spectrum \( \beta \)-lactamase mutants of these enzymes. Over thirty such variants are described. These have 1-4 amino-acid substitutions and hydrolyse all cephalosporins bar cephaprycins. Unknown before 1983, such enzymes now occur world-wide, predominantly in klebsiellae but also in other enterobacteria. Producers have been important in many outbreaks of nosocomial infection. Most TEM and SHV-derived extended-spectrum \( \beta \)-lactamases give clear resistance to ceftazidime; resistance to cefotaxime and cefuroxime, though clinically significant, may be inapparent in routine tests with certain enzyme subtypes. Recognition of producer strains is aided by double disc tests to show ceftazidime is potentiated by clavulanate. Carbapenems, inhibitor combinations and cephamycins remain active against producers. Although these TEM and SHV cephalosporinase mutants are the most important extended-spectrum \( \beta \)-lactamases, other types, presently much rarer, also deserve mention including: inhibitor resistant TEM and SHV mutants; cephalosporinase mutants of the OXA \( \beta \)-lactamase PSE-2 (OXA-10); AmpC (Class I) \( \beta \)-lactamases coded by plasmids and a few carbapenemases, some zinc-dependent, others not, that have been found in enterobacteria and pseudomonas. No currently available \( \beta \)-lactam nor inhibitor combination overcomes all these threats.

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GLYCOPEPTIDE RESISTANCE IN ENTEROCOCCI

N Woodford
Antibiotic Reference Unit, Laboratory of Hospital Infection, Central Public Health Laboratory, London NW9 SHT.

Vancomycin and teicoplanin inhibit cell wall synthesis in gram-positive bacteria by interacting with the D-alanyl-D-alanine group of the pentapeptide side-chains of peptidoglycan precursors. Two classes of acquired glycopeptide resistance are recognized amongst enterococci. The VanA phenotype, which is characterized by inducible high-level resistance to vancomycin (MIC >128mg/l) and cross-resistance to teicoplanin (MIC >4mg/l), is often plasmid-mediated and is usually transferable between strains. Resistant enterococci produce altered peptidoglycan precursors which terminate in D-alanyl-D-lactate (D-ala-D-lac) and which do not bind glycopeptides. Enterococci of the VanB phenotype are resistant to vancomycin, but usually remain sensitive to teicoplanin in vivo. Again, resistance is inducible and results from the production of peptidoglycan precursors that contain D-ala-D-lac. In some instances, VanB resistance may also be transferable, in association either with large fragments of chromosomal DNA or with plasmids.

A third class of resistance, VanC, is an intrinsic characteristic of Enterococcus gallinarum and E. casseliflavus / E. flavescens. These species have non-transferable, constitutive, low-level vancomycin resistance (MICs ≤32mg/l) and are sensitive to teicoplanin. However, the actual resistance gene of E. casseliflavus / E. flavescens differs from that of E. gallinarum.

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MOLECULAR BASIS OF DRUG RESISTANCE IN Mycobacterium tuberculosis

Stewart T. Cole,
Institut Pasteur, Paris, France

The potentially deadly association between AIDS and tuberculosis represents an enormous public health problem of global dimensions which has been compounded by the emergence in the US of strains of M.tuberculosis resistant to two, or more, frontline drugs. It is now known that, as in other eubacteria, resistance to rifampicin, streptomycin and fluoroquinolones results from missense mutations to essential chromosomal genes encoding the drug targets. In contrast, at least two novel mechanisms are responsible for resistance to the potent tuberculocidal drug, isoniazid. High level resistance is associated with mutations which inactivate the katG gene, or result in greatly reduced activity of its product, the haem-containing enzyme catalase-peroxidase which is believed to activate the drug inside the bacterium. Lower resistance levels, and cross-resistance to ethionamide, result from overexpression of the inhA gene, encoding a novel fatty acid synthase that may be involved in mycolic acid production. Unlike the situation in many bacteria multidrug resistance is not due to the acquisition of resistance plasmids or transposons but to the accumulation of mutations in the genes encoding the respective drug targets.

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HIV REVERSE TRANSCRIPTASE : MOLECULAR MECHANISMS OF IMMUNE ESCAPE

Rodney E Phillips
Wellcome Trust Senior Clinical Research Fellow
University of Oxford
Nuffield Department of Clinical Medicine
John Radcliffe Hospital
Headington, Oxford OX3 9DU

HIV reverse transcriptase (HIV-RT), like structural proteins of the virus, is processed to form antigens recognised by cytotoxic T lymphocytes (CTL). HIV-RT is also responsible for generating much of the genetic variation in the virus since the enzyme causes frequent errors during transcription. In human infection, the acquisition of mutations within the HIV genome produces a swarm of variants called a quasispecies.

In vivo, drugs such as zidovudine and the immune response, act to select forms of the virus able to resist these forces. Individuals with the Class I molecules, HLA A2 and B8, recognise well defined nonamer peptides formed by cytoplasmic degradation of reverse transcriptase. Genetic analysis of virus obtained from patients with these Class I restricted CTL responses has detected mutations in the RNA and DNA sequences encoding these epitopes. Some of these epitope variants are not recognised by the patients own cytotoxic T cells, although the variant nonamer peptides are still presented to the T cell antigen receptors by HLA B8. Other epitope variants act as antagonists and inhibit the capacity of specific CTL to recognise the prototype antigen.

Amino acid changes can arise in reverse transcriptase which allow the virus to evade these powerful selection forces while maintaining enzyme function. Evasion of immune pressure by mutation in T cell epitopes probably explains, in part, why the virus persists despite the antiviral response HIV elicits in man.

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INVESTIGATION OF POSSIBLE INTER-HOSPITAL SPREAD OF MULTI-RESISTANT E. FAECCIUM
Donald Morrison, Neil Woodford.
Laboratory of Hospital Infection, Central Public Health Laboratory, London, NW9 SHT.
Six isolates of multi-resistant E. faecium from four London hospitals were investigated by pulsed field gel electrophoresis (PFGE) for possible inter-hospital spread. The isolates were resistant to the recommended therapy for serious enterococcal infections and exhibited high level resistance to gentamicin, streptomycin, penicillin (except one isolate), vancomycin and teicoplanin.
Initial studies using Smal digested DNA and our standard pulse times of 1sec to 10sec for 30h followed by 15h revealed up to 4 band differences between these isolates. To investigate further their relatedness, DNA was digested with two other restriction enzymes and electrophoresed using extended pulse times.
The extended pulse times resulted in a larger number of band differences: a maximum of 9, 12 and 8 band differences was found with Smal, Apal and SfII respectively. Two groups of three isolates each were identified: group 1 differing by a maximum of 4 bands and group 2 by a maximum of 5 bands. Each group contained isolates from three different hospitals. Apart from the relatively close proximity of these hospitals, no other epidemiological evidence of inter-hospital spread is available to support the typing results.
The use of different restriction enzymes and extended pulse times has revealed some interesting observations on the much debated question of the number of band differences required to designate isolates as distinct using pulsed field gel electrophoresis. This will be discussed.

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INTERNATIONAL, INTER-HOSPITAL AND INTER-PATIENT SPREAD OF A MULTIRESISTANT STRAIN OF K. PNEUMONIAE
AP Johnson, B Cookson, B Azadian1, J Paul2, G Hutchison, MG Kauftmann, N Woodford, M Walde, B Walsh3, A Yousif4, JB Selkon1
Laboratory of Hospital Infection, Central Public Health Laboratory, London; Department of Microbiology, Charing Cross Hospital, London; Department of Microbiology, John Radcliffe Hospital, Oxford; 3Salamiya Medical Centre, Bahrain.
Clusters of infection and colonization with Klebsiella pneumoniae resistant to multiple antibiotics including third generation cephalosporins and aminoglycosides occurred in the ICUs of two British hospitals. Epidemiological investigations indicated that the outbreaks were associated with the transfer of a patient who had recently arrived in the UK from Bahrain. The patient had previously been treated in a hospital in Bahrain where multiresistant K. pneumoniae were endemic in the ICU.
Isolates from the ICUs of all three hospitals had identical antibiograms. Similar plasmid profiles, belonged to the same serotype (K2) and were indistinguishable by pulsed-field gel electrophoresis of chromosomal DNA. All the isolates exhibited identical patterns of beta-lactamase activity, having focussing and their resistance to cephalosporins in vitro was reduced in the presence of clavulanic acid. Resistance to both cephalosporins and aminoglycosides was encoded by transferable plasmids.
These observations indicate that patient carriage of multiresistant K. pneumoniae resulted in the international and subsequent inter- and intra-hospital spread of this strain, which was associated with significant patient morbidity and mortality.

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CIPROFLOXACIN RESISTANCE IN NEISSERIA GONORROEAE
C A Ison1, P J Woodford1 and R J Belland2
Department of Medical Microbiology, St. Mary's Hospital Medical School, London W2 1PG; Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, Hamilton, Montana, USA2.
Ciprofloxacin is now frequently used as first-line therapy for gonorrhoea. It has high activity against Neisseria gonorrhoeae and it is given orally. Therapeutic failure is still uncommon and mechanisms of resistance are unknown, although in other bacteria this has resulted from mutations in the DNA gyrase or reduced permeability of the outer membrane.
The relationship between therapeutic failure, MIC and dosage is still unclear for ciprofloxacin. In order to monitor the emergence of potentially resistant isolates and drifts in susceptibility we have used an agar dilution breakpoint technique. Three concentrations were chosen, 0.12, 0.03 and 0.008mg/l to categorise the isolates. Using this approach the susceptibility of gonococcal isolates from consecutive patients attending at St. Mary's Hospital since January 1988 has been monitored. Of more than 4,000 isolates tested, <1% had MICs of ≥0.12mg/l which would be regarded as potentially resistant and >90% of isolates were fully susceptible (MIC, ≤0.008mg/l).
To investigate possible mechanisms of resistance a series of seven mutants of strain FA19 were selected by passage on increasing concentrations of ciprofloxacin. The resulting mutants had MICs between 0.002mg/l and 16mg/l. Mutants exhibiting low or moderate levels of resistance were shown to have mutations within gyrA while those exhibiting higher levels had acquired analogous mutations in both gyrA and parC. Resistance due to these mutations was transferable and could be involved in high level resistance to ciprofloxacin.

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VANA GENES IN CLINICAL ISOLATES OF OERSKOVIA TURBATA AND ARCANOBACTERIUM HAEMOLYTICUM
E G M Power, Y A Abdulla, H G Talsania, W Spcie, G L French
Department of Microbiology, UMDS, Guy's Campus, London SE1 9RT.
We report the cloning and sequencing of vanA genes present in high-level vancomycin-resistant clinical isolates of Oerskovia turbata 892 and Arcanobacterium haemolyticum. The presence of vanA genes was detected by Southern blotting and PCR and confirmed by DNA sequencing. The A. haemolyticum 872 DNA sequence was identical to the published vanA sequence of vancomycin-resistant Enterococcus faecium BM4147. The O. turbata 892 DNA sequence showed three coding changes compared to the E. faecium BM4147 vanA sequence. Induction experiments suggested that vancomycin resistance in A. haemolyticum 872 and O. turbata 892 was constitutive. SDS-PAGE analysis of membrane proteins showed the presence of a ca. 39KD protein in both clinical isolates whose expression was unaltered in the presence of vancomycin unlike E. faecium. Mutations within gyrA were detected in both O. turbata 892 and A. haemolyticum 872 DNA sequences, suggesting that vancomycin resistance in the isolates described here is mediated by vanA. This is the first report confirming the presence of vanA in genera other than Enterococcus and suggests that dissemination of vanA-mediated resistance between organisms of different genera is possible in vivo.
RESISTANCE TO MUPIROCIN IN STAPHYLOCOCCI

C Needham¹, K.G.H. Dyke¹, W C Noble².
¹ Microbiology Unit, Department of Biochemistry, University of Oxford, Oxford OX1 3QU.
² Department of microbial Diseases, St John’s Institute of Dermatology, St Thomas’ Hospital, Lambeth Palace Road, London SE1 7EH.

The gene for high level resistance (MIC > 2mg/ml) to mupirocin, an analogue of isoleucine, in staphylococci has been characterised as that for an isoleucyl tRNA synthetase of non-staphylococcal origin. So far it has been reported only on a series of structurally diverse plasmids of differing EcoR I digest fragment patterns. Many of the MupF plasmids are self-transmissible and contain copies of the insertion sequence IS257 which has been shown to flank some staphylococcal transposons. There is no evidence for active transposition of mupR but the IS257 is involved in duplication of the region coding for mupR and in co-integrate formation between MupF plasmids and other staphylococcal plasmids such as pT181 that confers resistance to tetracyclines. It is concluded that recombination involving IS257 is very important in the multiplication of resistance genes and in the accumulation of resistance determinants on the same piece of transmissible DNA.

ANTIBIOTIC RESISTANCE CHARACTERISTICS OF COLIFORM BACTERIA FROM WATER AND GLACIAL ICE IN CANADA’S HIGH ARCTIC

S J Dancer and P Shears
Department of Medical Microbiology, Duncan Building, Royal Liverpool University Hospital, Liverpool L69 3BX.

Ellesmere island is the northernmost member of the Canadian Arctic Archipelago with over one third of the land mass covered by ice. A joint services expedition to the island’s Blue Mountains offered a unique opportunity for microbiological studies of resident bacteria in an environment uninhabited by man. Over one hundred samples of water and ice were collected from stream, lake and glacier and the filtrate cultured under canvas. Bacterial growth was harvested onto swabs for transport back to the UK, and fifty coliforms chosen for identification and antibiotic susceptibility testing.

POSTERS AND DEMONSTRATIONS

ORDER FROM CHAOS: A MECHANISTIC DEFINITION OF THE SPECIES CONCEPT IN BACTERIA

JT Magee
Dept. Microbiology and PHL, University of Wales College of Medicine, Cardiff CF4 4XW, UK

Definition of the species concept in bacteriology has proved a refractory problem. Definitions for eukaryotes assume sexual modes of reproduction and hinge on severe limitation of inter-species genetic transfer. This offers an inherent mechanism for conservation of species homogeneity; the species acts as an evolutionary unit, via the isolated gene pool. In contrast, bacteria reproduce asexually, but have mechanisms allowing transfer of genetic material between species or genera. Unlike the sexual eukaryotes, the bacterial gene pool is probably open, allowing broad slow exchange of genes throughout the bacteria. Further, in large populations of asexually reproducing organisms with short generation times, cumulative accretion of mutations in clonal lines should lead to rapid diversification. Clonal asexual reproduction combined with promiscuous gene exchange make the clone the most likely evolutionary unit in bacteria, and offers no apparent mechanism to restrict rapid diversification of the haploid bacteria into a continuous spectrum of diverse clones. No current definition of the bacterial species concept provides a feasible mechanism for the observed conservation of intra-species homogeneity.

The argument presented is that the modal properties of a bacterial species represent an optimal adaptation to an ecological niche; that severe Darwinian selection stringently limits diversification from the species mode; and that the biological discontinuities produced by this selection define the species. This results in a definition of the species concept based on an ecological mechanism for bacterial speciation. This mechanism is contrasted with the sexual mechanisms that define species of higher organisms.
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FORSAGING THE TOMB: PROBLEMS AT THE INTERFACE BETWEEN TAXONOMY AND MEDICAL MICROBIOLOGY

J T Magee
Dept. Microbiology and PHU, University of Wales College of Medicine, Cardiff CF4 4XW, UK

Systemsatics provides microbiologists with a common language that can convey a wealth of data in a species name. However, the rate of change in species nomenclature and of re-organisation at genus level is increasing rapidly. Concurrently, increasingly severe cash and time constraints have been imposed on diagnostic laboratories and library facilities. These factors, combined with the customary apathetic attitude to taxonomy in diagnostic laboratories, and difficulties in informing clinical staff of the changes, have ensured that most laboratories have abandoned any attempts to maintain an up-to-date nomenclature. Worse, these attempts have been abandoned at different times in different laboratories, with the result that mutual understanding of the nomenclature is being eroded, and whatever advantages exist in prediction of pathogenic properties from the latest classifications are lost. Taxonomists and medical microbiologists need to establish methods of overcoming these difficulties, and a better understanding of one another’s purposes and requirements, or the many advantages of the common language of systematics will be lost.

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THE GENE CLUSTER ENCODING COMPONENTS OF THE PYRUVATE DEHYDROGENASE COMPLEX OF NEISSERIA MENINGITIDIS: DETECTION AND SEQUENCE ANALYSIS

D A A Ala’Aldeen, V Weston, T Baldwin, S P Borriello

Microbial Pathogenicity Research Group, Department of Microbiology and the Institute of Infections and Immunity, Queen’s Medical Centre, Nottingham NG7 2UH.

Many prokaryotic cells produce a series of enzymatic subunits which together form a pyruvate dehydrogenase complex (PDHC), which catalyses the oxidative decarboxylation of pyruvate to acetyl-CoA + CO₂ with concomitant reduction of NAD⁺. In Escherichia coli three subunits (Elp, E2p and E3) which are encoded by three adjacent genes have been identified, sequenced and fully characterised. No such complexes so far have been reported in Neisseria species.

A Zap-11 expression library of Neisseria meningitidis was screened with a rabbit polyclonal antiserum raised against the meningococcal iron-regulated protein Fbp. The antigen was purified by elution from preparatory SDS-polyacrylamide gels and the antiserum was shown to contain antibodies against co-migrating proteins which were not identified.

Initial screening yielded large number of clones, many of which were selected for analysis. Selected clones were isolated, further purified and their recombinant pBluescript SKI plasmids were excised in vivo using the ExAssist helper pluge (Stratagene). The expressed proteins were partially characterised on SDS-PAGE and almost the entire gene cluster (5.8 Kb) is now sequenced. Four open reading frames have been identified. These of these reading frames show a high degree of homology with the Elp, E2p and E3 components of E. coli PDHC and related genes in a number of other prokaryotic and some eukaryotic species. An additional open reading frame, with a deduced peptide molecular weight of 16 kDa located between E2p and E3 components, is also identified.

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RABBIT AND MOUSE POLYCLONAL ANTISERA RAISED TO THE MENINGOCOCCAL TRANSFERRIN RECEPTORS ARE BACTERICIDAL.

D A A Ala’Aldeen, S P Borriello

Microbial Pathogenicity Research Group, Department of Microbiology and the Institute of Infections and Immunity, Queen’s Medical Centre, Nottingham NG7 2UH.

When grown in vivo or under iron-restriction in in vitro, Neisseria meningitidis express a number of iron-regulated outer membrane proteins, including two transferrin-binding proteins (TBPl and TBP2). The TBP3 are highly specific receptors for human transferrin and we have previously demonstrated their immunogenicity in humans and animals and their exposure on the surface of the organism. There is a growing interest in incorporating these TBP3 in future outer membrane-based meningococcal vaccines. Protection against meningococcal infection has been correlated with serum bactericidal antibodies. Therefore, it is important for these vaccine candidates to generate such antibodies. We have previously raised rabbit and murine polyclonal monospecific antisera against the TBP3 of strain SD (B:15:P1.16) which showed varying degrees of cross-reactivity on immunoblots between the TBP3 and/or TBP2 molecules of different heterologous strains from various serogroups, types and subtypes. The ability of these antisera to kill meningococci was tested by incubating live organisms (grown to log phase under iron-restriction) with the antisera in the presence of a human complement source (serum from an agammaglobulinaemic patient). The rabbit antiserum killed 10/11 strains and the mouse antiserum killed 7/10 strains. The sera showed varying degrees of cross-reaction on immunoblots with no obvious correlation with the identity of the strains or the TBP biotypes which vary between strains. The results indicate that the TBP3 could generate cross-protective immunity against a wide range of meningococcal strains.

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DETECTION OF LINEAR AND CONFORMATIONAL EPITOPES ON THE TBPl COMPONENT OF THE MENINGOCOCCAL TRANSFERRIN RECEPTOR, USING MONOCLONAL ANTIBODIES

K Bishop, D A A Ala’Aldeen, N B L Powell, A R Gottingel, S P Borriello

Microbial Pathogenicity Research Group, Department of Microbiology and the Institute of Infections and Immunity, Queen’s Medical Centre, Nottingham NG7 2UH, and ICAMR. Division of Biologics, Porton Down, Salisbury SP4 0RG

When grown in vivo or under iron-restriction in vitro, Neisseria meningitidis express transferrin receptors which consist of two transferrin-binding proteins (TBP1 and TBP2). We have previously demonstrated their immunogenicity in humans and animals and their exposure on the surface of the meningococcus. We have also shown that, unlike TBP2, the TBP1 loses its native biological and immunological properties when exposed to denaturing conditions, such as SDS-PAGE. We demonstrated that convalescing patients only produce antibodies to TBP1’s conformational epitopes, but it was not clear whether these epitopes are exposed on the surface of the organism.

In this study, mice were immunised with TBPl which was cloned and expressed in Escherichia coli. Fused spleen cells were screened using sonicated meningococci (strain SD, B:15:P1.16) and/or purified (biologically active) TBPl in Dot immunoblot and ELISA. Two monoclonal antibodies of different subclasses (IGG1 and IGG2a) were isolated and characterised. Both antibodies recognised the purified and biologically active TBPl, however, only the IGG1 antibody reacted with the protein on Western blots. Using gold immobilised under electron microscopy, the IGG2a showed significant surface-labeling on live organisms whereas the IGG1 antibodies failed to surface-label. These results indicate that the epitope recognised by the IGG2a antibody is conformational and surface-exposed whereas the IGG1 antibody recognises a linear epitope which is accessible on the purified biologically active TBP1 molecule but not on the intact organism.
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CAN Helicobacter pylori PRE-SCREENING BE USED AS AN AID TO THE EFFECTIVE DIAGNOSIS OF GASTRODUODENAL DISEASE?

M. A. Chapman, D. Moxon, R. D. Bardhan, A. M. A. Abbas

Department of Microbiology, St Mary's Hospital, London.

INTRODUCTION

Helicobacter pylori is closely associated with the development of duodenal ulcer disease. The organism may be readily detected without resorting to endoscopy by Enzyme Immunoassort Assay (ELISA).

It has been proposed by Sobala et al (Lancet 1991; 338; 94-96) that dyspepsia under diagnosis of age who are Helicobacter pylori positive (by ELISA) need within five days of hospital admission gastroscopy, without any concern of serious disease (unless they were taking NSAID's), and can be treated symptomatically. As a result of this policy a large cost saving could be made, reducing endoscopy workload by 23%.

AIM

To study the validity of a screening policy based upon Helicobacter pylori serological status.

METHOD

In this study 200 consecutive dyspeptic patients attending gastroscopy clinic at Rothemead General Hospital were studied. Blood samples were collected for Helicobacter pylori IgG ELISA (Helico-C, Shield Diagnostics, UK). A record each of patients diagnosis was made at gastroscopy.

RESULTS

Sixty six (33% of total) patients were <45 years old. Twenty eight (42.4%) were Helicobacter pylori seronegative, yet 18 patients (27.2%) were found to have clinically significant endoscopy findings (table 1), the remainder having a normal finding.

Significant Clinical findings missed if Helicobacter pylori status is considered as a tool for screening patients off endoscopy.

Table 1: Positivity rate of culture vs. gram stain from biopsies transported in: '3 hours and 24 hours in both liquid and complex transport medium.

Table 2: Detection of Patient Colonisation with Agrobacterium baumannii in an ITU

M. Crowe, K.J. Twomey, H. Humphries

Department of Microbiology & PRLE Laboratory, University Hospital, Queen's Medical Centre, Nottingham NG7 2ZU

Sporadic outbreaks of Acinetobacter infection have occurred in Nottingham hospitals, particularly in the intensive therapy unit (ITU), since at least 1977. These outbreaks have been separated by lengthy periods during which few or no cases of Acinetobacter infection were observed. The purpose of this study was to investigate whether colonisation of patients in the ITU with Acinetobacter was occurring during these intervening periods.

Over a six-week period, 37 random patients were screened (axilla, perineum, perianal, tracheostomy, tracheal aspirates and wounds) within ±2 h of admission for Acinetobacter carriage. No isolates from the above sites were inoculated on to CLED agar plates and an agar medium selective for Acinetobacter spp. Following incubation at 30°C for up to 7 days, presumptive Acinetobacter colonies were identified biochemically.

Of the 27 patients, six carried multi-resistant Acinetobacter baumannii, of whom five were post-operative neurosurgical and spinal patients, and one had a diagnosis of pancreatitis. Intera alia, the six positive patients yielded A. baumannii from all sites sampled (3/6 axilla, 5/6 perineum, 2/6 perianal, 3/6 tracheostomy, 3/6 tracheal aspirates, 1/3 wounds). All of these patients were receiving antibiotic treatment (4/6 broad spectrum antibiotics) at the time of first isolation of A. baumannii. The interval from admission to first isolation of A. baumannii varied from 2-10 days.

Detailed biochemical investigations of the A. baumannii isolates indicated that at least three different strains of A. baumannii were present in the ITU. Since the epidemic outbreaks of infection observed periodically in the ITU are invariably caused by a single strain, further investigations are clearly required to determine the factors leading to the onset of epidemic episodes of infection with this increasingly important nosocomial pathogen.

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THE EFFECT OF TRANSPORT MEDIUM AND TIME ON THE ISOLATION OF Helicobacter pylori FROM GASTRIC BISPECY SPECIMENS.

D. Moxon, R. D. Bardhan, A. M. A. Abbas

Department of Gastroenterology, Rothemead General Hospital, Rothemead, S. Yorks.

Recent work by Venemead, Ilichandska-Bednarska et al (1993), suggested that 0.9% saline may be used as an effective transport medium for gastric biopsies colonised with Helicobacter pylori. In this study we attempted to confirm the validity of these findings.

Seveny-two consecutive patients (15 men, 17 women), attending for diagnostic and/or follow-up endoscopy were studied, 78% were receiving H2-Receptor antagonists and/or proton pump blockers. A pair of biopsies were taken from both antrum and corpus. One biopsy from each pair was put in 0.9% saline and the other pair was placed in transport medium (median heat-labile meat broth). Biopsies from the antrum were cultured within 3 hours, whereas those from the corpus were kept at room temperature for 24 hours before culture. After culture the remainder of the biopsies were cultured onto a glass slide, stained with Gram stained (table 1).

Biopsies which were cultured within three hours produced similar positivity rates whether they were transported in complex medium (56%) or saline (56%). The corpus positivity rate was particularly low when samples were first kept in saline for 24 hours (28%).

Cultures were compared against Gram stain findings. At 3 hours slightly more of the antral biopsies were culture positive than Gram stain positive, but at 24 hours the reverse was true. Abnormal colony morphology was not seen when biopsies were cultured within 3 hours, irrespective of transport medium. However, after 24 hours 70% of isolates transported in complex transport medium demonstrated atypical growth, compared with 85% (17/20) of those in saline.

Venemead et al excluded all patients who had taken antibiotics, omeprazole, or bismuth-containing drugs during the 3 months prior to the study. However, the majority of our patients (78%) were taking either H2-Receptor antagonists or proton pump blockers. This perhaps is the cause of the low recovery rates from biopsies transported over 24 hours.

Table 1: Positivity rate of culture vs. gram stain from biopsies transported in: ‘3 hours and 24 hours in both saline and complex transport medium.

Table 2: Detection of Patient Colonisation with Agrobacterium baumannii in an ITU

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Detailed biochemical investigations of the A. baumannii isolates indicated that at least three different strains of A. baumannii were present in the ITU. Since the epidemic outbreaks of infection observed periodically in the ITU are invariably caused by a single strain, further investigations are clearly required to determine the factors leading to the onset of epidemic episodes of infection with this increasingly important nosocomial pathogen.

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AETIOLOGICAL AGENTS OF HIV-ASSOCIATED CHRONIC DIARRHOEA IN KENYA

B. I. F. Bachelor, C. Mwachari, M. Amir

Kenya Medical Research Institute, P.O. Box 43640, Nairobi, Kenya.

Seventy-five patients were recruited into a prospective study of chronic diarrhoeal disease and wasting among HIV infected adults in Nairobi, Kenya. Patients were enrolled within five days of hospital admission if they had diarrhoea of at least four weeks duration, had suffered marked weight loss and were HIV seropositive on testing by two different ELISA kits.

Stool samples were cultured for conventional bacterial pathogens associated with diarrhoea and Mycobacterium spp, and examined for the presence of Cryptosporidium parvum and other intestinal parasites, Clostridium difficile toxin and viruses. In addition, 36 samples (48.0%) were examined for microsporidia and 41 (54.7%) for diarrhoea-associated Escherichia coli.

The following putative pathogens were detected: Cryptosporidium parvum (17.3% of patients), Salmonellae inyumurium (13.3%), enteroaggregative E. coli (9.8%), diffusely adherent E. coli (9.8%), Shigella spp. (4.0%), Enterocytozoon bieneusi (2.8%), Campylobacter spp (2.7%), small round structured virus (1.3%), coronavirus (1.3%) and Aeromonas spp. (1.3%).

A broad spectrum of aetiological agents was seen but despite intensive analysis a possible pathogen was found in only thirty-one patients (41%). Cryptosporidium parvum is one of the commonest causes of HIV-associated diarrhoea in both Europe and Africa.
IMMUNOCYTOCHEMICAL LOCALISATION OF THE LACTOFERRIN RECEPTOR IN \textit{NEISSERIA MENINGITIDIS} AND IT'S TOPOGRAPHICAL RELATIONSHIP TO THE TRANSFERRIN RECEPTOR.

N.B.L. Powell$^1$, D.A. Ala'Aldeen$^1$, A.B. Schryvers$^2$, and S.P. Sorrell$^1$

$^1$Microbial Pathogenicity Research Group, Department of Microbiology, and Institute of Infections and Immunity Queen's Medical Centre, Nottingham, NG7 2UH, U.K.

$^2$Department of Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada.

Gold-labelled lactoferrin (Au-LF) was used to localise and quantify the distribution of lactoferrin binding receptors on live meningococci in vitro by electron microscopy. On most cells there was a uniform distribution of lactoferrin receptors, although the occasional cell had localised clusters of gold particles on the cell surface. A few cells also exhibited significant labelling of outer membrane blebs. Double labelling experiments with 5nm Au-LF and 15nm gold-labelled human transferrin (Au-HTF), indicated that lactoferrin and transferrin receptors in meningococci are separate and not closely situated on the cell surface. The lactoferrin receptor density in \textit{N. meningitidis} strain SD (B15P1.16) when labelled with 5nm Au-LF was 39 particles per cell. This compared with a transferrin density of 113 per cell when labelled with 5nm Au-HTF.

Isogenic transferrin binding protein (TBP) mutants of \textit{N. meningitidis} B16B6 (B2aP1.2) bound less Au-HTF and Au-LF than strain SD. The lower level of lactoferrin receptors was a surprise, and may indicate that lactoferrin receptor gene expression is also impaired.

EPIDEMIOLOGY OF NON-SEROTYPABLE \textit{HAEMOPHILUS INFLUENZAE} CAUSING INVASIVE DISEASE

C.A. Real, T.J. Falle, E.C. Anderson et al.
Oxford Public Health Laboratory, Level 6/7, John Radcliffe Hospital, Headington, Oxford, OX3 9DU.

The epidemiology of disease caused by non-capsulate strains of \textit{Haemophilus influenzae} in the UK has only been described for neonates and children in the Oxford region. Using standard serotyping methods, non-capsulate mutant \textit{H. influenzae} type b strains (b) are confused with the distinct and heterogeneous population of non-capsulate \textit{H. influenzae} (NC); both types are non-serotypable (NST). The epidemiology of such disease in all age groups caused by NC strains could be described from cases identified during an ongoing, population based, prospective survey of invasive \textit{H. influenzae} disease in the UK organised by the PHLS. Of a total of six hundred and twenty one strains of \textit{H. influenzae} received during the survey, 62 were NST strains. These 62 were all capsular genotyped by PCR and their rRNA gene restriction fragment patterns were determined by Southern blotting and probing of Eco R1 digested total cellular DNA. None of the NST strains were non-capsulate derivatives of type b strains. A wide variety of rRNA gene restriction patterns were detected. Of the 62 cases; 14 (26%) were in children less than 2 years of age and 21 (34%) in adults over 60 years of age. The pattern of diseases consisted of bacteremia (52%), pneumonia (19%), meningitis (10%), cellulitis (3%) and other infections (16%). The incidence of these infections, for all age groups, was 0.24 per 100,000 per year.

RANDOMLY AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS FOR THE CHARACTERISATION OF \textit{LEGIONELLA PNEUMOPHILA}.

LA Clarke, NI Leaves
Oxford Public Health Laboratory, Level 6/7, John Radcliffe Hospital, Headington, Oxford OX3 9DU, UK.

\textit{Legionella pneumophilia} is the causative organism of Legionnaires disease and Pontiac fever. Epidemiological investigations are necessary because the organism is environmentally acquired and can cause outbreaks of disease.

The present study investigated the potential use of Randomly Amplified Polymorphic DNA (RAPD) analysis in epidemiological investigations of legionellae. Initially, various species of the genus \textit{Legionella} were characterised using the RAPD method to select discriminatory RAPD primers. The selected primers were then used to characterise a collection of \textit{L. pneumophilia}, serogroup 1, subgroup Pontiac and subgroup Olda strains. The Pontiac subgroup has previously been shown to cause the majority of cases of disease caused by \textit{Legionella}. The RAPD results showed that subgroup Pontiac strains were easily distinguishable from each other, however subgroup Olda strains were highly related to each other (although there was no known epidemiological association).

The results of the present study are consistent with previously published data regarding the population genetic structure of these two subgroups of \textit{L. pneumophilia}. RAPD analysis provides a useful and rapid method of characterising of \textit{L. pneumophilia} serogroup 1, subgroup Pontiac, which may prove suitable for epidemiological investigations.

CEPHALOSPORIN SUSCEPTIBILITY TESTING OF \textit{HAEMOPHILUS INFLUENZAE}.

Helen Azopardi, J. Zoe Jordens, Mary P.E. Slack.
Haemophilus Reference Laboratory, Oxford Public Health Laboratory, Level 7, John Radcliffe Hospital, Headington, Oxford, OX3 9DU.

Cephalosporin susceptibility testing of ampicillin-resistant, non b-lactamase producing (ampRNBLP) \textit{Haemophilus influenzae} is often not reliable when disc diffusion susceptibility testing is used. Disc susceptibility tests were compared on different media in air and in 5% CO$_2$. Disc testing was also compared with break-point susceptibility tests and minimum inhibitory concentration (MIC) determinations by agar incorporation and E-Tests. Three control strains of \textit{H. influenzae} and 18 clinical strains, selected on the basis of previous routine disc susceptibility test results, were included.

Many strains failed to grow on susceptibility testing media when incubated in air and one strain failed to grow on Haemophilus Test Medium agar even when incubated in 5% CO$_2$. All strains grew on Nicotinamide Adenine Dinucleotide agar in 5% CO$_2$.

There was close agreement between the susceptibility results of the different testing methods for ampicillin susceptible strains and beta-lactamase producing, ampicillin resistant strains. Of six strains, previously thought to be ampRNBLP, five were susceptible to cefuroxime by disc diffusion and all six were susceptible by both MIC methods. Two of these six strains appeared susceptible to cefotaxime by disc diffusion but resistant by both MIC methods. All six strains were of intermediate susceptibility to cefoxime and cefotaxime by the break-point method.

The interpretation of cephalosporin susceptibility test results for ampRNBLP strains is difficult because their MICs are skewed to the upper limit of the susceptible range. Break-points have the advantage of ascribing these strains to an intermediate category but do not fully resolve the problems associated with cephalosporin susceptibility testing of ampRNBLP \textit{H. influenzae}. 

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THE MOLECULAR EPIDEMIOLOGY OF AMPICILLIN RESISTANT HAEMOPHILUS INFLUENZAE FROM THE OXFORD REGIONAL SURVEY

S.A. Kerridge, I. Dimopoulou, D.W. Crook
Oxford Public Health Laboratory, Level 6/7, John Radcliffe Hospital, Headington, Oxford, OX3 9DU.

The mechanism of spread of ampicillin resistance in a population of Haemophilus influenzae is poorly understood. It is unclear whether an ampicillin resistant strain of H. influenzae exists or spread has occurred by conjugal transfer of a plasmid.

The relatedness of 43 clinical isolates of ampicillin resistant H. influenzae from the Oxfordshire region was determined. Total cellular DNA was digested with EcoRI and probed with cDNA from E. coli ribosomal RNA. Seven ribotypes were identified with 29/43 isolates belonging to a single type. DNA extracts were also digested with PstI and probed with a plasmid containing the β-lactamase gene. Ten plasmid patterns were identified. The majority of the isolates (34/43) possessed one of two closely related plasmids. The plasmids of six isolates were transferred to a rec– deficient strain (nfl8). Bands produced by probing the transconjugants and their parents with a 1.2Kb probe containing the point of plasmid recircularisation suggested that plasmid was chromosomally integrated in the parents and extrachromosomal in the transconjugants.

Analysis of the ribotype and plasmid banding patterns suggests both conjugal transfer of plasmid and the person to person spread of a resistant strain in nature.

WHITE PIEDRA: AN OVERLOOKED MYCOSIS?

P. Howe, J Paul
Oxford Public Health Laboratory, Level 6/7, John Radcliffe Hospital, Headington, Oxford, OX3 9DU.

The yeast Trichosporon beigeli (T. cutaneum) causes white piedra, a seldom reported superficial mycosis of the hair shaft. In Europe, T. beigeli is recognised mainly as a rare invasive mycosis in immunocompromised patients.

We report a case of white piedra affecting the pubic hair of a 36 year old resident of the UK who had visited Sierra Leone. The patient presented to his GP with inconspicuous white nodules on the hairs, specimens of which were referred to our laboratory as possible nits. T. beigeli was identified by growth at 37°C, API 20C AUX, urease production and presence of arthroconidia and blastoconidia on Czapek dox agar with tween 80. The condition was treated by shaving and topical antifungals.

White piedra is possibly an overlooked mycosis in travellers returning from the tropics.