

Symposium on Pathology in Wales

Abstracts for posters on display

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THE USE OF WHOLE GENOME SEQUENCING TO SUPPORT EPIDEMIOLOGICAL INVESTIGATION OF TWO OUTBREAKS OF TUBERCULOSIS IN SOUTH WALES

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Background: The development of whole genome sequencing (WGS) promises a new era in molecular typing of *M.tuberculosis* isolates. Previous studies have shown that WGS provides outbreak resolution above and beyond the current international standard of MIRU-VNTR typing. We compare detailed molecular and epidemiological data, using a novel WGS analysis stepwise pipeline, in two outbreaks of tuberculosis in South Wales.

Material/methods: Twelve isolates from one outbreak in Neath Port Talbot (NPT) area, which had been identified as such using MIRU-VNTR, and 33 isolates apparently unrelated according to MIRU-VNTR from the same geographical area were sequenced using an Illumina Miseq. An adapted 60 SNP barcode method was then used to classify each isolate into a sub-lineage for initial filtering of isolates for downstream analysis, selecting only those isolates with the same sub lineage assignment as the outbreak isolates. Core genome MLST was then carried out on the isolates relevant to the outbreak. A transmission chain was then created based on common and unique mutations, using inkscape software. In a second outbreak, in the Gorseinon area, 13 isolates were studied. DNA was of sub-optimal quality but successfully sequenced using a novel DNA library build developed for ancient/fragmented DNA in collaboration with the University of Copenhagen.

Results: The results enabled a more detailed epidemiological picture to be built up. In addition the analysis found two isolates, apparently unrelated by MIRU-VNTR, to be directly linked to the outbreak. Analysis of the synonymous and non-synonymous mutations identified within selected outbreak isolates was then carried out with determination of the Ka/Ks ratio. Non synonymous mutations were analysed for their effect on protein function through a Provean analysis. This showed only 1 out of 16 nsSNPs to cause a functional difference, which was present in the DacB2 penicillin binding protein, known to be relevent to macrophage survival and meropenem susceptibility.

Conclusions: The results of this study support data from previous studies which have found WGS to be superior to MIRU-VNTR for outbreak analysis. In addition this study presents a novel pipeline for resolution of an outbreak through use of WGS data, gleaning both epidemiological and clinically relevant data within the same analysis.

AUDIT ON ADEQUACY OF LLETZ PATHOLOGY REPORTS AT BETSI CADWALADR UNIVERSITY HEALTHBOARD

Dr A Gunavardhan, Dr H Abdelsalam - Department of Cellular Pathology North Wales

Introduction:

Histopathology reporting plays a key role in the NHS Cervical Screening Programme (NHSCSP). Biopsy reports are a 'gold standard', against which findings from cytology and colposcopy are correlated. Therefore high standard of pathology reports are essential to link together various components of the programme and in multidisciplinary working.

It is also an indicator of quality, and is used to audit and monitor the effectiveness of each part of the service.

Aim of the study:

- 1. To assess the completeness of histopathology reports of LLETZ samples.
- 2. To obtain uniformity in reporting practice.

Materials and methods:

Pathology reports of LLETZ samples in the Department of Histopathology at Glan Clwyd hospital were reviewed over a period from 1/01/2015 to 30/06/2016. The total number of pathology reports reviewed were 180. No slides were reviewed. These reports were compared against a proforma based on standards set by NHSCSP.

Discussion and conclusions:

The audit showed that there is a wide variation in reporting patterns of LLETZ pathology reports. All the required informations were not available in some reports.

Action Plan:

We recommended that the Pathologists must have access to cytology report while reporting histology to allow correlation between cytology and histology. We also recommended introduction of proforma and distribution to all consultants including Locums and a re-audit against same standards after six months.

Re- Audit:

The re-audit of reports were done for the months of November and December 2016. Seventy reports were reviewed.

Results:

The data showed 95-100% compliance in reporting of excision margin status, comments on presence or absence of transformation zone component, CGIN and HPV related changes. Modifications are made in LIMS so that all pathologists now have access to cytology reports and a proforma is available in the reporting window.

Only 45% of reports were in proforma format .The possible explanations for this was sought and found that short term locums and new consultants were not aware of proforma.

Therefore the recommendation from re-audit is to prepare an introduction pack with all necessary information and SOPs to short term locums and newly appointed consultants and re-audit after 6 months.

AN INCIDENTAL RARE FINDING OF A MICROSCOPIC MUCINOUS CARCINOMA ARISING WITHIN A BENIGN BRENNER TUMOUR

Dr Anuya Bandecar (ST2 Histopathology), Dr Sally Ann Hales (Consultant Histopathologist), Dr Bianca Da Gama Rose (Consultant Histopathologist) – Department of Histopathology, The Countess of Chester Hospital

Background:

An 85-year old female presented for the chief complaint of stress incontinence. An ultrasound scan showed incidental cysts in both the ovaries, for which the patient underwent a bilateral salpingo-oopherectomy with an omental biopsy and peritoneal washings.

Method: The submitted ovarian and Fallopian tube tissue were assessed after staining with haematoxylin and eosin and performing the relevant immunohistochemistry panel. A detailed clinical history was obtained and a literature search was undertaken using Medline and PubMed. This yielded no other similar cases in English literature.

Results: The microscopic examination showed a focus of mucinous adenocarcinoma arising within a benign Brenner tumour.

This case was referred for an expert opinion to the Royal Group of Hospitals, Belfast, who confirmed the above diagnosis and the rarity of this presentation.

Conclusion:

An incidental rare finding of a microscopic mucinous carcinoma arising within a benign Brenner tumour.

QUALITY IMPROVEMENT: REDUCING THE BURDEN OF ROUTINE DERMATOLOGY SPECIMENS IN A UNIVERSITY TEACHING HOSPITAL

Dr Dan Hopkins (ST3 Histopathology) & Dr Adam Christian (Consultant Histopathologist)

Introduction:

Routine dermatology specimens account for a large amount of work undertaken by histopathology departments. Improved efficiency in process and examination of these cases is of significant importance, and may represent substantial cost benefit to a department.

Method:

Routine departmental practice involved the upfront cutting of three levels on all routine dermatology specimens. Education of biomedical staff and adjustment of standard operating procedures was undertaken to restrict multiple upfront levels to only curettage and smaller shave specimens. The impact of the change was assessed by monitoring the number of routine dermatology specimens, levels undertaken, and additional levels requested for a month prior to the change and after its implementation.

Results:

Similar numbers of routine dermatology cases were undertaken by the department over the periods of monitoring. A significant reduction in the number of levels undertaken was seen, and an insignificant increase in the numbers of additional levels was noted. This suggests that an improvement has been achieved.

INTRAOPERATIVE FROZEN SECTIONS DURING PANCREATIC SURGERY

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The principal aims of this study were to establish the different types of frozen sections (FSs) performed during pancreatic surgery and whether the FSs improve the outcome of pancreatic surgery in terms of completeness of surgical excision.

A retrospective analysis of 122 reports of elective cases performed in our institution between January 2011 and March 2014 was conducted. Reports of FSs and final pathological reports were examined.

Unexpected (peritoneal, liver etc.) nodules and transection margins (pancreatic neck and common bile duct) were the two main types of FSs. The former were performed in 28 cases (24%) and in 9 cases the surgery was abandoned due to the frozen section findings. Remaining 113 cases (93%), which proceeded to the main surgery, all had FSs of transection margins. The FSs of transection margins had limited impact on the outcome of pancreatic surgery with regard of achieving full excision.

While this study confirms value of FS of unexpected nodules during pancreatic surgery, there appears to be less evidence to support routine performance FSs of transection margins in order to achieve complete surgical excision. While these findings are essentially supported by results of other current studies published in the literature, there appears to be a consensus that in a small minority of cases, FSs of transection margins are still beneficial. If FSs of transection margins were not to be performed routinely in this institution, the questions remain how could these cases be accurately identified and whether the pathologists would be able to retain current levels of expertise if the numbers of these procedures were significantly reduced.

SUBSTANCE MISUSE DEATHS WITHIN ABERTAWE BRO MORGANNWG UNIVERSITY HEALTH BOARD: WHAT IS THE PATHOLOGIST'S ROLE?

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Drug and alcohol misuse causes profound morbidity and premature death. The pathologist has a key role at inquest, a fact finding process that answers key questions:-Who the deceased was? Where did they die? When did they die? How they came about their death?

In order to answer the final question in detail, essential to preventing further similar deaths, as much information as possible should be obtained prior to autopsy - detailed previous/current drug use and methods of delivery, significant medical history and any previous/current access to CDAT including maintenance programs.

It was our perception that such information gathering between pathologist and Coroner/Coroner's officers may be substandard, so with permission from the Coroner we have analysed autopsy reports over a 2 year period. How can this information sharing be improved and standardized within ABMU?

OUTCOMES OF CERVICAL LIQUID-BASED CYTOLOGY SUGGESTING A GLANDULAR ABNORMALITY. SOUTH WEST WALES EXPERIENCE

Olexandra Kozyar, Alison Finall - Histopathology Department, Abertawe Bro Morgannwg University Health Board (ABMUHB)

Introduction:

Usual type high-grade CGIN has strong association with High Risk (HR)-HPV infection but detection by cervical cytology remains low (0.1%-0.21%). The outcomes of cervical liquid-based cytology are presented.

Aim:

To ascertain the positive predictive value (PPV) of both 'Borderline change in endocervical cells' and '?Glandular neoplasia'. To examine the benefit of HR-HPV triage testing in the Borderline endocervical group.

Method:

Retrospectively identified cases in consecutive 12 months and correlated with HR-HPV results, histopathological diagnoses and clinical follow-up.

Results:

The incidence of cervical glandular abnormality in cytology was 0.007%.

PPV for CIN2+ and/or CGIN or worse in 'Borderline in endocervical cells' and '?Glandular neoplasia' was 0 and 40% respectively.

Discussion:

The incidence of glandular abnormality in cytology is lower than in published data. This may reflect the small sample size, impacting in turn on PPV.

HR HPV testing has a significant impact on patients in the Borderline endocervical abnormality category.

AN AUDIT TO EVALUATE PIPELLE BIOPSY ADEQUACY AS A TECHNIQUE OF ASSESSMENT IN POST-MENOPAUSAL BLEEDING: THE EXPERIENCE OF ONE CENTRE

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Endometrial pipelle is one of the first-line investigations for postmenopausal bleeding allowing a convenient method of endometrial tissue sampling in an outpatient setting. It does however have inadequacy rate ranging in the published literature from 12-22%. Insufficient samples have patient management implications and may lead to delays in diagnosis.

We present the adequacy rate of pipelle biopsy as an assessment technique in post-menopausal bleeding pathway in our centre.

The results have informed local clinical pathway decisions to improve patient evaluation in this clinical setting. Our gynaecology department have moved to outpatient hysteroscopy (OPH) and biopsy in all cases where an abnormality has been detected on transvaginal ultrasound (TVS) or where the TVS is inadequate. Obese patients and those on tamoxifen should have OPH as standard even where TVS is reassuring.

A RARE CASE OF LYMPHOCYTE-RICH CLASSICAL HODGKIN LYMPHOMA WITH MICROSCOPIC AND IMMUNOLOGICAL PROFILE SIMILAR TO THAT OF A NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA

Authors: Bandecar A¹, Forsyth L², Rubbi C³, Menon G⁴

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Background:

Classical Hodgkin lymphoma (CHL), including lymphocyte-rich CHL (LRCHL) comprises 95% of Hodgkin lymphoma cases, while nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL), accounts for approximately 5% of HL cases.

However, distinction of the two categories is not always straightforward, because in addition to similar infiltrating reactive cells in the background, 98% of LRCHL cases have LP cell-like atypical cells, whereas 55% of NLPHL cases have H/RS cell-like large atypical cells.

We present a case of a 13-year old patient who had left submandibular gland swelling of insidious onset for which an extra nodal resection was done.

Results:

- Nodular polymorphous infiltrate of small lymphoid cells with occasional eccentric germinal centers
- RS cells which are positive for CD30, CD20 and CD15
- Lymphoid cells strongly positive for CD3 with a weak expression of Pax 5
- An equivocal expression of OCT- 2
- Negative for Bcl-2, Bcl-6, CD45, EMA and EBV-LMP1
- CD3/CD4/PD-1 positive rosettes around many of the RS cells

Conclusion:

The overall morphological and immunohistochemical appearances were more in keeping with a classical Hodgkin lymphoma of lymphocyte-rich type. Due to their microscopic similarities and overlapping features, these cases often require detailed immunostaining and careful analysis by a specialist haematopathologist, to make a correct diagnosis.

It is important to study a large number of such cases to have a better understanding of the differential features between these two entities. Clinically, this has important implications with regards to the management of patients. While solitary presentation of NLPHL may be managed by active monitoring alone, LRCHL needs appropriate chemotherapeutic management.

GENETIC AND EPIGENETIC INTRA-TUMOURAL HETEROGENEITY IN COLORECTAL CANCER

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Colorectal Cancer (CRC) is a highly heterogeneous disease, with pathologically similar cancers having completely different responses to treatment and patient survival. Intra-tumour heterogeneity (defined as distinct morphological and phenotypic differences) has recently been demonstrated to be an important factor in the development and behaviour of cancer cells, and can be used to determine response to anticancer therapy.

Patients with resected CRC had DNA extracted from eight defined tumour areas which were analysed for two genetic mutations (BRAF and KRAS) and one epigenetic trait (CpG island methylator phenotype / CIMP). Normal adjacent tissue was studied as control.

Twelve patients with CRC were included. Intra-tumour heterogeneity for KRAS mutation was seen in 2 patients (17%). There was no statistical evidence of CIMP status heterogeneity (p=0.85), but 6 of the 12 patients (50%) demonstrated at least one heterogeneous area within the tumour.

Intra-tumoural heterogeneity for both genetic and epigenetic factors in CRC is more prevalent than previously thought. This study provides new insight into epigenetic heterogeneity of CRC, and supports development of a more targeted biopsy strategy to support expansion of personalised treatment.

SMALL CELL CARCINOMA OF THE RECTUM: A SYSTEMATIC LITERATURE REVIEW AND CASE SERIES

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Small cell carcinoma of the rectum is a rare entity that is usually metastatic at presentation and has a poor long term survival despite aggressive treatment schedules.

A ten year review identified three cases between 2004 and 2014. A systematic search through Embase, Pubmed and Google identified 112 articles of which 41 were used (121 patients). Optimal survival (37 months) was achieved with a combination of surgery, radiotherapy and chemotherapy.

Prospective tumour registries would optimise management of this unusual neuroendocrine tumour.

ANEUPLOIDY LEVELS IN PANCREATIC INTRAEPITHELIAL NEOPLASIA

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Pancreatic ductal carcinoma has an appalling survival rate of 4% at 5 years, with average survival after diagnosis of 3-6 months. A model of progression from PanIN 1 to adenocarcinoma has been proposed. In this study we examined levels of aneuploidy in the various stages using FISH and Centromeric enumeration probes for chromosomes 1, 6, 9 1 and 18. A total of 68 targets were selected for counting from 29 panceatic resections, from normal through PanIN 3 to adenocarcinoma, scoring 300 nuclei per target.

Conclusion: Amplifications are the most reliable index of an uploidy as truncation of nuclei by sectioning causes apparent loss of centromeres. Amplification levels were low in PanIN1 and 2 (>1%) rising to 12.7 and 20% in PanIN3 and carcinoma. This suggests a non-linear progression to cancer with major chromosomal changes occurring late in the sequence.

CAN A FINGER PRICK TEST BE USED TO PREDICT DISEASE PROGRESSION IN PATIENTS WITH BARRETT'S OESOPHAGUS?

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The PigA mutant phenotype assay is a novel flow cytometric assay neding only a pinprick of blood (10µml). PigA gene (X chromosome) codes for GPI-anchors which tether certain proteins to the red cell surface, its role being recognised in paroxysmal nocturnal haemoglobinuria. Screening red cells for the presence or absence of key proteins can identify mutant red cells and is a sensitive test for mutagens.

120 patients with gastro-oesophageal reflux disease, Barrett's oesophagus and adenocarcinoma of the oesophagus were studied using CD55 and CD59 as markers.

The Pig-A Gene Mutation Assay is a fast, reproducible and high throughput assay which is becoming increasingly popular as a potential alternative investigation for DNA damage utilising the power of flow cytometry.

There is a stepwise increase in mutant frequency along histological lines. Chemotherapy patients (positive control) show highest levels.

Age, diet and lifestyle may also play an important role in determining underlying mutant frequency. Aspirin users have lower mutant frequencies than non-users and may further underline the important chemoprotective role of aspirin.

The assay also appears to be associated with invasiveness of cancer, and good correlation of blood mutant frequency with tissue GPI anchor status is possible.

There may be the potential to utilise this assay in the investigation of patients with other pre-malignant conditions to aid diagnosis.

INVESTIGATING MECHANISMS THROUGH WHICH A HORMONAL THERAPY COULD BE USED TO TREAT PATIENTS WITH BARRETT'S OESOPHAGUS

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The NF-kB pathway is implicated in the dysplastic sequence of BO to OA.

Increased cytoplasmic p65 levels are seen in women compared to men (p=0.006).

In vitro studies have demonstrated Estrogen Receptors on commonly used OA cell types.

Investigations have also demonstrated that Estradiol reduces active p65.

This may be through maintaining higher levels of iKB in the cytoplasm, thus reducing p65's nuclear translocation.

Furthermore, estradiol maintains iKB levels even after DCA treatment.

A reduction in iKB levels appears to be associated with histological progression.

Patients with higher estradiol levels (e.g. those on hormone replacement therapy) show increased iKB levels.

These effects may, in part, explain the gender differences observed in Barrett's Oesophagus and Oesophageal Adenocarcinoma.

EBV POSITIVE MUCOCUTANEOUS ULCER WITH LYMPH NODE INVOLVEMENT CAUSING A SIGMOID STRICTURE. A CASE REPORT

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A 74 year old lady presented with rectal bleeding and a stricture with ulceration of the sigmoid colon was found. Atypical Reed- Sternberg –like cells in the infiltrate around the ulcer led to the diagnosis of this relatively new entity.

EBV positive mucocutaneous ulcer is an EBV associated B-cell lymphoproliferative disease first described by Dojcinov et al. (1) in the context of immunosenescence and iatrogenic immunosuppression and by Hart et al. (2) in post transplant immunosuppression

- Majority of the cases reported present in the skin and mucosal surfaces of the head and neck (1,2,3)
- Gastrointestinal sites such as oesophagus, small bowel and large bowel (rectum) are less common (1,2,4,5,6)

Histologically the presence of B-cell blasts often with HRS-like features and mixed inflammatory background raises differential diagnoses such as classical Hodgkin lymphoma (cHL), lymphomatoid granulosis (LyG) and T-cell rich large B-cell lymphoma (TCRBCL) (3)

Monoclonal IG rearrangements have been found in some cases (1, 2) as well as restricted or clonal TCR rearrangements (1), as seen in our case.

Good prognosis as majority of the cases published have had a benign course either spontaneously resolving or resolving on decrease/removal of the immunosuppression.

SMARCA4-DEFICIENT THORACIC SARCOMA

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We present a case of SMARCA4-deficient thoracic sarcoma arising in the left mediastinum of an 80 year old man.

This is an unusual malignancy transcriptionally related to epithelioid sarcoma, small cell carcinoma of the ovary (hypercalcaemic type) and rhabdoid tumours.

The histology, immunohistochemistry and molecular genetics are demonstrated, with clinical data and reference to current medical literature. Learning points from the case are discussed.

STAPHYLOCOCCUS AUREUS BACTERIURIA: A QUALITY IMPROVEMENT PROJECT

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Background:

Staphylococcus aureus bacteriuria is unusual and raises the suspicion of a deep seated staphylococcal infection.

Aims:

- 1. Assess the proportion of patients with *S. aureus* bacteriuria who had deep seated infections
- 2. Setup a new system to refer urines growing *S.aureus* to the microbiology registrars queue to facilitate early identification of deep infections

Methods:

An electronic database was used to identify urines growing *S.aureus*. The proportion of deep seated infections was assessed. Following the intervention, the number of *S.aureus* urines referred to the microbiologist and numbers of deep seated infections identified early was calculated.

Results:

- First stage (2015): 9% of 88 urines had deep seated *S.aureus* infections.
- Second stage (2017):
 - o 78% of 50 urines were referred to the registrar's queue
 - o 92% of urines referred had relevant comments
 - 8% of patients had deep seated *S.aureus* infections which were identified early- see below

Deep seated S.aureus infections	(second stage of the o	uality improvement project)
Deep seated staticas infections	Second stage of the c	

Cases	Age	Source of infection	Bacteraemia with <i>S.aureus</i>	Choice/duration of antibiotics
Case 1	63	Discitis and psoas	Y	Flucloxacillin IV
		abscess		Ertapenem IV and rifampicin PO
				Levofloxacin PO and rifampicin PO
				12 weeks in total
Case 2	80	Infected ureteric stents	Y	Vancomycin V
				Cotrimoxazole PO and rifampicin PO
				4 weeks in total
Case 3	92	Bacteraemia ?source	Y	Flucloxacillin IV
				patient RIP on day 4 of treatment
Case 4	22	Infected ureteric stents	N	Vancomycin IV
		(MRSA)		2 weeks

Conclusion

S.aureus bacteriuria may be a sign of deep seated infection and by identifying these early, appropriate management can be instituted in a timely manner.

THE PREVALENCE OF HEPATITIS B AND C INFECTION AMONG ASYLUM SEEKER POPULATION IN CARDIFF

Brendan Healy, Sian Jones and Catherine Russell

Objective of study:

This pilot project aims to establish the prevalence of Hepatitis B and C among asylum seekers, so as to determine whether screening of this population is warranted. The project also seeks to determine whether positive patients can be successfully linked to care from this service.

Methods

All asylum seekers that were assessed over the two week period from 27/2/17 - 10/3/17 in the Cardiff area were offered testing for HBV and HCV

Key Findings:

126 individuals were assessed over the period and all were tested. Of the 126 tested:

- 6 (5.77%) were found to be chronically infected with HBV and
- 1 (0.96%) was positive for HCV.
- Important differences in prevalence were noted according to the individuals country of origin
- The individual positive for HCV was originally from Egypt giving a prevalence of 25% in this group

Conclusion:

This pilot investigation gives the first data on prevalence of hepatitis B & C in asylum seekers in the Cardiff area. The pilot was extremely successful in capturing individuals that attended the centre and demonstrates that screening in this community is viable (100% uptake). Based on the results from the pilot we recommend indiscriminate testing for Hepatitis B in individuals seeking asylum and screening for Hepatitis C in individuals arriving from high prevalence countries (see map). These proposals will now be subjected to an assessment of the affordability and cost effectiveness of each strategy including assessment of targeted screening in hepatitis B. A business case for adoption of testing in this population will be constructed on the basis of that data.

The high prevalence of HBV also highlights a need to consider universal Hepatitis B vaccination in this population.

AUDIT OF JUNIOR DOCTORS UNDERSTANDING OF LABELLING OF "HIGH RISK" SAMPLES

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AUDIT OF PATIENTS THAT ARE INAPPROPRIATELY LABELLED 'HIGH RISK' WITH REGARDS TO BLOOD BORNE VIRUSES

Dr Brendan Healy1, Amber Bryce2

1Consultant in Microbiology and Infectious Diseases, UHW, 2MLA, Microbiology

