The Bulletin of the Sri Lanka College of Microbiologists

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Immunology

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of the
Sri Lanka College of Microbiologists
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The Sri Lanka College of Microbiologists

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The Sri Lanka College of Microbiologists

Council 2013 / 2014

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The Bulletin of the Sri Lanka College of Microbiologists
is published annually with the Scientific Sessions of the College.

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23rd Annual Scientific Sessions

The Sri Lanka College of Microbiologists

Inauguration

13th August 2014 at 6.00 pm
Sri Lanka Foundation
Colombo 7

Pre-Congress Workshop

Theme:

"Infection Control - Aiming for Success by Changing Practices"

13th August 2014

Scientific Programme

Theme:

"Rational Use of Antibiotics – Prepare Today for Success Tomorrow"

14th & 15th August 2014
Sri Lanka Foundation
Colombo 07

MESSAGE FROM THE PRESIDENT

As the president of the Sri Lanka College of Microbiologists I am writing this message with great pleasure. Since the inception of Annual Academic Sessions in 1991, Sri Lanka College of Microbiologists has come a long way under the able leadership of previous councils. Close to a year has gone since I have assumed responsibilities as the President of the College and we have come to the most important date in our academic calendar, the Annual Scientific Sessions.

In keeping with the daunting problem of antibiotic resistance, especially in our part of the world we have selected the theme of 'Rational use of antibiotics - Prepare today for success tomorrow' for the sessions. As clinical microbiology and infection control are two permanently fixed loops in a chain, the theme of the precongress workshop is 'Infection Control - Aiming for success by changing practices'.

The sessions are enriched with five international guest speakers spanning from USA through UK, India to Australia and eminent local guest speakers sharing their knowledge and experience. The comprehensive scientific programme will cover many aspects in our speciality and I am sure we would gain an invaluable wealth of knowledge which we could apply for the advancement of medical microbiology in Sri Lanka. I would like to take this opportunity to extend my sincere appreciation to all the guest speakers.

Adding colour to the inauguration ceremony is the Siri Wickremesinghe oration in memory of a great medical microbiologist, the late Dr. R. S. B. Wickremesinghe. I wish to thank Dr. Ranjith Perera, Head of the Department of Medical Microbiology, Faculty of Medicine, Ragama, for accepting the invitation of the council to be the orator.

As in the past, this year too was very active and my special thanks go to the council for the great deal of hard work rendered with happiness. Many members in the general membership too contributed actively. With this unstinted support we were able to take many important decisions regarding upgrading of Microbiology services in the country by finalising the list of hospitals for appointment of consultant Microbiologists and consultant Virologists, revising the list of hospitals for provision of offsite consultancy services to hospitals with no onsite consultant Microbiologists, conducting in-service MLT training workshops, workshop for infection control for private sector nurses and doctors, regular CME lectures and serving and contributing in advisory capacity in many forums including the Ministry of Health. I value the immense contribution of many active members in the College for letting us see an end in developing national antibiotic guidelines on empirical therapy in collaboration with all relevant professional colleges, as well as revising the Biosafety Manual for medical laboratories.

I appreciate very much the efforts of the Hony. Joint secretaries of the College, Dr. Rohini Wadanamby and Dr. Dushyanthie Athukorala and the College Office Secretary Mrs. Priyanga Opatha to make this event a success and for giving me strength to execute all the responsibilities.

This event would not have been possible without the generous contribution of our sponsors. On behalf of the council I would like to thank all the sponsors with a special note to AstraZeneca and State Pharmaceuticals Corporation for their valuable contributions.

Finally, on behalf of the Sri Lanka College of Microbiologists I would like to welcome all of you to the 23rd annual scientific sessions.

Dr. Kumudu Karunaratne
President
Sri Lanka College of Microbiologists

INAUGURATION PROGRAMME

13 - 08 - 2014 – Sri Lanka Foundation, Colombo 7

6.00 pm	Invitees take their seats
6.15 pm	Arrival of the Chief Guest Introduction of Members of the Council
6.30 pm	Ceremonial Procession
6.35 pm	National Anthem
6.40 pm	Traditional lighting of the Oil Lamp
6.50 pm	Welcome Address Dr. Dushyanthie Athukorala Hony. Joint Secretary
6.55 pm	Address by the Chief Guest Mr. S. Palitha Fernando Attorney General, Sri Lanka
7.10 pm	Address by the Guest of Honour Dr. Christopher Coulter Director, Queensland Mycobacterium Reference Laboratory and Infectious Diseases Physician and Microbiologist, The Prince Charles Hospital, Australia
7.20 pm	Address by the President Dr. Kumudu Karunaratne Consultant Microbiologist, LRH
7.50 pm	Introduction of the Orator Siri Wickremesinghe Memorial Oration Dr. Kumudu Karunaratne, President, SLCM
7.55 pm	Siri Wickremesinghe Memorial Oration **Notes of a Medical Microbiologist – A Journey through History, Mythology, Religion and Evolution** Dr. Ranjith Perera Senior Lecturer and Head of Department of Medical Microbiology, Faculty of Medicine, Ragama
8.30 pm	Vote of Thanks Dr. Rohini Wadanamby Hony. Joint Secretary
8.35 pm	Procession leaves the hall
8.40 pm	Reception

23rd Annual Scientific Sessions

The Sri Lanka College of Microbiologists

Pre congress workshop

Theme:

"Infection Control – Aiming for Success by Changing Practices"

13th August 2014

Sri Lanka Foundation
Colombo 07

Chairpersons: Dr. Pranitha Somaratne and Dr. Philomena Chandrasiri

8.30 am - 9.00 am	**Registration**
9.00 am - 9.45 am	**Management of outbreaks of health care assoiciated infections** Prof. Balaji Veeraraghavan *Professor and Head of the Department, Department of Clinical Microbiology* *Christian Medical College, Vellore, India.*
9.45 am - 10.30 am	**Prevention of healthcare acquired tuberculosis in the age of drug resistance** Dr. Chris Coulter *Director, Queensland Mycobacterium Reference Laboratory, WHO Collaborating Centre and Medical Advisor TB - CHRISP & Tuberculosis Control; Infectious Diseases Physician and Clinical Microbiologist, The Prince Charles Hospital, Chermside, Queensland, Australia.*
10.30 am - 11.00 am	**Tea**
11.00 am - 11.45 am	**Prevention of infections in intensive care units** Dr. David Jenkins *Lead infection prevention consultant, Leicester University Hospital, UK.*
11.45 am - 12.30 pm	**Preparing for mass infective emergencies** Dr. P. C. J. Fonseka *Medical Officer, Medical Disaster Management, Infectious Disease Hospital, Angoda.*
12.30 pm - 12.45 pm	**Discussion**

23rd Annual Scientific Sessions

The Sri Lanka College of Microbiologists

Scientific Programme

Theme:

"Rational Use of Antibiotics – Prepare Today for Success Tomorrow"

14th & 15th August 2014

Sri Lanka Foundation
Colombo 07

Day 1 – 14. 08. 2014

8.15 am - 8.45 am **Registration**

8.45 am - 9.30 am

Free paper session 1

Chairpersons: Dr. Kanthi Nanayakkara and Dr. Shirani Chandrasiri

OP 1 **Bacterial flora and their antibiotic sensitivity patterns in plastic biliary stents removed from patients through Endoscopic Retrograde Cholangiopancreatography (ERCP)**

Basnayake BMPI[1], *Kottahachchi J*[1], *De Silva M*[2], *Weerasekera MM*[1], *Wanigasooriya IWMP*[3], *Athukorala GIDDAD*[1], *Dissanayake DMBT*[1], *Nalaka ABMJ*[1], *Bogahawatta LBAE*[1], *Fernando SSN*[1]

[1]Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, [2]Professorial Surgical Unit, Colombo South Teaching Hospital, Kalubowila, [3]Endoscopy Unit, Colombo South Teaching Hospital, Kalubowila.

OP 2 **Antibiotic sensitivity of enteric bacteria in different food animals in relation to antibiotic use in the Colombo district**

Asanthi MAI[1], *Jayathilake K*[1], *Gunawardana GA*[2]

[1]Department of Microbiology and Infection Control, Sri Jayewardenepura General Hospital, Kotte, [2]Veterinary Research Institute, Gannoruwa, Peradeniya.

OP 3 **Salmonella serotypes isolated or serotyped at the National Reference Enteric Laboratory and their antibiotic sensitivity pattern**

Pathirage MVSC, Wijewardana N, Perera R, Katriarachchi K, Jayamaha C

Medical Research Institute, Colombo 08.

9.30 am - 10.15 am

Plenary 1

Getting the patient mobile – Diagnosis and management of prosthetic joint infections

Dr. Chris Coulter

Director, Queensland Mycobacterium Reference Laboratory, WHO Collaborating Centre and Medical Advisor TB - CHRISP & Tuberculosis Control; Infectious Diseases Physician and Clinical Microbiologist, The Prince Charles Hospital, Chermside, Queensland, Australia.

Chairperson: Prof. Nelun De Silva

10.15 am - 10.30 am **Tea**

10.30 am - 11.30 am

Free paper session 2

Chairpersons: Dr. Lilani Karunanayake and Dr. Kushlani Jayatilleke

OP 4 **Predictability of presence of Extended Spectrum Beta-Lactamase (ESBL) producing organisms in urine cultures using co-amoxyclav and cephalexin discs in the first line antibiotic susceptibility testing**
Goonewardene TDL, Nanayakkara GM
Provincial General Hospital, Ratnapura.

OP 5 **Development of a real-time PCR assay for intrapartum detection of Group B Streptococcal colonization**
Namalie KD, McIver CJ
Department of Microbiology, St George Hospital, Kogarah, NSW, 2217, Australia.

OP 6 **A comparison of qualitative and quantitative sputum culture methods**
Herath HMNC[1], Elwitigala JP[1], Dassanayake KMMP[2]
[1]National Tuberculosis Reference Laboratory, Welisara, [2]Colombo North Teaching Hospital, Ragama.

OP 7 **Rapid detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* culture isolates: an evaluation of a line probe assay**
Francis VR[1], Elwitigala JP[1], De Silva AD[2]
[1]National Tuberculosis Reference Laboratory, [2]Gene-Tech Research Institute

11.30 am - 12.30 pm

Symposium 1 – Emerging Infections

Moderators: Dr. Sunethra Gunasena and Dr. Malka Dassanayake

Melioidosis – Is it present in Sri Lanka?
Dr. Enoka Corea
Consultant Microbiologist and Senior Lecturer, Department of Microbiology, Faculty of Medicine, University of Colombo, Colombo 08.

New faces of dengue
Prof. Benjamin Alan Pinsky
Professor, Departments of Pathology and Medicine (Infectious Diseases), Stanford University School of Medicine; Director, Clinical Virology Laboratory, Stanford University Medical Center and Lucile Packard Children's Hospital, USA.

12.30 pm - 1.30 pm **Lunch**

1.30 pm - 2.15 pm

Plenary 2

Spectrum of cutaneous mycoses in Sri Lanka
Dr. Chalukya Gunasekera
Consultant Dermatologist, National Hospital of Sri Lanka, Colombo 10.

Chairperson: Dr. Maya Atapattu

2.15 pm - 3.00 pm

Free paper session 3

Chairpersons: Dr. Nalini Withana and Dr. Ajith Nagahawatte

OP 8 **Study on hepatitis B markers among family members in a group of hepatitis B virus infected patients**
Muthugala MARV, Galagoda GCS, De Silva S, Jeewanka IGI
Department of Virology, Medical Research Institute, Colombo 08.

OP 9 **Surveillance of rotavirus in three hospital settings of Sri Lanka 2007-2010**

Chandrasena TGAN[1], *Reagindrajith S*[2], *Gunawardane NK*[1], *Liyanarachchi N*[3] *Abeysekara CK*[4], *Matsomoto T*[5], *Yahiro T*[5], *Nishizono A*[5], *Ahmed K*[6]
Departments of [1]Parasitology and [2]Paediatrics, Faculty of Medicine, University of Kelaniya, Sri Lanka, [3]Department of Paediatrics, Faculty of Medicine, University of Ruhuna, Sri Lanka, [4]Department of Paediatrics, Faculty of Medicine, University of Peradeniya, Sri Lanka, [5]Department of Microbiology, Faculty of Medicine, Oita University, Yufu, Japan, [6]School of Medicine (Research Promotion Institute), Oita University, Yufu, Oita, Japan.

OP 10 **A study of invasive candida infections at a paediatric teaching hospital**

Karunaratne GKD[1], *Dinapala SK*[1] , *Kathriarachchi K*[1] , *Perera PD*[2] *Jayasekara P*[2]
[1]Lady Ridgeway Hospital, Colombo 8, [2]Medical Research Institute, Colombo 08.

3.00 pm - 3.45 pm

Plenary 3

Validation of serology and molecular tests

Prof. Benjamin Alan Pinsky
Professor, Departments of Pathology and Medicine (Infectious Diseases),
Stanford University School of Medicine; Director, Clinical Virology Laboratory, Stanford University Medical Center and Lucile Packard Children's Hospital, USA.

Chairperson: Prof. N. P. Sunil-Chandra

3.45 pm **Tea**

Day 2 – 15. 08. 2014

8.30 am - 9.30 am

Free paper session 4

Chairpersons: Dr. Sujatha Pathirage and Dr. Malika Karunaratne

OP 11 **Bacterial pathogens causing post-operative infections following abdominal surgery at a tertiary care hospital in Southern Province of Sri Lanka**

Palangasinghe S[1], *Vidanagama DS*[1], *Nagahawatte A*[2], *Perera B*[2]
[1]Teaching Hospital, Karapitiya, [2]Faculty of Medicine, University of Ruhuna, Galle.

OP 12 **Evaluation of bactericidal effect of three antiseptics on bacteria isolated from wounds**

Kottahachchi J[1], *Kumara DUA*[2], *Dissanayake DMBT*[1], *Athukorala GIDDAD*[1], *Chandrasiri NS*[3], *Damayanthi KWN*[1], *Hemarathne MHSL*[1], *Fernando SSN*[1], *Pieris H*[4], *Pathirana AA*[2]
[1]Department of Microbiology and [4]Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, [2]Professorial Surgical Unit, Colombo South Teaching Hospital, [3]Department of Microbiology, Colombo South Teaching Hospital.

OP 13 **A prospective study of causative organisms, antibiotic management and clinical outcome of patients with infective endocarditis treated at the National Hospital of Sri Lanka**

Asanthi MAI, Patabendige CGUA
Department of Microbiology and Infection Control, National Hospital of Sri Lanka, Colombo 10.

OP 14 **Determining minimum inhibitory concentrations of vancomycin for methicillin resistant *Staphylococcus aureus* isolates using an agar dilution method**

De Silva PHCJ[1], Wickramasinghe D[2], Vidanagama DS[2], Nagahawatte A[1]
[1] Faculty of Medicine, University of Ruhuna, Galle, [2]Department of Microbiology, Teaching Hospital, Karapitiya, Galle.

9.30 am - 10.15 am

Plenary 4

Pneumococcal infections in Southeast Asia: Past, present and future

Prof. Balaji Veeraraghavan
Professor and Head of the Department, Department of Clinical Microbiology
Christian Medical College, Vellore, India.

Chairperson: Prof. Jennifer Perera

10.15 am - 10.30 am **Tea**

10.30 am - 11.30 am

Free paper session 5

Chairpersons: Dr. Preethi Perera and Prof. Neluka Fernando

OP 15 **Analysis of data of urine culture isolates of 2013 sent from four laboratories of National Laboratory Based Surveillance of Sri Lanka College of Microbiologists**

Jayatilleke SK[1], Karunaratne GKD[2], Perera J[3], Perera RRDP[4], Wijesooriya WRPLI [4], Sunil-Chandra NP[4]
[1]Sri Jayewardenapura General Hospital, Nugegoda, [2]Lady Ridgeway Hospital for Children, Colombo 8, [3]Department of Microbiology, Faculty of Medicine, Colombo, [4]Department of Microbiology, Faculty of Medicine, Ragama.

OP 16 **Detection of antibacterial activity in cerebrospinal fluid**

Abeywardena HMW, Thevanesam V, Illangasinghe IMS, Kumari NRW
Teaching Hospital, Peradeniya.

OP 17 **Mupirocin resistance among isolates of methicillin resistant *Staphylococcus aureus* at National Hospital Sri Lanka**

Samaranayake WAMP[1], Karunanayake L[1], Patabendige G[2]
[1]Medical Research Institute, Colombo 08, [2]National Hospital of Sri Lanka.

OP 18 **Occurrence of KPC producing *K. pneumoniae* and associated factors in a selected hospital in the Colombo District**

Suranadee YWS[1], Perera AJ[1], Gamage S[1], Pathirage SC[2]
[1]Department of Microbiology, Faculty of Medicine, University of Colombo,
[2]Medical Research Institute, Colombo 8.

11.30 am - 1.00 pm

Symposium 2 – Antimicrobial Stewardship

Moderators: Prof. Vasanthi Thevanesam and Dr. S. D. Athukorala

Antibiotic stewardship

Dr. David Jenkins
Lead infection prevention consultant, Leicester University Hospital, UK.

An economic evaluation of antimicrobial stewardship programmes
Ms. Sonali Coulter
Scientist in Clinical Microbiology, Australia.

Implementation of antibiotic policies in developing countries
Prof. Balaji Veeraraghavan
Professor and Head of the Department, Department of Clinical Microbiology, Christian Medical College, Vellore, India.

1.00 pm - 2.00 pm **Lunch**

2.00 pm - 3.00 pm

Symposium 3 – Endocarditis

Moderators: Dr. Geethika Patabendige and Dr. Samanmalee Gunasekera

Clinical perspective on management of endocarditis
Dr. Duminda Samarasinghe
Consultant Paediatric Cardiologist, Lady Ridgeway Hospital for Children, Colombo 08.

Microbiology of infectious endocarditis: Do new tools solve old dilemmas?
Dr. Chris Coulter
Director, Queensland Mycobacterium Reference Laboratory, WHO Collaborating Centre and Medical Advisor TB - CHRISP & Tuberculosis Control; Infectious Diseases Physician and Clinical Microbiologist, The Prince Charles Hospital, Chermside, Queensland, Australia.

3.00 pm - 3.45 pm

Plenary 5

Pharmacology of antibiotics in rational use of medicines
Dr. Chandanie Wanigatunge
Consultant Physician and Senior Lecturer in Pharmacology, Department of Pharmacology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda.

Chairperson: Dr. Sujatha Mananwatte

3.45 pm - 4.15 pm **Award ceremony**

4.15 pm **Tea**

INTERNATIONAL FACULTY

Dr. Chris Coulter
Director, Queensland Mycobacterium Reference Laboratory, WHO Collaborating Centre and Medical Advisor TB - CHRISP and Tuberculosis Control; Infectious Diseases Physician and Clinical Microbiologist,The Prince Charles Hospital, Chermside, Queensland, Australia

Prof.Balaji Veeraraghavan
Professor and Head of the Department, Department of Clinical Microbiology, Christian Medical College, Vellore, India

Prof. Benjamin Alan Pinsky
Professor, Departments of Pathology and Medicine (Infectious Diseases), Stanford University School of Medicine; Director, Clinical Virology Laboratory, Stanford University Medical Center and Lucile Packard Children's Hospital, USA

Dr. David Jenkins
Lead Infection Prevention Consultant, Leicester University Hospital, UK

Ms. Sonali Coulter
Scientist in Clinical Microbiology, PhD Candidate, Centre of Research Excellence in Reducing Healthcare Associated Infections, Queensland University of Technology, Brisbane, Australia

NATIONAL FACULTY

Dr. Enoka Corea
Senior Lecturer, Department of Microbiology, Faculty of Medicine, University of Colombo, Colombo 08

Dr. Chalukya Gunasekera
Consultant Dermotologist, National Hospital of Sri Lanka, Colombo 10

Dr. Duminda Samarasinghe
Consultant Paediatric Cardiologist, Lady Ridgeway Hospital for Children, Colombo 08

Dr. Chandanie Wanigatunge
Senior Lecturer in Pharmacology, Department of Pharmacology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda

Dr. P. C. J. Fonseka
Medical Officer in Medical Disaster Management, Infectious Disease Hospital, Angoda

ABSTRACTS OF THE PLENARY LECTURES AND SYMPOSIA

Plenary 1

Getting the patient mobile: Diagnosis and management of prosthetic joint infections

Dr. Christopher Coulter

Total hip and knee joint replacement is considered a major achievement of modern elective surgery and offers the patient freedom from debilitating arthritic pain and restoration of function. Both procedures deliver a meaningful increase in quality adjusted life years gained and are considered to be highly cost-effective medical procedures. Other joints are amenable to arthroplasty but less well studied. The outcome for the small percentage of patients whose course is complicated by deep organs space infection is in the short term difficult as both revision surgery and prolonged intravenous and oral antibiotics are invariably indicated. Nonetheless favourable outcomes are possible in the majority of patients with arthroplasty infections. Such outcomes are improved by early and accurate diagnosis of pathogen specific infection, targeted antimicrobial therapy, prudent surgical decision making and a multi-disciplinary approach to management. Strategies to enhance diagnostic yield will be reviewed. Despite the large number of arthroplasty procedures performed worldwide, the quality of evidence to support surgical and medical approaches to the management of total joint infection is restricted and non-randomised trials, cohort studies, consensus and expert opinion remain prominent in international guidelines.

Plenary 3

Test validation

Prof. Benjamin Alan Pinsky

Test validation is the provision of objective evidence through a defined process that a test performs as intended. The validation of the performance characteristics of clinical tests is therefore critical to ensure the quality of reported laboratory results. In this talk, the process of test validation will be reviewed, including the components of analytical and clinical validation. Focus will be placed on the validation of molecular infectious disease tests, both qualitative and quantitative, as well as serologic tests. Validation resources and guidelines, including those of the College of American Pathologists (CAP) and Clinical and Laboratory Standards Institute (CLSI), will be discussed.

Plenary 5

Pharmacology of antibiotics in rational use of medicine

Dr. Chandanie Wanigatunge

Rational use of medicines refers to the correct, proper and appropriate use of medicines (WHO). Rational use requires that patients receive the appropriate medicine, in the proper dose, for an adequate period of time, and at the lowest cost to them and their community. This concept is even more important in the use of antibiotics as the world faces an epidemic of antibiotic resistance, the main culprit being the irrational use of antibiotics. Irrational use is also associated with an increase in treatment costs, interference with patient's normal flora and an increase in the incidence of side effects.

Pharmacokinetics is the study of what body does to a drug and pharmacodynamics is how a drug behaves in the body. While the traditional approach to use of medicines is based more on habit than science, the knowledge and application of the pharmacology of an antibiotic would enable selection of the antibiotic that is most appropriate to the individual patient. Knowledge of the concentration and time period of drug exposure needed to control infection, as well as understanding of how a drug is distributed in the body and comes into contact with the target organisms, can help refine drug selection and dosing to gain the maximum therapeutic effect with minimum cost and toxicity.

Symposium 1

Emerging Infections

Diagnostics for dengue and other pathogens that cause an undifferentiated systemic febrile Illness

Prof. Benjamin Alan Pinsky

Dengue is the most common mosquito-borne viral disease of humans with over half the world's population at risk for infection. Dengue has a wide spectrum of clinical manifestations, from self-limited febrile illness to fatal hypovolemic shock, and because of this, dengue is difficult to distinguish from many other infections based on clinical characteristics alone. Diagnostic testing is therefore critical to accurately identify dengue virus (DENV)-infected patients and also rule out dengue in patients with undifferentiated fever. Current diagnostics

for early DENV detection consist of point-of-care or laboratory-based antigen tests that lack sensitivity or molecular assays that are laborious to perform or lack the test characteristics necessary for routine use. In this presentation, methods for addressing these diagnostic limitations will be discussed. A new single-reaction, multiplex, real-time RT-PCR for the detection, quantitation, and serotyping of dengue viruses from patient serum or plasma will be described and it's performance compared to several reference diagnostics.

The presenting complaints of patients with dengue are non-specific and cannot reliably be differentiated from other causes of fever in endemic areas, including malaria and leptospirosis. *Plasmodium* species, the pathogens responsible for malaria, are the most common vectorborne parasites worldwide and are also the most common causes of systemic febrile illnesses in travelers returning from endemic countries. Leptospirosis, a bacterial zoonosis caused by spirochetes in the genus Leptospira, has emerged as another significant cause of an acute febrile illness. Molecular diagnostic testing for these and other pathogens with similar clinical presentations will be covered.

Symposium 1

Emerging Infections

Melioidosis – Is it present in Sri Lanka?

Dr. Enoka Corea

The story of melioidosis in Sri Lanka begins in 1927. Looking back through historical records we find that Sri Lanka was one of the first countries to report a case of melioidosis. This report of "Melioidosis in a European" was published in the Ceylon Journal of Science. Based on this case, the early editions of text books of tropical disease listed Sri Lanka (or rather Ceylon) as endemic for melioidosis. Even in 1971, in Howe's review of melioidosis and in 1991 in David Dance's review Sri Lanka is shown as endemic for melioidois.

However, other than for one tantalizing case of a Belgian tourist who may have been infected in Sri Lanka in 1994, there were no further reports of melioidosis and Sri Lanka was dropped from the text books and relegated to a country with sporadic case reports. In 2003 Sri Lanka had recorded its first case in an indigenous person, but it was published in a local journal and not accessible to the wider scientific community.

All this changed when Professor Vasanthi Thevanesam was asked to look at an unusual isolate from blood culture. She recognized it as *Burkholderia pseudomallei* and contacted Prof Tim Inglis who advised on therapy. This patient survived, but, unfortunately, this was not the case for the next patient who was dead before the blood culture isolate was identified.

Prof Tim visited Sri Lanka the following year and confirmed the identity of the two isolates and offered reference facilities at PathWest Australia. Since then the numbers of culture positive cases have increased exponentially, and it seems we are looking at an emerging disease. However, was it truly emerging, in the sense of a disease that has historically infected humans but was now appearing in a new location or had it always been there, unrecognised?

Evidence came with my visit to Perth and PathWest. Genotyping of the strains and mapping of the genotypes showed that there was a large diversity of genotypes, as many as 20 among the 35 isolates and that they were widely distributed across the country, more compatible with a bacterium that had been around a long time, than of one that had been introduced in the recent past.

The clinicians were clamouring for a case definition. So we had a look at the clinical presentations. They ranged from superficial abscesses to sepsis. All the clinical presentations were those that are usually caused by far more familiar bacterial pathogens. It was easy to understand why previous workers had termed melioidosis the "great mimicker". So there was no identifiable disease, 'melioidosis', just a multitude of syndromes that could involve any or even multiple systems, could present as an acute pyogenic illness or mimic tuberculosis, could give rise to a localized lymphadenitis or to acute septicaemia with no obvious focus, could grumble on for months or the patient could be dead within a matter of days. The only common feature was the isolation of *Burkholderia pseudomallei* from infected tissues.

We went on to look more closely at the data and ask question like "who was at risk of this infection"? The first answer that came to my mind was another question "who was not at risk"? because Sri Lanka is an agriculture based economy. You don't have to go very far from the urban centres to see paddy fields and just about everybody cultivates "home gardens"growing produce for domestic use. We did see the expected trends of a male and rural preponderance and, of course, a large proportion of diabetics.

The age range of patients was wide displaying the ubiquity of exposure to soil but mainly middle-aged reflecting the onset of diabetes at this age. When charting the seasonal trends it appeared that there was a trend of more cases presenting in Dec – March which roughly follows the dry zone monsoon where the whole country gets rain. There was a hint that the bacteraemia cases appeared earlier with the focal infections following a month or two later but the numbers are still too small to perform any statistical

analysis. The pattern of outcome was interesting, as initially we had a high mortality but in 2013/4, so far, most patients have survived. While there could be many explanations for this, such better ascertainment of less severe cases, my guess is that it is due to improved awareness leading to early diagnosis and effective treatment.

Things got even more interesting when we plotted the data on a series of maps from the National Atlas. The population density map does not show any direct relationship which is compatible with an infection acquired from the environment rather than for other people. The map of topography seemed to show an infection free area comprising the highlands above 500m where the climate is much cooler and the main crop is not rice but tea. There did not seem to be a difference in prevalence in these areas as wet, intermediate and dry zones had cases. Undisturbed areas of natural vegetation were free of infection and all cases occurred in rice growing areas which, of course, comprise the main extent of Sri Lanka excluding the tea gardens of the central highlands which were free of the infection.

More intriguing questions come to mind. How will melioidosis manifest in the soldiers, of both sides, who fought out a 30 year civil war? Why did we not diagnose increased cases after the devastating tsunami of 2004? Further research may hold answers to these questions.

Symposium 2

Antimicrobial Stewardship

An economic evaluation of antimicrobial stewardship programmes

Ms. Sonali Coulter

The goals of an antimicrobial stewardship (AMS) programmes are to optimizing clinical outcomes while minimising unintended consequences of antimicrobial use including toxicity, the selection of pathogenic organisms (such as *Clostridium difficile*) and the emergence of antimicrobial resistance.

Whilst there are many combinations of strategies available for the development of an AMS programme it is unclear which are optimal. There have been some studies that have reported cost savings in terms of pharmacy costs, but it is not clear that the cost-effectiveness of these programmes has been fully assessed. This information is essential for making credible arguments to decision makers about the value of funding these programmes.

This talk will present the decision analytic model (using TreeAge software) which I will use to assess the cost effectiveness of AMS programs and Matrix-assisted laser desorption/ionization – Time of Flight (MALDI-TOF) technology in the Australian setting. It will discuss the considerations required in using blood-stream infection data to calculate the costs and outcomes achieved pre and post implementation of specific strategies as part of the AMS intervention in two Brisbane Metropolitan hospitals. Finally it will consider the complexities of producing robust cost effectiveness estimates for these types of interventions and draw comparisons between strategies used in each of the hospital settings. Better informed prescribing will lead to better outcomes for patients, better use of limited resources and reduce the incidence of unintended consequences of antimicrobial use.

Symposium 3

Endocarditis

Microbiology of infective endocarditis: Do new tools solve old dilemmas?

Dr. Christopher Coulter

Infectious endocarditis (IE) remains an important clinical entity with significant morbidity and mortality. The epidemiology of IE has evolved over time with shifts in the relative frequency of the most usual pathogens, the challenge of antimicrobial resistance, the emergence of new pathogens and new host factors including intravenous drug use, in-hospital invasive procedures, immune compromised states and the increasing use of implantable cardiac devices such as pacemakers and implantable defribrillators. The microbiology laboratory plays a critical role in diagnosis and antimicrobial resistance detection. Challenges for the laboratory include pathogen detection, pathogen identification, resistance detection and providing information for clinicians in a clinically meaningful time frame. Continuously monitored blood culture systems, rapid diagnosis by protein analysis (MALDI-TOF) and molecular identification by genetic sequencing are all tools which can improve the contribution of the microbiology laboratory towards rapid and accurate diagnosis of IE. Advances in therapeutic drug monitoring and assessment of minimal inhibitory concentrations to specific agents may assist in optimal delivery of antimicrobial agents for treatment of IE.

Symposium 3

Endocarditis

Clinical perspective on management of endocarditis

Dr. Duminda Samarasinghe

"Few diseases present greater difficulties in diagnosis than endocarditis, difficulties which, in many cases are nearly insurmountable" was how Sir William Osler described endocarditis in the beginning of 20th century. That was an era where there were no blood cultures to diagnose or antibiotics to treat the infection. Today the diagnosis has become even more challenging due to partial treatment with antibiotics. In most cases, by the time endocardits is suspected, patient has already received many doses of antibiotics. Eventhough Duke Criteria clearly specifies when to diagnose endocarditis; the application has become difficult due to lack of microbiological evidence. In such a setting, treatment has become even more challenging and which antibiotic to use is mostly decided on best guess approach.

When blood cultures are negative but endocarditis is strongly suspected, it is important to rely more on clinical criteria, other laboratory investigations and echo-cardiography to diagnose and to treat endocarditis. The setting in which the patient has got the disease, appearance of vegetations on echocardiogram and other associated clinical findings will help to guess the responsible organism. These clinical features and echo-cardiographic evidence is even more important to assess response to treatment and to decide on duration of treatment. Echocardiography also helps in assessing the risk of complications like risk of embolisation and need for surgical intervention.

In 21st century, where we have excellent microbiological diagnosis and an array of antibiotics to treat, diagnosis and treatment of endocarditis is still a challenge. Close coordination of the clinician and the microbiologist is imperative to overcome this challenge and to successfully diagnose and treat endocarditis.

Pre-Congress Workshop

Preparing for mass infective emergencies, Infectious Disease Hospital (IDH), Angoda

Dr. P. C. J. Fonseka

Mass infective emergency is an event which requires the hospital to treat a number of infectious patients beyond its' conventional capacity. It creates a temporary mismatch between demand and supply of medical care in terms of manpower, accommodation, equipment and a potential threat to all healthcare workers.

A mass infective emergency is a catastrophic situation and therefore system response should be based on pre established agenda and a drill. Objective should be to improve hospital system to respond in an event of mass infective emergency, assuring occupational safety, preventing disease outbreak, saving maximum number of patients' lives and reducing their morbidities.

For the operational purposes, if more than 30 patients who are infected or suspected to have infections, get admitted within a period of two hour duration, is considered as mass infective emergency situation in the IDH hospital.

After preparation of complete preparedness and response plan to check the strength and weakness/gaps in terms of providing a mass infective care a drill was carried out and several weakness and gaps were identified. Medical instruments and other resources were inadequate, e.g.; ambu bags, wheel chairs, trolleys and PPE. Some system and procedure failures were identified, e.g.; incorrect PPE usage and deficiencies practicing standard precautions among some of the HCW, system of patient transportation and intra institutional communication. Identified weakness/gaps discussed with the relevant stake holders of the hospital and relevant recommendations were made to correct system errors and resource allocations for future purposes.

OP 1

Bacterial flora and their antibiotic sensitivity patterns in plastic biliary stents removed from patients through endoscopic retrograde cholangiopancreatography (ERCP)

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Introduction

Biliary stents provide effective relief to patients with obstructive disease of the biliary tract. With time biliary stent occlusion by biliary sludge and bacterial colonization are major complications of endoscopic biliary stenting leading even to cholangitis and sepsis. Hence it is important to identify the bacterial species and their antibiotic susceptibility profiles among patients with stent obstruction to ensure the appropriate choice and timely administration of antibiotics.

Objective

To identify the bacterial flora and their antibiotic sensitivity patterns in plastic biliary stents removed from patients through Endoscopic Retrograde Cholangio-pancreatography.

Method

Fifty plastic biliary stents removed from patients were collected over a period of 17 months commencing from September 2012. The stents were enriched in brain heart infusion broth and cultured on blood agar, MacConkey agar and chocolate agar. Identification of bacterial flora was done using appropriate biochemical tests. Antibiotic sensitivity tests were performed using Clinical Laboratory Standard Institute (CLSI) method.

Results

From the 50 stents 14 types of organisms were identified totaling 127 isolates altogether. Ninety one point three percent (116/127) of the organisms were Gram negative whereas 8.7% (11/127) were Gram positive. Eighty one point one percent (103/127) of the organisms were Enterobacteriaceae while 10.2% (13/127) were *Pseudomonas spp.* The commonest type of organism identified was *Klebsiella spp.* (21.3%) followed by *Escherichia spp.* (19.7%), *Proteus spp.* (18.9%), *Pseudomonas spp.* (10.2%), *Enterococcus spp.* (8.7%), *Enterobacter spp.* (7.9%), *Citrobacter spp.* (4.7%), *Serratia spp.* (3.1%), *Yersinia spp.* (3.1%), *Hafnia alvei* (1.6%) and *Morganella morganii* (0.8%).

Majority of isolated Enteribacteriaceae were sensitive to gentamicin, ciprofloxacin, imipenem and meropenem whereas isolated *Pseudomonas spp.* were sensitive to ceftazidime, gentamicin, imipenem and meropenem.

Conclusion

Klebsiella spp. was the commonest organism found in plastic biliary stents removed from patients with obstructive biliary disease following stenting, followed by *Escherichia spp.* Majority of the bacteria isolated from biliary stents were sensitive to gentamicin, imipenem and meropenem.

OP 2

Antibiotic sensitivity of enteric bacteria in different food animals in relation to antibiotic use in the Colombo District

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Introduction

Use of antimicrobial growth promoters in food animals is known to select resistant bacteria and they eventually reaches humans through the food chain. Surveillance of antibiotic resistance in bacteria of food animals allows detection of developing antibiotic resistance in food chain.

Objectives

To determine the prevalence of *Salmonella* spp., *Escherichia coli*, *Enterococcus faecium*, and *E.faecalis* in broilers, pigs and cattle, and antimicrobial susceptibility pattern (AMSP) of them in relation to, antibiotic usage in food animals in the Colombo district.

Methods

Faecal swabs from 485 animals from randomly selected 18 farms were collected. Specimens were inoculated on to brain heart Infusion broth supplemented with 6.5% saline, tetrathionate broth, XLD, MacConkey and Slanetz Bartley agar plates. *Salmonella spp*., *E. coli*, *E. faecium*, and *E. faecalis* were identified by biochemical tests. Four hundred and twenty two isolates were tested for antibiotic susceptibility by disk diffusion method, CLSI 2012. Data on antibiotic usage was collected through a questionnaire.

Results

Prevalence of *Salmonella*, *E. coli*, *E. faecium*, and *E. faecalis*, among broilers and cattle were 3%, 54%, 43%, and 21% and 2%, 48%, 16% and 30% respectively. Non susceptibility to multiple antibiotics were observed in 211(50%) of the isolates and majority were from poultry (48%). There was a statistically significant higher non-susceptibility among broiler *E.coli* to ciprofloxacin, ampicillin and cotrimoxazole and enterococci to vancomycin, compared to cattle and swine isolates. AMSP of isolates from the two farms where antibiotics were used routinely was not significantly different to isolates from others except that susceptibility to vancomycin of *E. faecium* from farm 1 and 11 was significantly low compared to other farms. This can't be explained with the enrofloxacin usage in these farms as it is from another class of antibiotic.

Conclusions

The significant higher resistance against antibiotics in *E. coli*, *Salmonella* and *Enterococcus* spp. strains isolated from broilers. Thus there is a necessity to conduct a properly designed research to study the situation in the whole country with widespread collection of data regarding antimicrobial use in farms, antibiotic sale from pharmacies and veterinary product outlets and food adulteration practices in companies producing commercial animal feed.

OP 3

Salmonella serotypes isolated or serotyped at the National Reference enteric laboratory and their antibiotic sensitivity pattern

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Medical Research Institute

Introduction

Salmonella associated infections are important food borne diseases. Most serotypes are capable of causing disease in both human and animals. *Salmonella* infections most often cause gastroenteritis, which can range from mild to severe. Invasive infections can be severe and potentially life threatening.

CDC is reporting resistance to ceftriaxone in about 3% of non-typhoidal *Salmonellae*, and some degree of resistance, i.e frank resistance or intermediate resistance, to ciprofloxacin in about 3%. Ciprofloxacin resistance or partial resistance in *Salmonella* Typhi is 67% (CDC).

The MRI enteric bacteriology laboratory receives stool samples for culture and *Salmonella* isolates from many hospitals for serotyping. In year 2013, 1121 stool samples were received. Of the stool samples received 189 were diarrhoeal samples and 879 were for food handler screening. In addition, 125 suspected *Salmonella* isolates were received for serotyping and identification.

Objective

To describe the pattern of serotypes isolated from different samples and to analyse the antibiotic sensitivity pattern.

Method

Laboratory data of all *Salmonella* isolates received at the Enteric Laboratory, MRI and all stool samples received for detection of pathogens from 1/1/2013 to 31/12/13 were analysed retrospectively.

Results

The number of isolates received for serotyping was 125, with 56 (44.5%) from blood cultures, 38 (30.4%) from stool cultures and 15 (12%) from food samples. Only 76 (60%) isolates sent to the reference laboratory for serotyping were confirmed as *Salmonella* spp. Of the 46 blood culture isolates confirmed as *Salmonella* spp, 36 (82%) were non-typhoidal Salmonella. *Salmonella enterica sub* sp *enterica* serotype Enteritidis was the predominant isolate and represented 20% of all none typhoidal *Salmonella* spp identified. All 10 isolates from food and all 7 isolates from stool were non-typhoidal *Salmonella* spp. Twenty five food handlers (3%) were positive for *Salmonella* spp and were nontyphoidal *Salmonella* spp. Ten stool samples out of 189 stool samples grew *Salmonella* spp.

Antibiotic sensitivity testing was performed using the CLSI method. Ciprofloxacin resistance among *S*. Enteritidis is 66.6%. Two non-typhoidal *Salmonella* isolates showed intermediate resistance to ceftriaxone.

Conclusions

The majority of non typhoidal *Salmonella* isolates were *Salmonella* Enteritidis. Ciprofloxacin resistance among *Salmonella* Enteritidis was 66.6%. Two non typhoidal *Salmonella* showed intermediate resistance to Ceftriaxone.

OP 4

Predictability of presence of extended spectrum beta-lactamase (ESBL) producing organisms in urine cultures using co-amoxyclav and cephalexin discs in the first line antibiotic susceptibility testing

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ESBL-producing organisms are Gram-negative bacteria that produce beta-lactamases, enzymes which mediate resistance to extended spectrum cephalosporins (3rd generation) and monobactams rendering them ineffective for treatment.

Objectives

To predict the presence of ESBL-producing coliforms from the first line antibiotic panel in urine cultures.

Methodology

All urine cultures that isolated a significant colony count of >10^5 CFU/ml of coliforms were tested.

In the first line antibiotic panel, co-amoxyclav (AMC) and cephalexin (Cl) discs were placed 15mm apart to detect augmentation of zones between them or resistance to either of them. The test was repeated placing the discs 25mm apart.

Second line antibiotic panels were run on samples which showed augmentation or resistance. The ESBL confirmatory test according to CLSI guidelines using cefotaxime/cefotaxime+clavulanic acid and ceftazidime/ ceftazidime +clavulanic acid discs was performed on all specimens that showed reduced zone diameters for cefotaxime and ceftazidime.

Only the results of ESBL confirmed specimens were analysed.

Results

Out of a total of 107 ESBL confirmed samples, augmentation was detected between AMC/Cl in 95. No augmentation was detected in 12 specimens. 88.8% of ESBL-producing coliforms showed augmentation between AMC/Cl placed 15mm apart.

All samples which showed augmentation between AMC/ Cl were confirmed as ESBL-producing coliforms. When AMC/Cl discs were placed 25mm apart, augmentation was not detected in all samples. Susceptible zones were detected for AMC in 55 samples (51.4%), Intermediate zones in 20 (18.6%) and resistant zones in 32 (30%). Resistant zones for Cl were detected in all samples.

Conclusion

There is an 88.8% possibility of predicting the presence of ESBL from the first line ABST, using AMC/Cl discs placed 15mm apart. Detection of augmentation between AMC/Cl has a 100% predictability of the presence of ESBL. Early identification of ESBL-infections reduces processing time, allowing minimum delay in commencing treatment with the most sensitive antibiotic. With discs placed 25mm or more apart, the probability of misreporting an ESBL as susceptible or intermediate to AMC is 51.4%, and 18.6% respectively. mTherefore the possibility of missing an ESBL by either placing AMC alone or with Cl, 25mm or more apart is 70%.

OP 5

Development of a real-time PCR assay for intra-partum detection of Group B Streptococcal colonization

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Introduction

Group B Streptococcus (GBS) is an important cause of early-onset neonatal sepsis. Maternal colonization with GBS is the primary risk factor and screening during late pregnancy and intrapartum prophylaxis are the main means of preventing this.

Some women are intermittent carriers of GBS, and the rate of GBS colonization can vary during pregnancy. Because of this fluctuation, intrapartum screening of pregnant women would be preferable and targeted antibiotic prophylaxis can be administered. This needs a sensitive and rapid method for the detection of GBS colonization.

Objective

To develop a rapid assay to screen mothers for GBS colonization in late pregnancy and labour.

Method

Eighty strains of GBS isolated from a variety of clinical specimens and 20 non-GBS strains including 3 fungal strains were tested. DNA extraction was done using instagene matrix (Biorad) according to the method described by the manufacturer. The cfb gene which codes for Christie-Atkins-Munch-Petersen (CAMP) factor was selected as the genetic target. Amplification products were analyzed using the conventional gel electrophoresis. The presence of 252 base pair band was interpreted as positive reaction. Each test had positive and negative controls. This assay was then adopted for the LightCycler format. The performance of the PCR was compared with the optimized culture. Using this method, sixty vaginal swabs sent to the Microbiology laboratory at St. Georges Hospital for screening for GBS were tested after the routine cultures were performed.

Results

Out of the 80 GBS isolates tested, 76 became positive by real time PCR. Sensitivity was 95%. All 20 non GBS strains were negative and specificity was 100%. The limit of detection was 3 colony forming units per assay.

The conventional assay needed 6 hours and the real time assay needed 2 hours.

17 vaginal swabs gave positive results (17/60). This included 2 culture negative samples but missed 1 culture positive. Sensitivity was 88% and the specificity was 95%.

Conclusion

The PCR described is a fast, sensitive and specific method and has the potential for intrapartum detection of GBS colonization.

OP 6

A comparison of qualitative and quantitative sputum culture methods

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Introduction

Sputum culture is one of the most difficult to interpret specimens in diagnostic microbiology. Routinely performed qualitative method of culturing is unable to differentiate colonizers of the respiratory tract from actual pathogens. Quantitative culture after homogenization and dilution of sputum has been suggested as a solution to this problem.

Objectives

To compare the qualitative sputum culture method with quantitative method.

Design, settings and methods

A descriptive analytical study was carried out in a patient population of community acquired pneumonia and patients who had acute exacerbations of chronic obstructive pulmonary disease. The sputum samples were collected prior to antibiotic treatment in the wards. A total of 155 samples belonging to Murray and Washington grades 3, 4 and 5 were processed by qualitative and quantitative methods.

Results

S. pneumoniae, coliforms and pseudomonads were isolated as pathogens. The qualitative method without homogenization or dilution isolated 19 coliforms (12%), 24 pseudomonads (15%) and 5 *S. pneumoniae* (3%) as pathogens. Pathogens could not be isolated in 70% of the samples. The qualitative method with homogenization but without dilution isolated 21 coliforms (14%), 24 pseudomonads (15%) and 5 *S. pneumoniae* (3%) as pathogens. A pathogen was not isolated in 68% of samples. Fourteen (9%) coliforms, 22 (14%) pseudomonads, 5 (3%) *S. pneumoniae* were isolated as pathogens in the quantitative method after homogenization and dilution. Seventy four percent of samples did not yield any pathogen.

Conclusions

The homogenization and dilution did not show an improvement in the isolation of typical respiratory pathogens. There was no difference in the types of isolates between qualitative and quantitative methods in the population studied. There was a reduction of the rates when considering an organism, a pathogen, in the quantitative method when compared to qualitative method. This needs to be further tested correlating the clinical outcome with the culture result in larger populations of patients who are not on antibiotics. The study did not reveal sufficient information to conclude that one method used is superior to the other in pathogen isolation

OP 7

Rapid detection of rifampicin and isoniazid resistance in *Mycobacterium tuberculosis* culture isolates: an evaluation of a line probe assay

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Background

Molecular methods allow rapid detection of drug resistance in *M. tuberculosis*. One such commercially available method is the Line Probe Assay (LPA). The aim of this study was to assess the performance of a commercial line probe assay the GenoType MTBDR*plus* test (Hain Lifescience GmbH, Nehren, Germany) for rapid detection of rifampicin and isoniazid resistance in Sri Lankan *M. tuberculosis* culture isolates.

Objectives

- To identify the sensitivity and specificity of the Geno Type MTBDR*plus* assay in detecting rifampicin, isoniazid and multidrug resistance in *M. tuberculosis* culture isolates in Sri Lankan epidemiological settings.
- To identify the pattern of *rpo B, kat G* and *inh A* gene mutations associated with rifampicin, isoniazid and multi drug resistance in Sri Lankan *M. tuberculosis* strains.

Methodology

A total of 74 *M. tuberculosis* culture isolates, consisting of 17 multi-drug resistant (MDR), 4 rifampicin mono-resistant, 15 isoniazid mono-resistant and 38 susceptible strains tested by conventional method were collected from the National Tuberculosis Reference Laboratory of Sri Lanka. The collected isolates were tested with the GenoType MTBDR*plus* test and the performance was compared with the conventional phenotypic drug susceptibility test results.

Results

All seventy four strains tested with the GenoType MTBDR*plus* test gave interpretable results. The sensitivity of the assay in this study was 90.5%, 90.6% and 88.2% and the specificity was 98.1%,100% and 98.2% in detecting rifampicin resistance, isoniazid resistance and multi-drug resistance (MDR) respectively. Mutation at codon 516 of *rpo B gene* is the commonest mutation detected in rifampicin resistant isolates. Mutation at codon 315 in the *kat G gene* is the commonest mutation detected in isoniazid resistant isolates.

Conclusion and Recommendation

The GenoType MTBDRplus test is very user friendly and is easy to perform. It is highly specific and sensitive in detecting rifampicin resistance, isoniazid resistance and multidrug resistance. As our MDR-TB rate is low, we recommend performing this assay on culture isolates or on direct specimen of high risk patients who can harbour MDR-TB. The conventional DST should be performed routinely.

OP 8

Study on hepatitis B markers among family members in a group of hepatitis B virus infected patients

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Introduction

Incidence of hepatitis B viral (HBV) infection in Sri Lanka is low compared to other countries in South Asia. The risk of infection is high in close contacts of HBV infected patients. Prevention of transmission to spouse and other family members is an important aspect in the management of HBV infections.

Objective

To study the HBV serology markers among family members of chronic HBV carriers who were diagnosed and followed up by Department of Virology, MRI.

Methodology

Laboratory data of serological markers of HBV was analyzed in 230 family members of 78 index cases. Index cases include all diagnosed and followed up chronic HBV carriers at the Department of Virology, MRI from November 2008 to December 2012 available for contact screening. Family members were screened for HBs antigen, HBe antigen, HBe antibody, HB core total antibody, HB core IgM and HBs antibodies.

Results

Among screened family members, 55 (23.9%) had evidence of exposure to HBV at the time of screening. Fourteen (6.1%) were positive for HBs antigen at the time of testing and 12 (5.2%) had active infection (HBe antigen positive/ HBe antibody negative). Among HBs positives, only 02 had sero-conversion to HBe (HBe antigen negative / HBe antibody positive). Forty one (17.8 %) had serological evidence of past exposure to HBV infection (HBcore total antibody positive) and 36 (15.6%) had HBs antibodies at the time of screening.

Out of screened 51 spouses, 19 (37.2%) had evidence of exposure to HBV and among other 179 screened family members, 36 (20.1%) had evidence of exposure. At least one member of the family was affected in 40 (51.28%) index cases.

Conclusion

Prevalence rate of HBV infection among family members (23.9%) was significant when compared to general population (0.3-2.3%) in this study. Screening of family members and vaccination of non immune members should be encouraged. Correct health education and counseling should be given to HBV infected patients and to their family members about the disease, mode of transmission and preventive measures.

OP 9

Surveillance of rotavirus in three hospital settings of Sri Lanka 2007 – 2010

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Introduction

Rotavirus is an important aetiological agent of childhood diarrhoeas in Sri Lanka.

Objectives

To study the rotavirus epidemiology and genotypic diversity of cases hospitalized in three geographical locations of Sri Lanka, Ragama, Galle and Kandy.

Materials and Methods

The study was approved by the ethical review board of the Sri Lanka College of Paediatricians. Stool samples were collected from children < 5 years, hospitalized at the Teaching Hospitals at Ragama (RTH) (November 2007 – October 2010) Galle (GTH) and Kandy (KTH) (mid and late 2008) respectively for acute gastroenteritis. Rotavirus was detected using EIA kit, Rotaclone®. A subset of rotavirus positive samples was genotyped by reverse-transcription(Rt)-PCR and polyacrylamide-gel-electrophoresis (PAGE).

Results

Stool samples of 1245 children (69.2%, 23.3% and 7.3% from RTH, GTH and KTH respectively) were screened for rotavirus. Of them, 476 were positive by EIA. The overall

rate of prevalence of rotavirus infection was 38.2%. The median age of infection ranged from 13-20 months. Rotavirus genotyping was done on 375 (78.8 %) samples. G1[P8] was the overall dominant strain (44.8%) followed by G9[P8] (10.1%), G2[P4] (5.3%), G3[P8] (3.2%), G1[P6] (2.1%), G12[P6] (1.3%), G2[P8] (1.06%) and 0.26% of G4[P6], G4[P4] and G4[P8]. The G or P serotype was untypable in 25.6% of samples and 5.6% were of mixed-G and P type. PAGE yeilded 25 electropherotypes (E1-E12 and E16-E29), with E5 and E20 causing 19 and 14 percent of infections respectively. The electropherotype could not be determined in 26%.

Conclusions

Rotavirus continues to be an important cause of childhood diarrhoreas in Sri Lanka. Strain G1P8 predominated in all areas during the surveillance period with a notable percentage of mixed-G and P infections. Multiple E types identified indicate increasing strain diversity

OP 10

A study of invasive candida infections at a paediatric teaching hospital

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Introduction

Candidaemia is a major cause of morbidity and mortality in the nosocomial setting, and the epidemiology of candidaemia is changing.

Objectives

To describe the clinical and laboratory data of paediatric patients diagnosed to have candidaemia and to determine the species of candida isolates from blood.

Design, setting and methods

The data collected on blood cultures which were performed during a period of two years from January 2012 to December 2013 were analysed. The cultures were processed using both the manual in-house blood culture bottles and BACTEC automated system. Species identification was done at the Department of Mycology, Medical Research Institute. Clinical significance of the cultures were decided based on the clinical condition of the patients as well as when two consecutive blood cultures yielded positive for *Candida* spp. in the same patient.

Results

A total of 120 positive blood cultures were analysed. Seventy eight cultures were considered significant (65%) while others were considered as contaminants. Out of the significant isolates 73 (93.5%) are from automated blood cultures. Thirty of seventy three (41.1%) BACTEC bottles became positive within 24 hours while thirty one (42.5%) became positive between 24 – 48 hours. All manual cultures were positive after 48 hours. Fifty six of seventy eight (71.7%) samples were from ICU patients. Most cultures (56.4%) were from patients in the age group of 1 month to 1 year. Sixty four of seventy eight (82%) isolates were non-albicans *Candida*. Out of the isolates tested for speciation, 51 were identified as *Candida tropicalis*, 1 *Rhodotorula* spp, and 1 *Geotricum* spp. congenital heart disease was the clinical presentation in a majority. Other common clinical presentations were pneumonia and sepsis. Seven catheter related blood stream infections were present.

Conclusions

Most of the candidaemia patients were detected from intensive care setting where invasive devices and broad spectrum antibiotics were used commonly. Time taken to be positive in automated blood cultures was significantly shorter than that of the manual cultures. Majority of infections were due to non-albicans candida. *Candida tropicalis* was the most common species causing infections at LRH.

OP 11

Bacterial pathogens causing postoperative infections following abdominal surgery at a tertiary care hospital in Southern Province of Sri Lanka

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Introduction

A prospective study was carried out at a tertiary care hospital from January to April 2012 to study postoperative infections among patients undergoing abdominal surgery.

Objectives

To find out the incidence of postoperative infections following abdominal surgery, to identify the common bacterial pathogens causing postoperative infections and their antibiotic sensitivity pattern and to determine the most effective empirical antibiotic/s for the treatment.

Method

Three hundred and eighty five patients were included in the study. They were followed up for 30 days postoperatively to detect postoperative infections. Relevant specimens for microbiological cultures were collected accordingly.

Results

Thirty patients (7.8%) developed postoperative infections and total episodes of postoperative infections were 37 (9.6%).

Incidence of surgical site infections (SSIs) was 5.97% followed by urinary tract infections (UTI) 1.82%, hospital acquired pneumonia/ventilator associated pneumonia (HAP/VAP) 1.56% and catheter related blood stream infections (CRBSI) 0.26%.

Coliforms were the commonest pathogens isolated in SSIs (36.36%) and 62.5% were ESBL producers. Equal numbers of *Staphylococcus aureus* and *Acinetobacter* isolates were seen (18.18% each). *Acinetobacter* isolates were resistant to almost all antibiotics.

Among seven pathogens causing UTI were 2 ESBL producing coliforms, 1 *Acinetobacter* species, 1 *Enterococcus* species and 3 *Candida* species.

Among 5 pathogens isolated in HAP, there were coliforms (3/5), *Pseudomonas* species (1/5) and *Streptococcus pneumoniae* (1/5).

Acinetobacter species were isolated in both patients with VAP and were resistant to all tested antibiotics.

Pseudomonas aeruginosa isolated in CRBSI was resistant to carbapenems.

Conclusions

Surgical site infections were the commonest postoperative infections. UTI, HAP, VAP and CRBSI were also detected. Gram negative organisms were the predominant pathogens.

Gram negative organisms were the commonest pathogens causing SSIs. Majority of SSIs were superficial incisional SSIs in which wound cleaning alone may play an important role in the management. If antibiotics are indicated carbapenems and amikacin are the most effective empirical treatment.

ESBL production among coliforms (61.5% of all coliforms) is a significant problem in this population.

Infections caused by carbapenem resistant organisms (37.5% of all Gram negative bacilli) are treatment challenges detected in this study.

OP 12

Evaluation of bactericidal effect of three antiseptics on bacteria isolated from wounds

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Introduction

Antiseptics are widely used in wound management to prevent or treat wound infections due to proven wound healing properties regardless of their cytotoxicity.

Objective

To determine the bactericidal effects of three antiseptics on pathogens causing wound infections.

Design, settings and methods

Study was done at Colombo South Teaching Hospital and Department of Microbiology of University of Sri Jayewardenepura in 2013. Forty eight stored bacterial isolates from wounds, standard strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were tested for bactericidal effects of 3 acids (acetic acid, ascorbic acid and boric acid) in three concentrations (0.5%, 0.75% and 1%). Bacterial suspensions were equivalent to 0.5 McFarland standard.

Results

There were 33 (68.8%) Coliforms, 10 (20.8%) *Pseudomonas* species, and 5 (10.4%) *Staphylococcus aureus.* Acetic acid at concentration of 0.5% inhibited growth of 37 (77%) and 42 (87.5%) of tested isolates when exposed for 30 and 60 minutes respectively. However 100% inhibition was achieved at 4 hours. At concentration of 0.75%, 40 (83.5%) and 44 (91.6%) were inhibited when exposed for 30 and 60 minutes respectively. Similarly 100% inhibition was achieved at 4 hours. At concentration of 1%, 46 (95.8%) inhibition was seen at 30 minutes and 100% inhibition at 60 minutes.

Ascorbic acid, at 0.5% and 0.75% concentrations, inhibited growth of 45 (93.7%) and 47 (97.9%) of isolates respectively when exposed for 30 minutes. At these two concentrations, 100% inhibition was achieved when exposed to one hour. At 1% concentration, 100% inhibition was achieved at 30 minutes.

Boric acid did not show bactericidal effect at concentrations of 0.5%, 0.75% and 1%. Ascorbic acid was bactericidal for all organisms tested within the shortest exposure time at the lowest concentration compared to other 2 acids. *Pseudomonas* species were inhibited at 30 minutes by 0.5% acetic acid.

Bactericidal effect against all six standard strains was seen with three acids at each concentration tested from 30 minutes onwards.

Conclusion

Despite promising bactericidal effects, further studies warrant, as ongoing debates on toxicity of acids on tissue epithelialisation. Application of antiseptics for a shorter duration could overcome this problem without losing bactericidal activity.

OP 13

A prospective study of causative organisms, antibiotic management and clinical outcome of patients with infective endocarditis treated at the National Hospital of Sri Lanka

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Objectives

To determine causative micro-organisms, antibiotic management and the clinical outcome of infective endocarditis (IE) patients.

Methodology

Patients who were referred to the Department of Microbiology for advice on management and patients with positive blood cultures in whom diagnosis of definite IE was made according to the Modified Duke Criteria from 01.06.2013 to 30.11.2013 were included in the study.

Results

Thirty seven patients were followed up. Six (16%) were late onset prosthetic valve IE (PVE $\geq$ 4years) and out of rest 23 (62%) had defective valves. Majority (84%) were within 21- 59 years of age and males (60%). All presented with fever and 4 (11%) were with complications; cerebrovascular accident 3 (8%) and one with heart failure. Out of 24 (65%) blood cultures positives 17 (71%) had $\geq$ 2 positive cultures. Organisms were viridians group of streptococci 10 (40%), enterococci 4 (16%), *Staphylococcus aureus* 5 (20%), a coagulase negative staphylococcus (CNS), a group G streptococcus species, a *Salmonella* Typhi, a *Candida tropicalis*, and a *Trichosporon* spp. Of PVE, blood cultures were positive for *Streptococcus milleri* 2 (32%), a viridans group streptococcus, a CNS and a *Candida* spp. Out of 15 streptococci and enterococci, 11 were tested for penicillin minimum inhibitory concentration and 10 (91%) were highly sensitive to penicillin and one intermediately sensitive, but only one patient was able to complete the treatment with penicillin; 10 (91%) were treated with ceftriaxone and vancomycin, and of them 5 (50%) had to undergo valve replacement (VR) and one died. Overall mortality was 8%. Out of overall 15 (41%) VR, 6 (40%) developed ventilator associated pneumonia and 2 (13%) bacteraemia with multidrug resistant coliform, one complicated with early onset PVE and one died.

Conclusions

Culture positive IE due to viridians group of streptococci, staphylococci and enterococci predominates. Detection of fungal and gram negative IE is an interesting finding.

Viridans group streptococci still show good susceptibility to penicillin. There is high rate of valve replacement. Development of nosocomial infections further complicated the outcome in some. Considerable mortality too was detected.

OP 14

Determining minimum inhibitory concentrations of vancomycin for methicillin resistant *Staphylococcus aureus* isolates using an agar dilution method

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Introduction

Infections caused by *Staphylococcus aureus* resistant to methicillin (MRSA) have become an important health care burden. Its resistance profile severely limits treatment options leaving vancomycin as the main alternative in many cases. Though *Staphylococcus aureus* strains with resistance or intermediate susceptibility to vancomycin have been described, disc diffusion susceptibility testing cannot be used to detect such strains.

Objective

The aims of this study were to determine MIC values of vancomycin and the resistance profile of MRSA strains isolated from clinical samples sent to the Microbiology Laboratory at Teaching Hospital, Karapitiya.

Methods

Fifty four clinical isolates of MRSA were tested with cefoxitin and confirmed the resistance profile using guidelines of CLSI (Clinical Laboratory Standards Institute, USA) disc diffusion method. BHI agar plates with vancomycin concentrations of 6 µg/mL, 3 µg/mL, 1.5 µg/mL, 0.75 µg/mL, 0.38 µg/mL and 0.19 µg/mL were prepared. Highest dilution and test method was decided according to the vancomycin agar screening method described by CLSI.

Results

Of the 54 MRSA isolates 83.3% were from patients at TH Karapitiya and 16.7% from specimens sent from TH Mahamodara. They were from different specimens including wound swabs (31), pus (17), HVS (3), blood (2) and ET aspiration (1).

Only two isolates (3.7%) grew on 3 µg/mL but were inhibited by 6 µg/mL. Both were isolated from patients at TH Mahamodara. Other (96.3%) isolates had vancomycin MICs of 3.0µg/mL or below.

More than 50% of the MRSA isolates were resistant to erythromycin (87.03%), clindamycin (79.62%) and ciprofloxacin (57.41%). Resistance to c-trimoxazole (33.33%) and fusidic acid (35.85 %) was observed.

Conclusion

Two (3.7%) isolates with highest detected vancomycin MIC of 6.0 µg/mL may fit in to vancomycin intermediate *Staphylococcus aureus* (VISA) category according to CLSI criteria and needs confirmatory testing. Reduced susceptibility to vancomycin and resistance to other antibiotics are concerns when treating infections caused by MRSA.

OP 15

Analysis of data of urine culture isolates of 2013 sent from four laboratories of National Laboratory Based Surveillance of Sri Lanka College of Microbiologists

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Objectives

1. To determine the aetiological agents of midstream urine cultures with a colony count of > 10^5 CFU/ml.
2. To analyse the antimicrobial susceptibility of those isolates.

Method

The National Laboratory Based Surveillance on Antimicrobial Resistance is a collaborative project of the Ministry of Health and the Sri Lanka College of Microbiologists. At the initial phase decided to analyse midstream urine cultures with a colony count of $\geq 10^5$ CFU/ml. The specimens were processed according to the standard protocol specified in the laboratory manual in microbiology. Antibiotic susceptibility tests were performed according to the method established in the centre which is either by CLSI method or by Stoke's comparative disk diffusion method. Data of 2013 sent by the participating laboratories were analysed using WHONET software.

Results

The data was received from four centres. They were Sri Jayewardenapura General Hospital, Lady Ridgeway Childrens' Hospital, Faculty of Medicine, Colombo and Faculty of Medicine, Ragama.

A Total of 1175 significant isolates were analysed. The majority were Gram negative enteric organisms, commonly known as coiforms, with 922 (78.5%) isolates. The others were *Enterococcus* species 83 (7%), Candida species 60 (5.1%), *Pseudomonas* species 38 (3.2%), *Acinetobacter* species 21 (1.8%), Group B beta-haemolytic *Streptococcus* 20 (1.7%), coagulase negative *Staphylococcus* species 10 (0.85%), *Streptococcus* species 9 (0.8%), *Staphylococcus aureus* 7 (0.6%), and *Staphylococcus saprophyticus* 5 (0.4%).

The susceptibility of coliforms were 11.6% (92/795) to ampicillin, 71.1% (621/873) to nitrofurantoin, 25.9% (223/862) to cephalexin, 46% (392/853) to cefuroxime, 29.4% (255/866) to nalidixic acid, 47.8% (422/883) to cefotaxime, 92.6% (665/718) to meropenem, 70.3% (601/855) to gentamicin, 41.6% (341/819) to amoxicillin-clavulanic acid and 38.4% (318/829) to ciprofloxacin. None of the 13 isolates of *Acinetobacter* species tested were sensitive to meropenem while only 55% (16/29) of *Pseudomonas* sp. were sensitive to meropenem.

74% (60/81) of *Enterococcus* species were sensitive to ampicillin.

Conclusion

Coliforms constitute the commonest organism causing urinary tract infections (UTI). A high resistance rate was noted in coliforms for broad spectrum antibiotics like cefotaxime and ciprofloxacin. *Acinetobacter* sp. shows a very high resistance rate even for carbapenems. Ampicillin can be recommended as empirical therapy to treat UTI due to enterococcus species.

OP 16

Detection of antibacterial activity in cerebrospinal fluid

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Introduction

Meningitis is caused by a wide variety of infectious and non-infectious agents. Even though majority of infectious cases are caused by viruses, bacterial meningitis remains as a significant problem. It needs prompt and precise management with correct antibiotics which is dictated by accurate microbial identification. In Sri Lanka, microbial diagnosis of meningitis is mainly based on Gram staining and positive culture, for limited availability of antigen detection. This has a low sensitivity when associated with prior antibiotic usage.

Objective

To find out whether antibacterial properties are present in the CSF samples sent for culture to the laboratory, which may give rise to negative culture results. If this is proven, to highlight the use of other methods rather than only Gram staining and positive culture for the microbial diagnosis of meningitis.

Method

A prospective clinical study was conducted in a Tertiary care Hospital, Sri Lanka from 1st of January to 31st June 2012.Two sets of Müller-Hinton Agar plates were inoculated with *Escherichia coli* NCTC 10418 and *Staphylococcus aureus* NCTC 6571, and 6 mm diameter sized 6 wells per plate were made and 20 micro litre of CSF per sample was placed into each respective well of both plates. All plates were incubated overnight at 37°C and zones of inhibition were noted. Samples which give zone of inhibition in either plate or both were taken as positive for antibacterial activity.

Results

Out of 287 CSF samples tested, antibacterial activity was positive in 147 (51.21%) and negative in 140 (47.79%). One of the antibacterial activity free samples resulted culture positive with *Haemophilus influenzae*. The rest of the samples (286) did not yield bacterial growth.

Discussion and conclusion

Probable prior antibiotic usage seems to have caused antibacterial activity in CSF which might have affected negative culture results. Hence, the use of culture as the main laboratory method to detect the aetiology of meningitis is questionable. Therefore, use of non-culture diagnostic tools as molecular techniques or antigen detection tests would provide management friendly service for CSF samples which is the most valuable sample received at microbiology laboratory.

OP 17

Mupirocin resistance among isolates of methicillin resistant *Staphylococcus aureus* at National Hospital of Sri Lanka

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Introduction

Mupirocin is widely used to eradicate carriage and prevent infection with MRSA. Current clinical and epidemiological trends have shown increase usage of mupirocin could lead to increase in resistance.

The emergence of mupirocin resistance has been increasing worldwide. e.g. USA 13.2%, China 6.6%, India 6%, Turkey 45% and Korea 5%.Two levels of mupirocin resistance are known. Low level resistance (LLR) (MIC8 µg/ml – 256 µg/ml) mediated via mutation in the native *ile*Sgene. The clinical significance is unclear. High level resistance (HLR) (MIC $\geq$ 512 µg/ml) mediated by a plasmid-encoded *mupA* gene has been associated with decolonization .

Objective

To determine the prevalence of phenotypes of mupirocin resistance of MRSA isolates at National Hospital of Sri Lanka.

Design, setting and methods

A prospective descriptive study was carried out for 3 month duration from November 2013. Hundred (100) consecutive, non-duplicative MRSA isolates (clinical and screening samples) were tested.

All isolates were identified with standard biochemical tests and cefoxitin 30µg disc. Methicillin resistance was confirmed by latex agglutination test (PBP2' Oxoid).

Disc diffusion test was performed with mupirocin 5 µg and 200 µg disks and incubated at 35±2 °C for 18-24 hrs.

No zone around mupirocin 200µg and 5 µg disc indicate HLR, any zone in 200 µg disc with no zone around 5 µg disc indicate LLR and any zones of inhibition around both discs indicate susceptibility.

Results

MRSA isolates	Number of isolates	Percentage
Susceptible to mupirocin	94	94%
HLR of mupirocin	4	4%
LLR of mupirocin	2	2%

n=100

Conclusion

Prevalence of mupirocin resistance among the MRSA isolates is high according to our study.

The use of mupirocin as decolonization therapy for MRSA carriers and use as an antiseptic should be re-evaluated before setting guidelines.

Standard test methods and interpretative breakpoints for mupirocin susceptibility should be improved.

Multicentre studies are needed to assess the prevalence of mupirocin resistance in Sri Lanka.

OP 18

Occurrence of KPC producing *K. pneumoniae* and associated factors in a selected hospital in Colombo District

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Introduction

Carbapenem resistance poses a challenge in the management of infections caused by Klebsiella pneumoniae. Carbapenemase production is the most common method in resistance and Klebsiella pneumoniae carbapenemase (KPC) and metallo beta lactamase (MBL) type carbapenemase production are the most common mechanisms.

Objectives

To study the occurrence of KPC gene and associated factors in carbapenem resistant Klebsiella pneumoniae strains in a selected hospital in the Colombo District.

Method

Thirty two isolates resistant to carbapenem by CLSI disk diffusion method were identified as *K. pneumoniae* using the API 20 E system. Minimum inhibitory concentrations to meropenem were determined using E strips. The modified Hodge test and EDTA inhibition test were performed. Identification of KPC gene was done using a conventional PCR method.

Data regarding associated factors were collected using a data extraction form, only from 26 patients each in carbapenem resistant and carbapenem sensitive group as 6 patients from the carbapenem resistant group were lost to follow up. Analysis was done using the Fisher extract formula.

Results

All isolates were positive for Modified Hodge test. Twenty (62.5%) isolates gave positive results in EDTA inhibition test. All were negative for KPC gene by PCR. Admission to an ICU, having an invasive catheter or device and prior carbapenem and glycopeptide exposure were associated with infection with carbapenem resistant strains.

Conclusions

No KPC producers were found in this sample but continued surveillance is needed to detect cases.

PP 1

Self-reported practices of hand hygiene among the medical students

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Introduction

Hospital acquired infections are a major problem among hospitalized patients. Hand hygiene is the single best measure for infection control.

Objectives

1. To evaluate the self-reported practices and the sources of knowledge about hand hygiene among medical students.
2. To assess whether further educational programmes in hand hygiene are necessary for medical students.

Design, setting and methods

Self-administered, pre-tested validated questionnaires from previous publications, based on hand hygiene guidelines laid down by the World Health Organization were distributed among all 177 medical students at the Sir John Kotelawala Defence University. The students were given 15 minutes to answer the questionnaires.

Results were analyzed by comparing the knowledge of WHO guidelines with the appropriate hand hygiene behaviour of the medical students.

Results

All 177 students participated in the study. The age range was 17 to 26 years and 74.58% were males.

Of the participants 85.88% agreed that hand hygiene is the single most important factor in the prevention of nosocomial infections.

The sources of knowledge about hand hygiene were from school, medical faculty, multimedia and other sources were 93.78%, 56.49%, 76.66% and 78.53% respectively. The percentage of students who knew the importance of hand washing before direct contact with patients, after direct contact with patients, moving from a contaminated body site to a clean body site in the same patient, before aseptic procedures, after contact with body fluids and after contact with the patient's immediate surroundings were 55.93%, 67.79%, 46.89%, 70.05%, 90.96% and 40.67% respectively.

Only 34.46% of students knew that the duration of hand washing procedure should be 40 to 60 seconds. The percentage of students who knew the importance of single use of a clean towel to dry after washing was 55.93%. Of the participants 72.88% agreed that they need further education in hand hygiene.

Conclusion

Most of the students had acquired knowledge about hand hygiene from school. The medical students were found to be deficient in their knowledge and practices. Therefore, further education with regard to hand hygiene is necessary.

PP 2

An audit on hand washing practices among health care workers at Colombo North Teaching Hospital, Ragama

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Introduction

Hospital acquired infections (HAI) are a major challenge to patient safety. Effective hand washing is the single most important procedure for preventing transmission of HAI.

Objectives

To assess the hand washing technique practiced by health care workers (HCW) and the availability of facilities for hand washing.

Methods

An observational check list was prepared including steps in hand washing and availability of facilities for hand washing. 120 hand washing episodes were assessed including 28 doctors, 72 nurses and 20 minor staff employees (MSE) at Teaching Hospital, Ragama from 20th January 2014 to 20th February 2014.

Results

Soap was used by 93.3% (112/120) to wash hands. Others washed only with water. Out of the 42 HCW who were wearing jewellery only 33.6% (14/42) removed them prior to hand washing. None of the MSE and only 22.2% doctors removed jewellery. Rubbing hands systematically covering all surfaces especially finger tips, thumbs and finger webs was performed by only 11.7% (14/120). Only 65.8% (79/120) dried their hands using towels after

washing hands. All the steps were followed accurately by 3.3% (4/120) nurses only. None of doctors and MSE followed all the steps in spite of displaying the poster demonstrating the proper hand washing technique in all places.

Uninterrupted water supply was available at all places in all hand washing episodes. Soap and single use towels were available in 95% and 80% episodes respectively. Availability of soap and single use towels were satisfactory in special care units compared to general wards. No foot operated bins were seen in any place.

Conclusions

A high percentage of health care workers did not adhere to recommended technique of hand washing. In general, nurses practiced recommended hand washing technique better than the other two categories. Although the availability of soap and running water was adequate, availability of single use towels and foot operated bins was not up to the requirement in many places. Improving the compliance of adhering to recommended hand washing technique and supplying facilities required, are mandatory to improve hand washing practices among HCW.

PP 3

Three attacks of *Salmonella* Typhi bacteraemia in an adult returned from India

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Introduction

Salmonella enterica serotype Typhi causes typhoid fever resulting in severe illness and serious complications.

Case report

A 56 year old male was admitted to Teaching Hospital, Ragama with high fever, chills for 9 days and loss of appetite, loose stools for 5 days. He had been in India for 20 days. Investigations revealed WBC count-5800/mm^3, neutrophils-75.9%, lymphocytes-22.8%, platelets-141000/mm^3 and CRP-153 mg/l. SAT was negative initially but gave a titer of 1/320 for *Salmonella* Typhi H anti-bodies after 10 days. He was commenced on oral ciprofloxacin. After 4 days, ceftriaxone was started after taking cultures as fever did not respond. A non lactose fermenting coliform was isolated from the blood and identified as *Salmonella* Typhi by serotyping. The isolate was sensitive to ampicillin, chloramphenicol, ceftriaxone and resistant to ciprofloxacin. Patient was discharged after treating for 10 days with ceftriaxone.

Three weeks after discharge, he presented with similar symptoms of 3 days duration. Investigations revealed a similar picture. Cultures were taken and ceftriaxone was re-started empirically. Blood culture revealed *Salmonella* Typhi. SAT titer was 1/320 for the same antibodies. Anti-Vi antibodies were negative. Ultrasound scan abdomen, 2D Echo were normal. Social history was not suggestive of re-infection. Patient was treated with 14 days of ceftriaxone on the basis of relapse due to inadequate treatment.

Three weeks after discharge, he presented again with fever. Stool and blood cultures were positive for *Salmonella* Typhi. Ultrasound scan abdomen, 2D Echo were repeatedly normal. He was treated with azithromycin for 7 days and was decided to give a course of amoxicillin for 3 months presuming a chronic carrier state.

Discussion

The rate of treatment failure in quinolone-resistant *Salmonella* Typhi infections is higher with a shorter duration of treatment. The optimal treatment for these infections has not been determined, but azithromycin and ceftriaxone are effective drugs. 3-5% of patients who are treated for acute illness become carriers harbouring the pathogen in gallbladder or kidney. The majority of carriers can be cured by a prolonged course of oral antibiotics.

Conclusion

Quinolone- resistant *Salmonella* Typhi infections should be treated with 14 days of ceftriaxone to prevent relapse. A chronic carrier state should be suspected in patients with recurrence of infection after adequate treatment.

PP 4

Importance of active case detection in a malaria elimination programme

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Introduction and objectives

Malaria surveillance methods routinely used in Sri Lanka are passive and active case detection (PCD, ACD) and activated passive case detection (APCD). Active case detection is carried out by mobile malaria clinics. Tropical and Environmental Diseases and Health Associates (TEDHA) an implementation partner of the Anti Malaria Campaign (AMC) carries out APCD and ACD in four districts in Sri Lanka namely Trincomalee, Batticaloa, Ampara and Mannar, complementing the parasitological surveillance activities carried out by the AMC.

Design, setting and methods

The ACD programme of TEDHA involves screening of populations irrespective of the presence of fever or any other signs or symptoms of malaria to detect infections and residual parasite carriers. TEDHA screens a) high risk populations using ACD through mobile malaria clinics including armed forces personnel and b) pregnant females who visit antenatal clinics for asymptomatic malaria infections during their first trimester. Populations are selected in consultation with the Regional Malaria Officer of the AMC thus avoiding any overlap with the population screened by the government.

Results

TEDHA screened 387,309 individuals between January 2010 and December 2012, for malaria by ACD including high risk groups and pregnant women and diagnosed eight malaria positive cases (7 *Plasmodium vivax* infections and one mixed infection with *P. vivax* and *Plasmodium falciparum*). All these cases were from the Mannar district amongst resettled populations and army personnel. During this period 125 cases were detected in the Mannar district by the Anti Malaria Campaign by passive case detection. No cases of malaria were detected by ACD by the AMC.

Conclusions

The progress made by Sri Lanka in the malaria elimination drive is largely due to increased surveillance and judicious use of control methods. The country now needs to focus on enhanced surveillance to be malaria free and to prevent re-introduction of malaria into the country. As highlighted here, ACD played a major role in interrupting malaria transmission in the country.

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PP 5

Proportion of vulvovaginal candidiasis and antifungal susceptibility pattern of the isolates from patients attending gynaecology clinic at Colombo South Teaching Hospital (CSTH)

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Introduction

Vulvovaginal candidiasis is the second most common cause of vaginal inflammation. Although a short course of topical formulations or a single oral dose of antifungals are adequate in the treatment of uncomplicated disease, a longer course of treatment is recommended for recurrent infection.

Objectives

1. To determine the proportion of vulvovaginal candidiasis among patients attending gynaecology clinic at CSTH.
2. To detect the antifungal susceptibility of isolates.
3. To identify selected risk factors associated with the disease.

Design, setting and methods

Hundred and fifty eight patients attending gynecology clinic CSTH from August 2013 to February 2014 having vaginal discharge were included. High vaginal swabs from patients were collected aseptically and processed on Sabourouds dextrose agar. Direct smears, Gram stain were done followed by culture isolation and identification. Antifungal sensitivity tests were performed using fluconazole (25µg), clotrimazole (10µg), itraconazole (10µg), mconazole (10µg) and ketoconazole (10µg) disks according to Clinal Laboratory Standard Institute (CLSI) guidelines May 2004. Probable risk factors for the infection were assessed using an interviewer administered questionnaire.

Results

Out of 158 patients, 34 had *Candida albicans* while one had non *Candida albicans* species. All direct microscopy positive specimens were recovered by culture and all the isolates were exclusively sensitive to antifungals tested.

Fourteen (40%) culture positive patients had diabetes mellitus ($P<0.05$) and 7 (20%) were practicing hormonal contraceptive methods. Among culture positive patients, 2 (5.7%) had used antibiotics in past 6 months. When enquired the past episodes, 6 culture positive patients had vaginal discharge related to pregnancies.

Conclusion

Candida albicans is the commonest pathogen responsible for vulvovaginal candidiasis. The infection can be safely treated with commonly used antifungals. Diabetes mellitus is an associated risk factor.

Recommendations

Vulvovaginal candidiasis can be safely treated with antifungals used in routine clinical practice.

PP 6

Prevalence of anti *Helicobacter pylori* antibodies in patients with dyspeptic symptoms

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Introduction

Serology is a minimally invasive and an easy method in detecting anti *H. pylori* antibodies as an aid in diagnosing *H. pylori* infection. However widely used anti-IgG antibody, does not discriminate past infection from the current one.Sero-prevalence of anti *H. pylori* IgG of 10.3% has been reported among healthy volunteers in Sri Lanka.

Objective

We conducted the following study to assess the prevalence of anti *H. pylori* antibodies among patients with dyspeptic symptoms and endoscopic mucosal abnormalities.

Methodology

This is a cross sectional study of 205 patients who had dyspeptic symptoms and endoscopically visible mucosal erythema with or without erosions/ulcers at Teaching Hospital, Peradeniya from 2012 to 2013. Anti *H. pylori* antibodies were tested using venous blood using an immune-chromatographic rapid assay (SD BIOLINE *H. pylori* rapid test) which detects IgG, IgM and IgA anti *H.pylori* antibodies. The sensitivity, specificity of the test was 95.9% and 89.6%. Histology based haematoxylin and eosin, toluidine blue staining and immune-histochemistry (DAKO0471) assay were performed to detect active *H. pylori* infection on endoscopic gastric biopsies. Cases which showed positivity with ≥ 2 histology based tests were regarded as active *H. pylori* infections.

Results

Anti-*H.pylori* antibodies were present in 10 (10/205) cases with a prevalence of 4.9%. Of the 10 cases, active *H. pylori* infection was present in 3 (30%) cases by histology.

Conclusion

Prevalence of anti-*H.pylori* antibodies was low (4.9%) in the sample, which demonstrates the low exposure rate to *H. pylori*. However, detection of specific anti-*H. pylori* antibody response using an ELISA is would be useful for confirmation. Active infection was even less in number in the study cohort, suggesting the symptoms and endoscopic findings in most cases might be due to other causes.

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PP 7

Profile of extended spectrum beta lactamase (ESBL) producing enterobacteria isolated from clinical specimens in a private teaching hospital in Sri Lanka

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Objectives

Data from the private hospitals in Sri Lanka regarding antibiotic resistance is lacking. This study was done to determine the profile of ESBL producing enterobacteria isolated from clinical specimens in a private hospital laboratory of Sri Lanka and to recommend appropriate empiric antibiotics to treat such infections.

Methods

Gram negative bacteria isolated from clinical specimens received at the microbiology laboratory of a private teaching hospital from January 2013 to Jan 2014 were analysed retrospectively using WHONET. The isolates were identified by colony and microscopic morphology and preliminary conventional biochemical tests. Screening for ESBL was done using double disc synergy method (CLSI 2007). Ethical clearance was obtained from the ERC of the Faculty of Medicine, SAITM.

Results

There were 386 enterobacteria isolated from 95 clinical specimens comprising of 75 urine specimens, 3 SBF, 10 wound and ulcer swabs and 7 HVS. Of these, 101 isolates were ESBL producers (26%). There were 146 isolates of *Escherichia coli* of which 68 were ESBL producers (47%). Of 36 *Klebsiella* spp., 5 were ESBL producers (14%). The remaining 155 isolates were identified as coliforms, of which 28 were ESBL producers (18%).

The ESBL producing *E.coli* were susceptible to meropenem, imipenem, ertapenem, netilmicin and amikacin. Among the urinary isolates, nitrofurantoin and gentamicin susceptibility of *E. coli* was 61% (34/55), 97.2% (44/45) respectively. All 3 blood culture isolates were susceptible to gentamicin. Wound swab isolates of *E. coli* were 60% (6/10) susceptible to gentamicin. Similar resistant patterns were seen in klebsiella and coliform isolates.

ESBL producing enterobacterial isolation rates between males and females was not significantly different. However the majority of ESBL producing enterobacteria were isolated from patients above 40 years of age (66%).

Conclusions

In the present study, prevalence of ESBL producing enterobacteria in this hospital is 26%. In a teaching

hospital in Sri Lanka, ESBL rates were documented as 32.87%. Though moderate, when compared to neighbouring countries such as India (60%), our rate of ESBL resistance is much higher, compared to developed countries (USA 3%). Nitrofurantoin can be recommended as empiric therapy for UTIs caused by ESBL producing *E. coli.*

PP 8

Audit on blood culture contamination rates and missing clinical information on blood culture request forms in a tertiary care hospital in Sri Lanka

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Introduction

Blood cultures (BC) are among the most important laboratory tests performed during diagnosis of life threatening infections, which help targeted therapy. BC are costly and contamination leads to waste of resources and time, and further, builds up an unnecessary anxiety among the clinician as well as the patient. Relevant clinical history on the request will reduce the time taken to inform significant blood cultures to clinicians.

Objectives

1. To determine blood culture contamination rates over a 3 month period in the hospital.
2. To determine the proportion of missing clinical information on the positive blood culture request forms over a 3 month period in the hospital.

Design, settings and methods

A retrospective study carried out on blood cultures received in the microbiology laboratory from 1/12/2013 to 28/02/2014. Data was extracted on to a pre-checked data extraction sheet and data was analysed using the SPSS computer software. Only positive blood cultures were analysed to get the missing clinical details on request forms.

Results

Laboratory has received 363, 424 and 422 blood cultures in December 2013, January 2014 and February 2014 respectively. Thirteen, 30 and 36 were positive for an organism in the relevant months and 9, 20 and 14 were considered contaminated, giving a 2.4%, 4.7% and 3.3% contamination rate accordingly.

Considering the missing clinical information, of the positive blood cultures, 4 of 13 (30.76%) in December 2013, 9 of 30 (30%) in January 2014 and 18 of 36 (50%) in February 2014 did not have a clinical history on the request forms of which 1, 1 and 13 were respectively from clinically significant blood cultures.

Conclusion

Even in resource poor settings, blood culture contamination rates were within acceptable limits in 2 of the 3 months, however, the proportion of missing clinical information was over 30% in first 2 months which has increased to 50% in the last month. Unavailability of microbiology request forms may have partly contributed to this. Guidance for doctors is necessary in completing clinical history on blood culture requests.

PP 9

Bacterial pathogens and their antibiotic susceptibility pattern of neonatal eye swab cultures during the year 2013 at a tertiary care hospital in Sri Lanka

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Background

Eye infections are common during neonatal period. Identifying the causative pathogens and the antibiotic sensitivity pattern will help guiding the clinician to decide on empirical antibiotic therapy.

Objectives

1. To identify the common pathogens isolated in neonatal eye swab cultures.
2. To identify the antibiotic sensitivity pattern.
3. To recommend empiric local antibiotic therapy for neonatal superficial eye infections.

Methodology

Results of all neonatal eye swab cultures of one year (Jan 2013-Dec 2013) were obtained from the laboratory record book and analyzed. The isolation and identification of pathogens were performed according to the Microbiology Laboratory Manual. The antibiotic susceptibility test was done using Joan Stokes method.

Results

One hundred and eleven eye swabs were cultured. Among them 64% (71/111) were culture positive and 36% (40/111) were culture negative. Coagulase negative *Staphylococcus* spp were the commonest isolates, representing 60% (43/71) of the positive cultures. *Staphylococcus aureus* grew on 27% (19/71) of positive cultures.

Other organisms isolated were coliforms (3/71), *Pseudomonas* spp (2/71), *Streptococcus* spp (2/71), *Streptococcus pneumoniae* (1/71), Haemophilus *influenzae* (1/71) and *Acinetobacter* spp (1/71).

Antibiotic susceptibility test results were available only for 45% (32/71) of isolates. Susceptibility test was not done on scanty growths and some susceptibility test results had not been recorded in the laboratory record book. Chloramphenicol susceptibility test result was available only on 23 isolates and 20 (87%) isolates were susceptible. One MRSA, 1 multi drug resistant coagulase negative *Staphylococcus* spp and 1 *Acinetobacter* spp were among the 3 Chloramphenicol resistant isolates. Among the 20 *Staphylococcus* isolates with Chloramphenicol susceptibility test results available,18 (90%) were susceptible. Gentamicin had been tested on 36 isolates and 21 (58%) isolates were sensitive. *Staphylococcus* spp showed 58% (18/31) susceptibility to Gentamicin. Sixty seven percent of isolates (24/36) including 66% (20/30) of *Staphylococcus* spp were susceptible to Norfloxacin. Fusidic acid had been tested only on 9 *Staphylococcus* spp and 4 (44%) were susceptible.

Conclusion and recommendation

Staphylococcus spp were the commonest pathogen isolated. Chloramphenicol eye drops might be used as empiric local antibiotic therapy for neonatal superficial eye infections.

PP 10

Antibiotic susceptibility pattern of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from different microbiological specimens in a tertiary care children's hospital in Colombo

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Introduction

Isolation of multi drug resistant MRSA is a frequent occurrence in hospital practice.

Objectives

To determine occurrence and antimicrobial susceptibility pattern of MRSA in different clinical samples from both in and out patients.

Methodology

Information on request forms of all specimens (blood, respiratory, pus, wound swabs, ear, eye and screening swabs) received to the microbiology laboratory, Lady Ridgeway Hospital from 1/12/2013 to 28/02/2014 were collected retrospectively. The antibiotic susceptibility was performed by Joan-Stokes technique. Methicillin resistance was detected using cefoxitin discs.

Results

There were total of 118 (4%) MRSA isolates from 4742 specimens. Seventy six (64%) were from clinical specimens and 42 (36%) were from screening swabs. Of clinical specimens 21 (18%) were from surgical wards, 18 (15%) from medical wards; 17 (14%) from intensive care units (ICU) while only 2 (2%) were from out patients. All the isolates were sensitive to vancomycin. Screening swab isolates had been tested only for vancomycin and cotrimoxazole and of them 41 (95%) were sensitive to cotrimoxazole. Out of clinical isolates majority were resistant to erythromycin (72%) and clindamycin (67%). Sensitivity to fusidic acid (FD) and cotrimoxazole was 77% and 93% respectively. Of 12 isolates from ear discharges, sensitivity to ciprofloxacin, gentamicin, chloramphenicol and FD were 100%, 90%, 80% and 75% respectively. Out of 19 isolates from abscesses, 100% were sensitive to cotrimoxazole and FD and 59% and 65% were resistant to erythromycin and clindamycin respectively. All four blood culture isolates were susceptible to erythromycin, FD, cotrimoxazole and vancomycin.

Conclusions

Four percent of samples tested yielded MRSA. Majority of MRSA positive samples were from in-patients. Of them a high percentage of resistance was noted to erythromycin and clindamycin while majority were sensitive to cotrimoxazole and FD. Ciprofloxacin, gentamicin, chloramphenicol and FD are good options for empirical treatment to ear infections. This study provides an institute level initiative to understand antimicrobial susceptibility pattern among clinical isolates of MRSA and provide clinicians with information to guide antimicrobial prescribing practices.

PP 11

Salmonella enterica serotype Paratyphi A with an unusual biochemical pattern

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Introduction

We present a report of *Salmonella enterica* serotype Paratyphi A with an unusual biochemical pattern.

Case presentation

A nine year old girl was admitted to Teaching Hospital, Karapitiya with fever, loose stools and vomiting of four

days duration. On admission, she had fever with chills and rigors and right hypochondrial abdominal pain. Watery stools were passed about three times a day and she had a few episodes of vomiting. Examination revealed soft, tender hepatomegaly. She gave a history of travel to India three weeks prior to this episode. During that stay she had developed blood and mucous diarrhoea which resolved after treatment.

Blood culture, after 24 hours of incubation, revealed a non-lactose fermenting Gram negative bacterium. Kligler Iron agar pattern showed an alkaline slant and an acid butt with H_2S production. The isolate was negative for urease production. Serotyping, performed according to the Kaufmann-White scheme, using O and H antisera identified the isolate as *Salmonella enterica* serotype Paratyphi A. The identity of the isolate was confirmed by Matrix Assisted Laser Desorption/Ionization Time of Flight mass spectrometry (MALDI-TOF) at PathWest Laboratory Medicine Western Australia. Patient was successfully treated with intravenous ceftriaxone.

Conclusion

This is the first documented isolate of an H_2S producing variant of *Salmonella* serotype Paratyphi A from Sri Lanka. Salmonella identification schemes stress that Paratyphi A does not produce H_2S or decarboxylate lysine. Variants, such as in this case, may present a diagnostic dilemma unless full serotyping is performed.

Acknowledgements

Staff of the Department of Microbiology, Teaching Hospital, Karapitiya and MRI.

PP 12

Study of surgical site infections after coronary artery bypass grafting (CABG) at the Teaching Hospital, Karapitiya

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Introduction

Despite advances in operative techniques and wide spread use of prophylactic antibiotics, surgical site infections (SSIs) continue to be a major source of morbidity and mortality for patients undergoing operative procedures. The patients undergoing CABG are at a risk of developing SSIs both at the sternal and the harvesting sites.

Objectives

1. To determine the incidence of SSIs in Cardiothoracic Unit (CTU) at Teaching Hospital, Karapitiya (THK).
2. To identify risk factors of SSIs including *S. aureus* colonization.
3. To identify the causative micro-organisms and their antibiotic susceptibility patterns.

Design, setting and methods

A prospective study was carried out in the CTU at the THK from January to April 2010 among fifty-five patients admitted for CABG.

Pre-operative MRSA screening was performed in all patients and repeat screening was done after decolonization for those colonized with *S. aureus*.

Post-operative clinical samples were collected on clinical suspicion of SSIs. Antibiotic susceptibilities were performed for the isolated pathogens.

Results

The 55 patients included 40 males and 15 females. Twenty percent were colonized with *S. aureus* and MSSA to MRSA ratio was 10:1. Incidence of SSIs was 18.2% (10/55). Infection rates at sternum and leg were 10.9% and 7.3% respectively. All were superficial infections and none had deep infections or mediastinitis. Sixty percent males and 40% females had SSIs. Among the patients with SSIs, 50.0% had *S.aureus* colonization while 13.3% of patients who did not develop SSIs had *S.aureus* colonization.

Of all the SSIs, 50% (5) had no bacterial growth while an *Acinetobacter* spp. was isolated in 30% (3) and a *Pseudomonas* spp. and a coagulase negative Staphylococcus was isolated in 10% (1) each of the SSIs. All except the *Pseudomonas* spp. were multiresistant organisms.

Conclusion

S.aureus colonization, diabetes and chronic obstructive pulmonary disease seem to be associated with the development of SSIs. Female sex, age $\geq$65 years, obesity, hypertension, prolonged hospital stay ($\geq$7 days) and smoking did not show any association with the development of SSIs. Commonest organisms causing SSIs were Gram negatives. Antimicrobial resistance among those pathogens was a problem.

Financial assistance by Research and Higher Degree Committee, University of Ruhuna is acknowledged.

PP 13

Analysis of blood cultures processed in a paediatric tertiary care hospital during year 2013

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Objectives

1. To determine the clinically significant and contamination rate.
2. To determine the aetiological agents in bacteraemic patients.
3. To analyse the antibiotic susceptibility pattern of common bacterial pathogens.
4. To determine the clinical conditions leading to bacteraemia.

Method

Blood cultures received to the laboratory were processed both manually and by Bactec automated blood culture system according to the standard protocol. Culture identifications were performed using conventional methods as well as by rapid test kits for Gram negative bacteria. Antibiotic susceptibility tests were performed according to Stokes comparative disc diffusion method. MIC for penicillin and cefotaxime was detected by E-test in *Streptococcus pneumoniae*. The data for year 2013 were analysed by WHONET software.

Results

The total blood cultures processed in year 2013 was 9637 and 1182 (12.3%) blood cultures yielded a positive growth. Out of the positive cultures 548 (46.4%) were significant. Contamination rate of total cultures were 6.6% (634/9637). Coagulase negative staphylococcus was the predominant contaminant.

These 548 significant isolates were detected in 370 patients out of which 72 were neonates. Commonest isolate is neonates was coliform comprising 47.2% (34/72) out of which 47.1% (16/34) were ESBL producers. Commonest isolate in children over 1 month too was coliforms comprising 50.0% (134/298), out which 35.8% (48/134) were ESBL producers. Next common isolate in this age category were non fermenters comprising 24.5% (73/298)). *Streptococcus pneumoniae* was isolated in 18 patients while 4 patients had *Haemophilus infuenzae*. ABST was analysed on per patient basis. Over 1 month age group amicakcin and ciprofloxacin were the only antibiotics which showed over 65% sensitivity for coliforms. Out of *Pseudomonas* sp. 81.8% were sensitive to ceftazidime. Sepsis of unknown source was the commonest clinical presentation while catheter related blood stream infections were the next.

Conclusion

A high percentage of blood cultures are growing skin contaminants. Coliforms are the commonest aetiological agent in bacteraemic neonates as well as in children. A high percentage of ESBL producers are causing blood stream infections specifically in neonates. Sepsis of unknown source is the leading clinical diagnosis in bacteraemic children.

PP 14

Fungal keratitis – Sri Lankan picture

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Introduction

Fungal keratitis is a rare but severe cause of corneal infection. This infection is difficult to treat and can cause severe visual impairment or blindness. It has a worldwide distribution but more common in developing tropical countries. The commonest fungi that cause fungal keratitis are *Fusarium* spp. and *Aspergillus* spp. Also yeasts like *Candida* spp. can cause fungal keratitis.

Objectives

To determine the species of fungi isolated from corneal buttons and corneal scrapings from 2004-2013 in Sri Lanka.

To determine the changing pattern of species during the 10 years.

Methodology

One thousand sixty nine samples were analysed for this study. Of them 1058 were corneal buttons and 11 were corneal scrapings. Specimens were processed using 10% KOH for direct microscopy and cultured on Sabouraud's Dextrose Agar supplemented with antibiotics. Fungal species identified, morphologically and biochemically, were plotted against time.

Results

Out of 1069 samples, fungal aetiological agents were identified in 432 samples (40.41% isolation rate) by direct microscopy. Among the 432 positive samples, 407 samples grew fungi. Of them 402 were moulds while 5 isolates were yeasts.

Among the moulds, majority were *Fusarium* spp. followed by *Aspergillus flavus* and *Aspergillus fumigatus*. In addition,*Curvularia* spp., *Penicillium* spp. and *Paecilomyces* spp. were isolated. Among the yeasts, the most common isolate was *Candida tropicalis*.

Fusarium spp. and *Aspergillus flavus* show higher isolation rates than other organisms. Over the past decade, the number of *Fusarium* spp. isolates had decreased from 2005-2010, but after that it had started to increase. The number of *Aspergillus flavus* isolates was steady over the decade.

Conclusion

During the past decade, *Fusarium* spp. was the most commonly isolated mould from fungal keratitis patients in Sri Lanka. *Candida tropicalis* was the most commonly isolated yeast species.

Presidential address delivered at the Inauguration of the 22nd Annual Scientific Sessions of the Sri Lanka College of Microbiologists on 24th July 2013

Dr. Sunethra Gunasena
Consultant Virologist

Chief guest Dr. Palitha Mahipala, Director General of Health Services, Guest of honour Professor Ravi Vasanthapuram, Officials from the Ministry of Health, Mrs Rangani Wickramasingha, Family members and the friends of late Dr. Siri Wickramasingha, Distinguished invitees, Council and the members of the College, Ladies and Gentlemen.

It is with great sense of pride and achievement I stand here to deliver the Presidential Address 2013 to this august gathering. During the first part of my address I would like to introduce the Sri Lanka College of Microbiologists. It started as the Ceylon Association of Microbiologists in 1969. The name was changed to the Sri Lanka Association of Microbiologists in 1974. The Association evolved into the present the Sri Lanka College of Microbiologists in 1979.

The Sri Lanka College of Microbiologists is an organization of professionals working in Medical Microbiology in Sri Lanka. The College started with few members and has grown with a membership of about 160. Our members serve in the Ministry of Health, Universities and in the Private sector as Specialists in Microbiology, Virology, Parasitology, Mycology and in Immunology. If I take the ''Role of a Microbiologist'', it has expanded enormously over the years and has become one of multifaceted careers in medicine.

As the Specialist in-charge of a Laboratory, working with different categories of staff, Microbiologist should be a good Team Leader. In addition as the demand is made, he or she should be a good Administrator, Trouble shooter and Peace maker, Negotiator or an Advisor.

The College is closely linked with postgraduate training in Sri Lanka through representation in the Board of Study in Microbiology of the Post Graduate Institute of Medicine, University of Colombo. Our members are actively engaged in the training of future microbiologists of Sri Lanka through the postgraduate training programs of the Post Graduate Institute of Medicine.

Microbiologists also serve as members of the Task Force in Microbiology, National Advisory Board on Infection Control of the Ministry of Health Services and contribute to the development of guidelines, policy plan which guide the delivery of health services in Sri Lanka.

Specialists in Microbiology also extend their services as External Evaluators for the Cosmetics, Drugs, Devices Regulatory Authority at the Registration or at the Technical Evaluation of these items. With more and more emphasis on the demand for quality health care services, Microbiologists are called upon to serve as External Assessors for the Sri Lanka Accreditation Board for accreditation of medical laboratories.

Ladies and gentlemen, now let me introduce you to some of the activities conducted by the College. A Continuing Medical Education activity for the members is the most regular and one of most important activities of the College. These include lectures on a variety of topics, case presentations, workshops, training programmes. College also

conducts training sessions both formal and informal for members of the other specialties, medical officers and other categories of staff.

The Annual Scientific Session is the most exciting and most looked forward activity of the College calendar. We have organized 2013 Annual Scientific Sessions on the theme ''Emerging infections: meeting the challenges'' with a Pre-congress workshop, 6 free paper sessions, 5 plenary lectures and 3 symposia.

The Siri Wickramasingha Memorial Oration is another important event included in the inaugural ceremony of Annual Scientific Sessions. It is conducted in the memory of a much respected and valued member who has contributed to the field of microbiology. Late Dr Siri Wickramasingha was a long standing member and served as a Secretary and as a President of the College.

Currently the College has undertaken 3 major projects:

Development of National Antibiotic guidelines with collaboration of other professional colleges was one of the important projects undertaken by the College. Development of antimicrobial resistance has been identified as one of the emerging problems today. Rational use of antibiotics has been recognized as one of the key factors to control this emerging problem. We believe with rational use of antibiotics as per the guidelines we will be able to reduce the emergence of antibiotic resistance.

The Antibiotic Resistance Surveillance project in collaboration with the Ministry of Health, Sri Lanka and funded by GlaxoSmithKline Pharmaceuticals is another on-going project of the College. Following the successful completion of the Phase 1 of this project, Phase two was initiated with the increased number of participating microbiology laboratories and an expanded range of bacterial pathogens. Data obtained from microbiology laboratories in this project will be entered into the National Antibiotic Data base developed by the College. This data will be invaluable in the implementation of the antibiotic guidelines and regarding the use of antibiotics in this country.

Revision of the Bio safety manual is our third project. With expansion of microbiological services and introduction of newer technology, revision of the manual became a priority. The College planned to complete project in the third quarter of this year during the tenure of my presidency. I take this opportunity to thank the previous Presidents and their councils for initiation of these projects and laying the ground work.

With that short introduction to the Sri Lanka College of Microbiologists now I like to move to the second part of my address. When I went for the selection interview for the Diploma in Medical Microbiology, the very first question panel asked was whether I would be able to work in a laboratory, after working in a Paediatric Surgery ward for six years to which I think the answer is obvious. I was released to the Medical Research Institute 1989 to join the Diploma training program. Virology seemed to be the popular choice for our batch as six out of eight diplomats selected to join the MD Virology programme. My entry to the Polio Regional Reference Laboratory was quite unexpected. Experience gained from working with polio for several years was one of the reasons for selection of the topic tonight ''Poliomyelitis: Towards Eradication.''

India has been taken out of the list of Polio endemic countries and South East Asia Region is getting ready for Polio-free certification, I think it is appropriate to talk about Polio and the Eradication. Paralytic Poliomyelitis commonly known as Polio is caused by the Poliovirus. Earliest records of Polio came from Egyptian paintings and carvings that included pictures of adults with withered limbs and children walking with canes.

The first clinical description is provided by a British physician named Michael Underwood in 1789. He referred to polio as ''Debility of the lower extremities.'' Poliovirus is transmitted faeco-orally and young children are the most vulnerable to the infection. Paralysis is rare in young children occurring 1 in 1000 infected patients. However it is commoner in older children and in adults occurring in 1 in 75 patients. Prior to the 20th century, polio infection occurred mainly in young children 6 months to 4 years of age. Most of them developed mild infection with long term immunity. However with the improved sanitary conditions in developed countries in late 19th and early 20th century, the age of infection shifted from young children to older children and adults, who developed the paralytic disease more frequently. There was a dramatic increase of polio cases during early 20th century thus it became one of the most feared diseases.

Poliovirus the causative agent of polio, was first identified by Karl Landsteiner in 1908. Major breakthrough came when John Enders and his team from Childrens Hospital, Boston were successful in cultivating the poliovirus in human tissues in the laboratory in 1948. This development greatly facilitated the vaccine research that led to the development of vaccines against polio.

Other important advances that led to the development of polio vaccines were:

1. Identification of three poliovirus serotypes (Poliovirus type 1, or Mahoney, Poliovirus type 2, Lansing; and Poliovirus type 3, Leon).
2. The finding that the virus must be present in the blood prior to paralysis.
3. The demonstration that administration of antibodies in the form of gamma-globulin protects against paralytic polio.

The first effective polio vaccine was developed in 1952 by Jonas Salk at the University of Pittsburgh. The Salk vaccine, or inactivated poliovirus vaccine also referred as IPV, is based on three wild polioviruses grown in Vero cell line which is then inactivated with formalin. The injected Salk vaccine confers IgG-mediated immunity in the blood, which prevents polio infection from progressing to blood protects the nervous system .Salk vaccine was licensed for use in 1957. Jonas Salk refused to commercialize the vaccine saying that he developed it for the children. Oral Polio vaccine was developed by several groups. After careful evaluation of the performance, safety features, special committee selected Oral polio vaccine or better known as OPV developed by Albert Sabin in 1957 and licensed in 1967 for worldwide distribution. Oral polio vaccine produces an infection that mimics natural infection and confers IgG-mediated immunity in the blood, as well as mucosal immunity in the gut.

Impact of the use of vaccine was immediately seen. Following the licensing of IPV in 1957, United States of America used it in a mass campaign with dramatic reduction of polio cases from 58,000 cases on average per annum to just 5,600 cases. With licensing of OPV, the second mass immunization campaign was carried out with further reduction in polio cases. The last case of Polio due to the wild poliovirus in USA was reported in 1979.

In spite of the availability of vaccines, polio was a major public health problem in many countries. In 1988 the total number of Polio cases reported exceeded 350,000 from more than 125 countries. The World Health Assembly, first committed to polio eradication when it adopted resolution 41.28 in 1988 calling for global eradication of the disease by the year 2000. It was marked with the launch of Global Polio Eradication Initiative or GPEI by national governments, World Health Organization, Rotary International, US Centers for Disease Control and UNICEF.

GPEI adopted four strategies:

1. Maintaining high immunization coverage through routine immunization was the main strategy adopted.
2. Supplementary immunization was the second strategy adopted to fill gaps in routine immunization.
3. Third strategy was laboratory investigation of patients with acute flaccid paralysis the clinical manifestation of paralytic polio.
4. Fourth strategy was to implement vigorous outbreak control in the event of detection of wild poliovirus from a patient or from any source.

With adoption of these four strategies, there was a significant reduction of wild polio cases from 350,000 cases in 1988 to 222 cases in 2012, more than 99.9% reduction within 24 years. Three of the six WHO regions are certified “Polio-free” American region in 1994, Western Pacific region in 2002. European region in 2002, Wild poliovirus type 2 was last detected in 1999. Since then only vaccine poliovirus type 2 has been detected. South East Asia is polio-free for 28 months. Six countries remaining polio-free for more than 10 years: Bhutan since 1986, Sri Lanka since 1993, Maldives since 1994, Korea since 1996, Timor Leste since 1996, Thailand since 1997. Four countries were able to eliminate the virus following importations. Bangladesh in 2006, Indonesia in 2005, Myanmar in 2007, Nepal in 2010. The greatest achievement, India is remaining free of polio since January 2011. It is very important that Poliovirus circulation is closely monitored. This activity is carried out in all countries including Sri Lanka with Acute Flaccid Paralysis Surveillance (AFP) the clinical manifestation of Polio Contact Surveillance.

In this surveillance, AFP patients less than 15 years of age are investigated with two stool samples and maximum of five contacts with a single stool sample. Poliovirus isolation is performed at the Polio Regional Reference Laboratory using two cell lines (L20B and RD). Once virus is isolated in cell cultures, identification, typing and differentiation performed by real time PCR and isolates are referred to the Polio Global Research Laboratory for genetic sequencing.

Polio RRL has processed 500 to 700 stool samples annually from 100 to 150 AFP patients and their contacts. Polioviruses were isolated singly or in mixtures. Wild poliovirus was last isolated in a laboratory in 1993. Since then only vaccine viruses were isolated. In spite of these major achievements, some challenges to the success of the eradication initiative has been identified. Major challenge identified is the persistently infected countries. Since India has been taken out of the list in 2012, Afghanistan, Pakistan and Nigeria are the three polio endemic countries.

Incomplete surveillance of AFP cases, low routine immunization coverage leading to immunity gaps, large scale population displacement due to civil unrest, natural disasters and lack of commitment and accountability are some of the factors identified for this situation. Risk of importation of wild poliovirus from endemic country to a polio-free country is another serious challenge. Importation of wild virus can be a threat to any country and it is really dangerous for a country with low immunization coverage. Importation of wild poliovirus to Somalia and to Kenya from Nigeria in 2012 caused outbreaks in those two countries. Wild virus from Pakistan was detected in environmental samples in Israel has become a concern

for the programme. Oral polio vaccine (OPV) contains attenuated poliovirus named Sabin poliovirus or vaccine polioviruses. Following the attenuation these viruses have lost the ability to infect the nervous system. However they replicate in the gut of the vaccine recipient and are excreted into the community (Sabin like poliovirus). Poliovirus being a RNA virus acquire mutations during replication. Vaccine poliovirus also acquires mutations during replication in the vaccine recipient or in the community. Vaccine derived polioviruses (VDPVs) are vaccine viruses that acquired mutations and differ from their parent vaccine virus by more than 10 nucleotides in the VP1 sequence. In communities with inadequate routine immunization coverage and absent natural immunity to the circulation of wild virus, these VDPVs can recombine with other enteroviruses. Following recombination, these VDPVs behave as wild poliovirus. They infect nervous system causing polio. They circulate in the community causing outbreaks of polio.The VDPVs responsible for outbreaks of Polio is identified as ''circulating VDPVs'' or ''cVDPVs''. During the year 2011 and 2012, polio outbreaks due to circulating VDPVs have been reported from several countries in the African region, from Afghanistan and from Pakistan. Important fact to remember is that most of the outbreaks were caused by circulating VDPV type 2.

Another concern for the polio eradication program is the patients with primary immune-deficiency disorders (PIDDs). Poliovirus replicates for a longer period in patients either with antibody deficiency or with combined immune-deficiency. During replication, vaccine poliovirus acquires mutations and can become a VDPV. VDPVs detected from patient with immune-deficiency disorders are called ''Immune-deficient VDPVs'' or ''iVDPVs'' iVDPV can cause paralysis in the patient or they can replicate without any harm to the patient. iVDPV register maintained by the GPEI, has 46 patients with immune-deficiency disorders who have been excreting the virus for more than six months. They are identified as ''Prolonged excreters''.

Seven patients have been excreting the virus for more than 5 years and are called ''Chronic excreters''. It is important to note that Thirty one out of fifty three patients that is 59% excrete iVDPV type 2. Department of Immunology and Department of Virology, MRI performed a study to find the ''Prevalence of prolonged and chronic poliovirus excretion among persons with primary immune deficiency disorders (PIDDs) in Sri Lanka''. This study was carried out during the period May 2009 to September 2011 and follow up samples were collected till May 2012. 942 patients were investigated for immunodeficiency at the Department of Immunology, from which 51 with PIDDs were identified. Fifty patients were investigated for poliovirus excretion at the Polio RRL, Department of Virology. We identified five patients who excreted poliovirus in their stools. Three had vaccine viruses. One patient died and one patient was lost to follow up. Other patient cleared the virus during follow up. Two patients excreted VDPVs. An eight month old baby with severe combined immune-deficiency or SCID with VDPV type 2 expired few weeks after collecting the samples.The other patient with common variable immune-deficiency has excreted VDPV type 3. Long term follow up of this child shows the chronic excretion of poliovirus and the development of VDPV in a patient with immune deficiency disorder.

This girl child when first presented in May 2008 was excreting vaccine poliovirus type 2. Samples collected few months later and 1 year later showed non-polio enterovirus but no poliovirus. When she came back in July 2011 she was excreting vaccine poliovirus type 3. Over the next few months she started excreting the VDPV type 3 showing the change of vaccine virus to VDPV with acquiring mutations. She continued to excrete the VDPV type 3 till Mach 2012 for minimum of 8 months.

This child has managed to control the infection evidenced by the absence of poliovirus in her stools in May 2012. This is most likely due to the increasing the dose of IVIG which she receives regularly. She is remaining free of poliovirus evidenced by the absence of virus in stool samples collected regularly until June 2013.

Ladies and gentlemen, in spite of all these challenges, Global Polio Eradication Initiative remains optimistic as the situation of Poliovirus situation in 2013 is most promising. Total of 69 Wild Poliovirus cases were reported in 5 countries. Three endemic countries, Afghanistan, Pakistan and Nigeria account for 63.7% of these cases. Two countries, Somalia and Kenya showing Wild Poliovirus circulation following importation. All 5 countries were having cases due to Wild Poliovirus type 1. Wild Poliovirus type 3 has not been detected so far in 2013. That is Zero cases due to Wild Poliovirus type 3 reported for the last 8 months and the program is keeping the fingers crossed. The year 2012 ended with least number of polio cases reported from least number of countries. This situation has been recognized as the best opportunity to finally put an end to this feared but preventable disease.

In May 2012 World Health Assembly has declared ending polio as a ''Programmatic Emergency for Global public Health'' and requested the Director General of WHO to develop and finalize a comprehensive polio end game strategy. WHO has prepared ''The Polio Eradication and Endgame Strategic Plan 2013-2018'' which was endorsed by the World Health Assembly in May 2013. Endgame Strategic Plan 2013-2018 identified strategies for four major objectives.

- Polio detection and interruption by 2014.
- Immunization systems and OPV withdrawal by 2016.
- Containment and Certification (by 2018).
- Legacy Planning to ensure world remains polio free.

Unlike in the previous plan, in which the strategies were implemented in phase based or sequential manner, strategies in the end game plan is implemented in parallel with four major achievements identified. Interruption of circulation of wild poliovirus by end of 2014. Withdrawal of vaccine poliovirus type 2 from oral polio vaccine by 2016. Containment and global certification of "Polio-free" status by 2018. Withdrawal of bivalent OPV in 2019. Ending one of the most feared diseases will extend the benefits of a "Polio-free world" to all children everywhere protecting them from this debilitating preventable disease. I have a long list to acknowledge and I like to mention a selected few of them.

- Global polio Laboratory Network and the contributors whose presentations I have used today.
- WHO HQ, SEARO.
- Director and the staff at Enterovirus Research Center, Mumbai, India.
- WR and team at Colombo office.
- Epidemiology Colleagues.
- Chairperson and members of National Certification Committee for Polio Eradication.
- Chairperson and members of National Expert Committee on Polio.
- Director MRI.
- Colleagues and staff at MRI.
- My appreciation to Past and Present staff at the Polio RRL Dr. Nalini Withana, Dr. Vasanthi, Dr. Chamari, Upali, Lalitha, Thalatha, Akash, and the Current team Dr. Thanuja, Wasantha, Uditha, Wimal.
- To my teachers and trainers.
- To my family husband Harsha, sons Ravindu and Bharatha.
- To my parents, brothers and sisters and to my extended family.

Ladies and gentlemen, finally I like to thank all of you for your presence today and for your attention.

Dr. Geethani Wickramasinghe
Consultant Virologist

Thank you Madam President for your kind words of Introduction and good evening ladies and gentlemen. Cheif Guest, Dr Palitha Mahipala, Director General Health Services (DGHS), Dr. Sunethra Gunasena, President of the Sri Lanka College of Microbiologists, Mrs. Ranganie Wickremesinghe, Family members and friends of the late Dr. Wickremesinghe, Past presidents, members of the council, members of the Sri Lanka College of Microbiologists and distinguished invitees.

It is with pleasure and modest pride that I stand before you today, to deliver the Siri Wickremesinghe oration for 2013, at the invitation of the Council of the SLCM. I thank the President and the Council of the College for inviting me to do so.

Rakitha Sirimal Bandara Wickremesinghe whose photograph you see here was born on the 28th of November 1937 to Dr. Artie and Helen Wickremesinghe and had his school education at Royal College, Colombo. He entered the Medical College in 1959. He qualified as a doctor in1963 from the Faculty of Medicine, Colombo and held various appointments in the state medical sector before proceeding to UK for post graduate studies. Dermatology Unit Kandy and MRI were the places where he loved most. He obtained the Diploma and Master of Science in Microbiology from the University of Manchester, and MD with Board Certification in Microbiology from the Postgraduate Institute of Medicine, University of Colombo. After joining MRI, he dedicated the major part of his life to the life and soul of this Institute where he always belonged. Although he got a post at Fairfield Hospital/ Victorian Infectious Disease Reference Laboratory (VIDRL), Melbourne, Australia, he was there only for a short period. His interests were mainly towards his homeland and microbiology work at MRI. Hence he left his wife there to support his children's education and returned back to rejoin MRI and worked as a consultant microbiologist in charge of the bacteriology division until his retirement. Thus we all benefitted from his teaching. He was an excellent teacher to most of us who are seated in this audience today. He was loved and respected by all those who knew him. He was the Director of MRI from 1996 to 1998. After retirement he took up the challenging post of Resident Pathologist and Laboratory Manager at Durdans Hospital, Colombo.

Dr. Wickremesinghe was actively involved in the Sri Lanka College of Microbiologists and was a member of the Council even at the time of his death. I can still remember and hear his encouraging words made as a senior judge on our oral presentations at College Scientific Sessions. He was a long standing member of the Health Department Sports Club taking part in cricket, tennis and billiards. He made his mark in whatever he did. He extended his kindness and love to turtles too. You can witness this by visiting the Kosgoda turtle hatchery. He was a well read man and a walking encyclopaedia for most of us. Although he was such a knowledgeable person he was very simple. I have seen him doing marketing at Delkanda Pola with his wife Ranganie.

When Dr. Wickremesinghe was the Secretary to the of the Board of Study in Microbiology in 1992, it was late Dr. Sathasivam / Consultant Virologist, Rabies Department who brought out the fact that selection of trainees for the Diploma in Microbiology was not according to the criteria laid down. Dr. Wickremesinghe took immediate steps to rectify this and as a result I was selected as a trainee. Therefore I am grateful to both these late doctors Dr. Sathasivam and Dr. Wickremesinghe. If not for them I would not be here today delivering this oration.

My topic today for this Oration is **Challenges and Achievements Faced as a Virologist.**

I initiated studies on respiratory viruses in 1997. I selected a topic on Respiratory Syncytial Virus as the dissertation

for my MD virology as there were hardly any studies on viruses affecting the respiratory tract. My topic was 'A Study in Respiratory Syncytial Virus infection in children below 2yrs admitted to Children's Hospital Colombo with Lower Respiratory Tract Infection. I was fortunate to have Dr. Mrs Nalini Withana as my supervisor and head of the Dept, who provided me the necessary reagents when the National Science Foundation (NSF) refused to fund my study quoting as there are numerous studies in this field in Sri Lanka. I received my Post MD training in VIDRL/ Melbourne where Dr. Siri Wickremesinghe also worked as a Microbiologist. During this period, because of my interest in Influenza I was fortunate to obtain training at the WHO CC for Influenza in Southern hemisphere at Commonwealth Serum Laboratory (CSL) / Melbourne. There I met Raki – son of Dr. Wickremesinghe who worked at CSL and was a good companion to me.

Influenza surveillance was established in Sri Lanka in 1968 with the pandemic of Influenza due to H3N2 by Dr. Yvette E Hermon (Virologist), and Dr. N. M. P. Mendis (Epidemiologist) (1,2). During this pandemic two Influenza virus strains were isolated from Sri Lanka which were confirmed by the WHO Collaborating Centre (CC).

After several years of silence in influenza surveillance, I was able to do several outbreak investigations of Influenza in the country. In 1998 I handled the first outbreak in this era before I got the training in this field. Thereafter in 2002, 2004 and 2006 in various provinces in the country. During this period MRI Virology Dept did not have a proper laboratory space for Influenza work. There were no trained staff and the lab lacked necessary equipment, such as Fluorescence Microscope, Bio-Safety Cabinet and centrifuge. No commercial swabs and media to collect specimens as today.

In 1998 there was an outbreak of fever with Ryes syndrome like illness associated with high morbidity and mortality in the country. I must thank Prof. Lamabadusuriya for the encouragement given to me to investigate this outbreak. First we identified Influenza A virus infection for the first time in Sri Lanka by Direct Fluorescent Test (DFT) from the samples we collected. Mr. Upali Hettiarachchi our SMLT during that time, who was the only trained person to do egg isolation technique, isolated the virus by this technique from these positive samples. Following the shipment of these isolates to WHO CC centres in London and Australia for sequencing we identified the strain as very much similar to Influenza A (H3N2) Sydney 5/97. This strain of influenza virus was responsible for similar outbreak in other countries in the same year. Then in 2002 there was an outbreak of fever in Jaffna district.

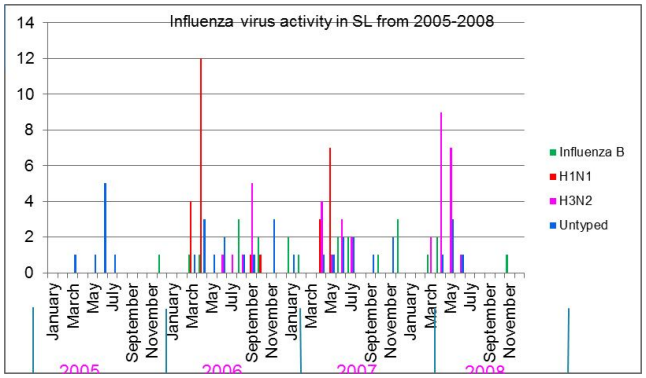
I am grateful to Dr. Mrs. Maya Athapattu who was then attached to WHO Colombo office for making arrangements for me to go there, for sample collection and do the investigations. The aetiological agent was identified as Influenza A H3N2.

Then in 2004, just several months after the identification of Avian Influenza H5 N1 infection in Hong Kong, there was an outbreak of fever and pneumonia in Embilipitiya, adjacent to a chicken farm. Most of the patients and some staff members vacated the hospital due to the fear of infection. We detected Inf B virus and eliminated the fear of Avian Influenza.

We initiated influenza like illness surveillance in 2005 from the OPD /LRH after several outbreaks of influenza occurred in the country. In 2007, this was extended to sentinel surveillance to involve 20 sites to include all the provinces of the country as shown by red drops in the map.

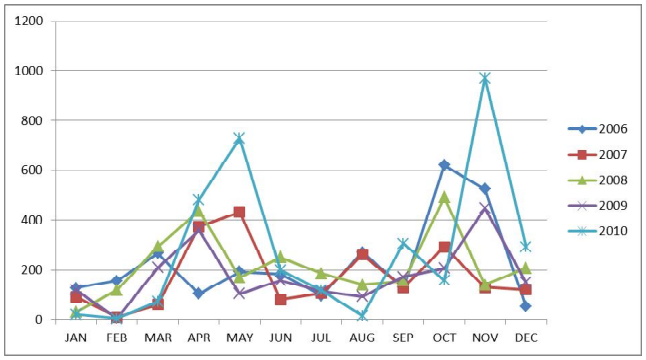
National influenza network

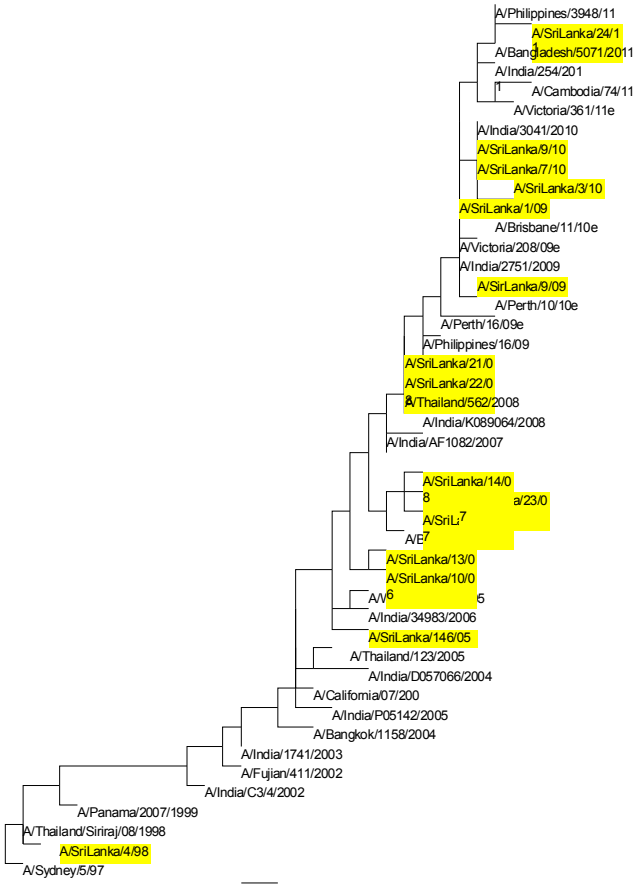
Infection Control Nurses (ICN) in these sites were trained to collect samples and transport to the National Influenza Center (NIC)/ MRI. Thus the outbreak investigations and surveillance activities were initiated by the NIC, more focused influenza surveillance was started under the avian influenza preparedness plan of Global Influenza Surveillance Network (GISN) in 2007. I am thankful to the Ministry of Health and the Epidemiology Unit for the support given. In addition to the ILI surveillance we collected specimens from patients who were admitted with Severe Acute Respiratory tract Infection which is known as SARI surveillance. We have 3 sites for SARI Surveillance – Lady Ridgeway Hospital, Borella, General Hospital, Matara and Teaching Hospital, Peradeniya. All these specimens are tested at the national laboratory at MRI. We wanted to start 2 regional laboratories (Galle and Kandy). Although we trained laboratory personnel, due to various constrains we were unable to do so. In addition to submitting daily reports to patients, we analyse all the surveillance data monthly and disseminate information to the Epidemiology Unit, Ministry of Health and also to the GISRS in Geneva via the Flunet. This shows the influenza virus activity in Sri Lanka from 2005-2008. Different types, subtypes and strains of Influenza viruses circulate each year. Usually the highest incidence was seen around April – May season in Sri Lanka.



There is a higher rain fall in April, May season and October, November season in Sri Lanka depending on the monsoon. Thus there is a relationship of the viral activity to the rain fall which is also seen in most of the neighbouring countries.

Rain fall pattern in Colombo 2006 -2010

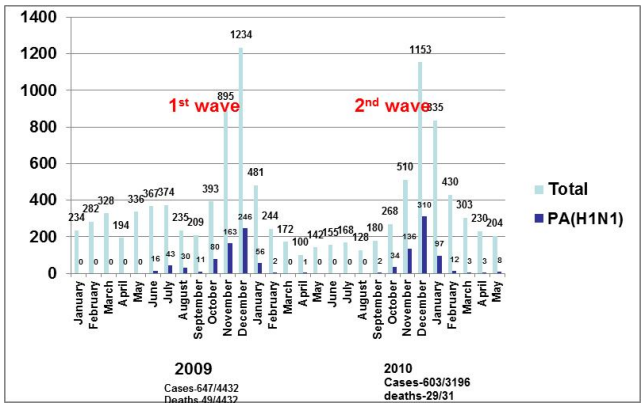




Phylogenetic Analysis of Influenza A viruses isolated in Sri Lanka from 1998 to 2011

This is the phylogenetic analysis of Sri Lankan isolates of Influenza viruses from 1998 - 2011. They were similar to the strains circulated in other parts of the countries in South East Asian and Australian regions.

Pandemic (H1N1) cases from June 2009 - May 2011



Facing the pandemic of swine flu was really a challenge. We identified the first laboratory confirmed case on the 16th June 2009, just 5 days after the declaration of pandemic flu by the WHO. By this time we were equipped with a real time PCR machine, hence all the cases were diagnosed by this technique. We had 2 waves and in the first wave 647 cases were diagnosed with 49 deaths. In the second wave 603 cases with 29 deaths.

There were 7 maternal deaths in the first wave and 4 maternal deaths in the second wave. Complications and deaths were associated with pregnancy and co-morbidities such as neurological, respiratory, haematological disorders, malignancy and immune-compromised states.

Studies on antiviral resistance

There are 2 groups of antiviral drugs, M2 inhibitors (Amantadine and Rimantadine) and neuraminidase inhibitors (tamiflu and Zanamivir) which are active against Influenza. The studies done on Srilankan isolates by the WHO Reference Laboratory. Australia showed that our isolates are also resistant to M2 inhibitors similar to the global situation, and all virus strains tested are sensitive to Neuraminidase inhibitors – Tamiflu except the seasonal H1N1. Unlike in other countries there are no resistant pandemic flu viruses detected from Sri Lanka.

Ultimately we achieved a well equipped Laboratory (NIC) which has a very good recognition in the global influenza programme with facilities to do real time PCR and a good laboratory influenza surveillance system. During the pandemic NIC/ Sri Lanka accepted samples from Maldives too which was highly recognized by the WHO. Now we are capable of detecting the viruses by DFT, virus isolation with typing and sub typing, identifying the strains by

molecular techniques. And also we share our data with global influenza surveillance response system (GISRS) via Flu net. Depending on our laboratory data, Influenza vaccine was registered in 2011 for the 1st time in Sri Lanka.

I really appreciate the support given to me by the Ministry of Health, Epidemiology Unit, the staff of Influenza laboratory/ MRI, the international coloborations (WHO, World Bank, CDC) for the achievement of this state.

Next I will speak on Measles and Rubella Surveillance.

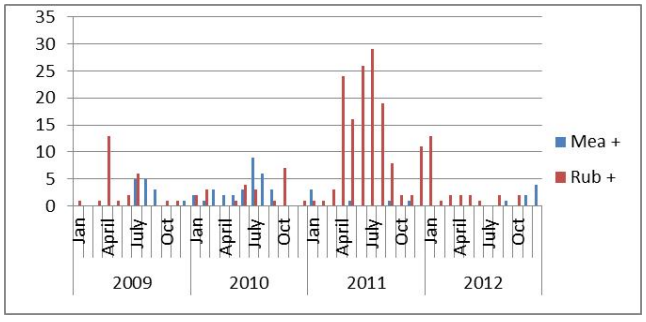
Both these are two important viral diseases causing Fever and Rash which are preventable by vaccination. Both are responsible for outbreaks and the WHO is targeting the elimination of these diseases from the world.

- Both diseases are diagnosed by serological techniques. Such as detecting virus specific IgM Antibodies by ELISA technique (There are commercial test kits to do this test).

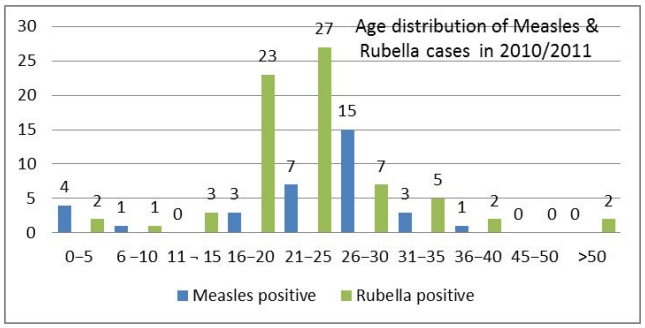
What are the new developments in this field? Newer sampling techniques-collection of oracol (gingival) fluid from the gingival margin using a special device, collection of Naso-Pharyngeal Aspirate (NPA), and obtaining Nasal and Throat swabs similar to collection of samples for influenza surveillance. These are all non invasive procedures that cause minimal discomfort to the patient. Using these samples we can detect the positive cases by serological as well as by several other techniques.

Newer laboratory procedures were adopted. Using the above samples we are able to isolate the virus on Vero h-slam cell line, do molecular techniques – Conventional PCR,- Real time PCR, Geno typing and Gene sequencing. Positive samples with Measles virus show cytopathic effect with syncytia formation on Vero h slam cell line. In the early stage you can see the giant cell formation with multiple nuclei and at later stages you can see the syncytia formation. These cell lines can be scraped and stained with Measles specific fluorescent reagent to show the green fluorescence for the confirmation. Negative cell lines do not show any Fluorescence. Rubella viruses do not produce CPE on cell culture. Hence Fluorescence techniques are used always for the confirmation.

Total Number of positive Measles and Rubella cases 2009-2012



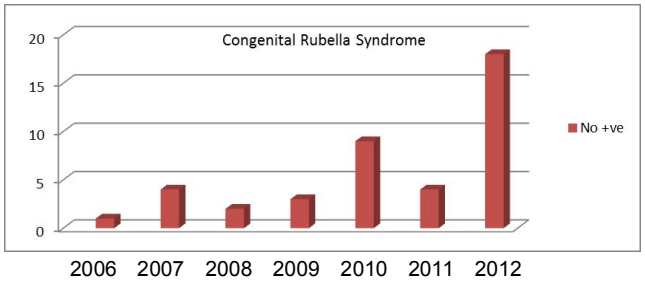
This graph shows the total number of positive Measles and Rubella cases from 2009 and 2012. In 2009 and 2010 mainly Measles cases were seen (Blue bars) and in 2011 mainly rubella cases were seen (green bars). This was due to several outbreaks which occurred in 2011.



Most of these positive cases were seen among males in both these diseases. Both diseases were seen mainly among young. 20-35 year age group.

Different genotypes circulate in different countries. In India Measels Genotype D8, in China genotype H5 and so on. The importance in detecting genotypes is to identify the imported cases. This is important in elimination of these diseases. Genotyping showed that the Measles viruses circulating in Sri Lanka belong to the genotype D8 and Rubella viruses are all geno type 2B.

All these Sri Lanka genotypes are now stored in the WHO Genebank for future references. Elimination of rubella is important to prevent infection of pregnant mothers as it causes congenital rubella syndrome of the affected foetus.



No of positive CRS cases from 2006-May 2012

The above graph shows the rise of congenital rubella syndrome cases in 2012. Probably due to a breakdown in vaccination programme following a sudden death of a school girl in the Matara district following rubella vaccine.

My last achievement as a virologist at MRI was the establishment of diagnosis of diseases caused by CytoMegalo Virus (CMV).

It is not significant in immune competent population (causes CMV mononucleosis syndrome). But it causes latent infections in 60-80% of population. So in immune compromised population such as organ transplants,

leukaemic, cancer and AIDS patients, it is highly significant as it causes CMV disease.

- These diseases can be either direct effects such as CMV mononucleosis syndrome, Colitis, Pneumonitis, Hepatitis and Retinitis or
- indirect effects such as Immune mediated graft rejection, Opportunistic infections

From Serological tests (IgM, IgG) it is impossible to detect these disease states.

To detect the disease status in these patients I established a CMV antigenaemia test which I learnt in Australia. It is based on indirect immuno fluorescence assay to detect pp65 Ag on leucocytes when there is active replication of the CMV. It is Negative if there is no active viral replication. It is a semi-quantitative assay; usefull to quantify Ag level and demarcate between CMV infection and disease. Peripheral blood leucocyte positivity rate >0.02% is clinically significant especially in renal transplant recipients. Hence as the antivirals against CMV is now available you can use these drugs as pre-emptive therapy and halt the progression of the disesae.

- There are several steps in the test. First you have to isolate peripheral blood leukocytes from the sample of blood you collected and then prepare the microscopic slides. After the fixation of slides with cold acetone, Immunoperoxidase / IF staining with Monoclonal antibodies to CMV IE Ag is done.
- Later evaluation under a fluorescent microscope for green fluorescence on CMV antigen positive cells and semi quantitative scoring is done.

As the supervisor, I was able to encourage (Dr Nadeeka Janage) one of the post graduate trainee in MD virology to do his MD virology dissertation in 2010 on CMV PCR which is a Qualitative / Quantitative assay. A compa-rative study was done on CMV antigenaemia assay and molecular techniques on different samples. It is useful to demarcate the CMV infection and CMV disease.

- Ultimately we were able to establish the quantification of viral load at MRI by PCR in April 2013. This will benefit the immune compromised patients in SL such as organ transplant patients, Leukemic and AIDS patients where the CMV disease is more common.

There was a well established course for MD medical microbiology in the Post Graduate Institue of Medicine (PGIM) to produce satisfactory number of hospital based medical microbiologists. The Ministry of Health and the PGIM recognised the need for specialists in the field of virology as there were several outbreaks of emerging viral infections in the world. Under the guidance of Prof Lalitha Mendis and Prof Manel Wijesundara I was able to initiate a separate MD medical virology programme in 2006 with the help of my colleagues. I am happy to say altogether nine post graduate trainees have undergone this training programme and the country can now face the emerging viral infections in future. Thus finally I was able to follow the foot steps of late Dr Siri Wickramasinghe and pay tribute to him.

Acknowledgments

I would like to acknowledge all my teachers, staff of the National Influenza Center, National Measles and Rubella Laboratory and all staff members of Department of virology/ MRI. Prof. Sriyal Malik Peiris and staff of the Hong Kong University – for conducting training sessions in Sri Lanka and helping me in numerous ways, giving valuable advice, sending reagents etc in emergencies. Dr. Ian Barr and staff of the WHO CC in Australia, for confirming our isolates, doing sequences and antiviral studies on our isolates. Pasteur Institute France – They helped me in doing 2 post tsunami projects in 2007 and 2008 conducting training work shops and donating equipment.

All my colleagues who helped me in numerous ways in presenting this oration and finally my husband and two daughters for their encouragement and tolerance.

INTRODUCTION TO GEOGRAPHIC INFORMATION SYSTEM

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What is geographic information system

There is no single definition for geographic information system (GIS). There are many working definitions and most of them are acceptable for understanding purpose.

A geographic information system is a computer system that incorporates hardware, software, and data for capturing, managing, analyzing, and displaying all forms of geographically referenced information (1).

There are three W's in geography

1. What is where?
2. Why it is there?
3. Why do I care? (Implications of above two points)

The concept that place and location can influence health is a very old and familiar idea in medicine. As far back as the time of Hippocrates (3rd century BC), physicians have observed that certain diseases seem to occur in some places and not others. Even within the human body, many diseases and organisms are known to have a predilection for, or to exclusively affect specific body organs or systems (anatomico-physiological ''locations'' within the human body) (2).

Spatial nature of epidemiological data has long been understood. In 1854, there was a cholera outbreak in Soho district of London and nearly six hundred people died from cholera in just 10 days. Dr. John Snow, a London physician and anaesthesiologist who mapped (Figure 1) the locations of water pumps and the homes of people who died of cholera, Snow was able to show that one pump, the public pump on Broad Street, was causing most of the disease. Snow suspected that infected water from the pump was the cause. He instructed the authorities to remove the handle to the pump, making it unusable; the number of new cholera cases dropped dramatically. The Broad Street pump proved to be the source of contaminated water and hence cholera, just as Snow had thought (3). Since then, epidemiology has played an increasingly important role in providing scientific evidence to support animal and human health policy development.

Spatiotemporal distribution

Distribution of disease or any phenomenon in earth surface (geographicaly) called spatial distribution. In the case of infectious diseases like influenza, Dengue and Malaria, the study of their geographic distribution frequently involves examining the diffusion of the disease through space over a given period of time (spatio-temporal mapping).

Transmission of infectious diseases is closely associated with concepts of spatial and spatio-temporal closeness of at risk individual. In the case of non-communicable diseases transmission, environmental risk factors may play important role.

The most basic GIS approach is to examine maps of disease occurrence visually to answer the question ''WHAT IS WHERE'' (4). This method has inherent

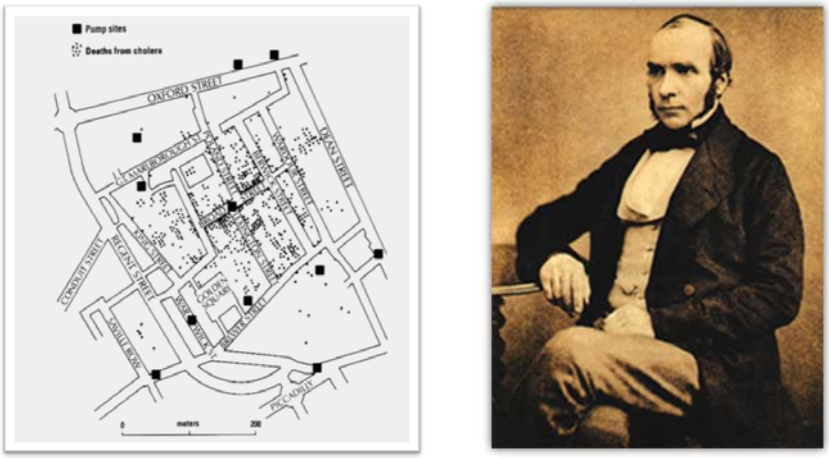

Figure 1. Dr. John Snow mapped the locations of water pumps in Soho district of London.

weakness as this does not involve statistical testing. It needs to be followed by statistical assessment and experimental challenge of hypotheses before inferences in relation to cause and effect can be drawn. Spatial epidemiology provides the necessary tools for such statistical assessment. Although the field of spatial epidemiology has a large number of techniques, deciding which one to use can be challenging.

Spatial epidemiological analysis has three main objectives

1. Describe the spatial patterns
2. Identify disease clusters
3. Explore or predict the disease risk

To achieve these objectives in addition to the traditional attribute data describing the characteristics of the entity studied (demographic and other characteristics related to the disease), geo-referenced feature data (location information) are required.

Specific analytical objectives in three groups of analytical methods

1. Visualisation
2. Exploration
3. Modelling

First two focus solely on examining the spatial dimension of the data.

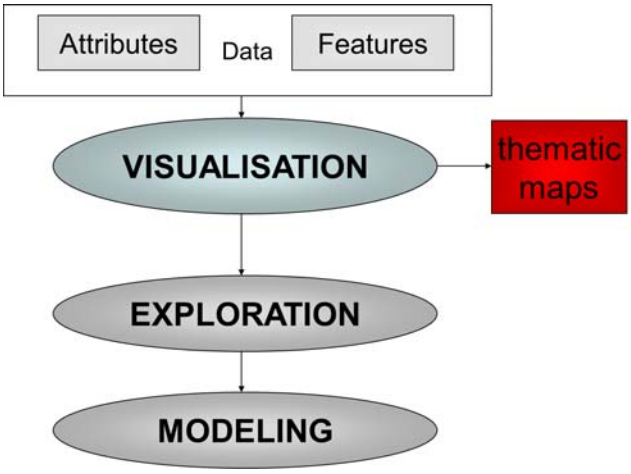

Figure 2. Framework of spatial analysis.

Visualisation is the most commonly used spatial analysis method, resulting in maps that describe spatial patterns Figure 2. Exploration of spatial data involves the use of statistical methods to determine whether observed patterns are random in space. Modelling introduces the concept of cause-effect relationships using both spatial and non-spatial data sources to explain or predict spatial patterns. It needs to be emphasised that none of these approaches allows definitive causal inferance.

Spatial visualisation

In the last two decades, we have seen an explosion of interest in disease mapping, with the recent developments in advanced spatial statistics and the increasing availability of computerized geographic information system technology. One of the first steps in any epidemiological analysis is to visualize the spatial characteristics of dataset (5).

Mapping vs Analysis of disease data

Although the mapping of disease data can be relatively straightforward, interpreting spatially referenced disease data can sometimes be challenging, particularly for non-infectious and chronic diseases For example, a researcher might map the distribution of people with schizophrenia in urban areas and find that they tend to reside in low-income, inner-city areas. At this stage, the researcher can understand how the data is distributed (patterns or clusters - mapping), but explaining ''why it is there'' as such is another story and requires further research (analysis).

Spatial analysis of epidemiology

Epidemiology is about the quest for knowledge in relation to disease causation, and this can be about understanding risk factors or about the effects of interventions. To determine cause and effect relationship, need to develop a theoretical hypothesis based on observed data. In most epidemiological investigations definitive causal inference is difficult, if not impossible, to obtain through analysis of epidemiological data.

Visualisation helps to:

- Identify errors
- Identify potential patterns
- Generate hypotheses about factors influencing patterns

Visualisation also serves as an excellent tool for communicating findings to the target audience.

Type of data

Data collected for the purpose of epidemiological investigations typically focus on the attributes of observations such as the disease status of individual.

Representation of spatial data depends on the map scale.

E.g. A school may be represented as a polygon in large scale (1: 10,000) and the same school becomes a point in small scale maps (1: 10,000).

Point data

E.g. Location of disease outbreaks, school survey data (Figure 3) (6).

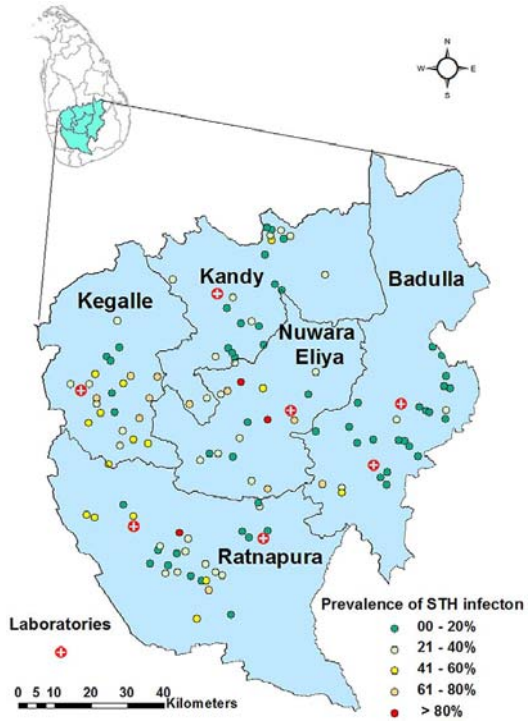

Figure 3. Geographical location of study schools and laboratories in the districts of Kandy, Kegalle, Nuwara Eliya, Badulla and Ratnapura, together with prevalence of infection with any one or more soil-transmitted helminth infection at each school (6).

Aggregated data

E.g. Disease incidence by geographic boundaries (Figure 4) (7).

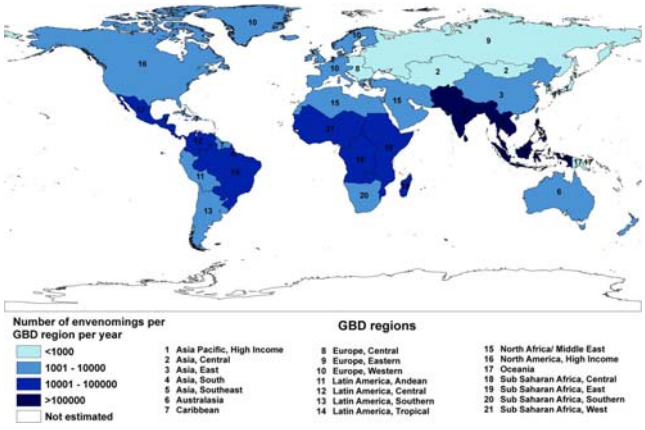

Figure 4. Regional estimates of envenomings due to snakebite (low estimate) (7).

Continuous data

E.g. Rainfall, air pollution, predicted prevalence of infection (Figure 5) (6).

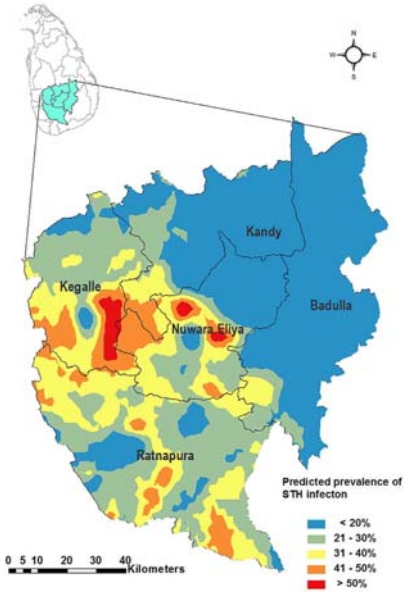

Figure 5. Predicted prevalence of infection with any one or more soil-transmitted helminth infection among children in the plantation sector in five districts of Sri Lanka (6).

Spatio-temporal analysis is concerned with cluster validation, e.g. that a detected cluster is not due to mere chance factors, and with attribution of detected clusters to the appropriate factors that played a role in their occurrence. Analysis also includes doing comparisons with other relevant patterns/clusters (in the same place at different times and in other places) and again trying to methodically explain any spotted differences or trends. Thus, in the case of the schizophrenia example mentioned above where a cluster or pattern has been detected, many questions arise (new hypotheses) that need to be addressed. For example, does the stress of urban poverty cause mental illness, or are the mentally ill forced to live in cheap housing because their illnesses prevent them from earning a stable income? Or is there a circular relationship between poverty and mental illness? Even though GIS is used widely in infectious diseases like Malaria (8), Filariasis, Helminth infections (9) and Dengue etc in other countries its use is very primitive in Sri Lanka. Most of the countries share their health related data freely and anybody can access through internet. One of such is onemap.sg maintained by Singapore government. If you need to find either Dengue clusters or Breast cancer screening centers in Singapore you are just a few clicks away.

In the west GIS is used extensively for disease prevention and control for example: Recently CDC has developed an interactive disease atlas(10). Annually 800,000 people die from cardiovascular disease in the US. Centers for disease prevention and control (CDC) is aiming at reducing Annual Heart Diseases and Stroke-Related deaths in the US by 200,000 using interactive disease Atlas which uses GIS throughout, from data collection to presentation. According to the report many of these deaths could be prevented by improving health care systems, creating healthy places to live and play and supporting healthy lifestyle choices. Hope is that by

providing this data in an easy to use format (through a map) it will be clearer to see where cardiovascular disease is more prevalent and which population groups are at high risk for the problems such as hypertension, myocardial infarction and heart failure. Their goal is to help doctors, health care administrators and public health officials as well the as general public better focus health education and other preventive programs in those areas and groups and reduce the mortality (11).

1. Wikipedia. Geographic information system 2014 [updated 24 May 2014]. Available from: http://en.wikipedia.org/wiki/Geographic_information_ system.
2. Centre M. Medical Geography City University, London, UK: School of Informatics City University, London, UK; 2002 [cited 2014 2014-06-05]. Available from: http://healthcybermap.org/HGeo/pg1_1.htm.
3. Stolley PDLT. Investigating Disease Patterns. New York: Scientific American Library; 1995.
4. Theobald DM. GIS CONCEPTS AND ARCGIS METHODS. 3 ed: Conservation Planning Technologies; 2007.
5. Dirk PU. Spatial Analysis in Epidemiology. Oxford University Press; 2008.
6. Gunawardena K, Kumarendran B, Ebenezer R, Gunasingha MS, Pathmeswaran A, de Silva N. Soiltransmitted helminth infections among plantation sector schoolchildren in Sri Lanka: prevalence after ten years of preventive chemotherapy. PLoS neglected tropical diseases. 2011 Sep; 5 (9):e1341. PubMed PMID: 21980549. Pubmed Central PMCID: 3181244.
7. Kasturiratne A, Wickremasinghe AR, de Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, et al. The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS medicine. 2008 Nov 4;5 (11): e218. PubMed PMID: 18986210. Pubmed Central PMCID: 2577696.
8. Hay SI. Malaria Atlas Project 2014 [cited 2014 5-6-2014]. Available from: http://www.map.ox.ac.uk/.
9. School L. Global Atlas of Helminths Infections: London School of Hygiene and Tropical Medicine; 2014 [cited 2014]. Available from: http://www.thiswormyworld.org/
10. CDC. Division for Heart Disease and Stroke Prevention: Interactive Atlas 2014. Available from: http://nccd.cdc.gov/dhdspatlas/.
11. Ankersen R. CDC Aims at Reducing Annual Heart Disease and Stroke-Related Deaths in the US by 200,000: ESRI; 2014 [cited 2014]. Available from: http://www.esri.com/esri-news/~/media/Files/Pdfs/news/arcnews/spring-2014/ spring-2014.pdf.

SEPSIS AND WOUND INFECTION FOLLOWING SNAKE BITE A REVIEW INCLUDING THE SRI LANKAN PERSPECTIVE

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Introduction

There are about 7 medically important venomous snakes in Sri Lanka, namely cobra, common krait, Sri Lankan krait, Russels' viper, saw-scaled viper, green-pit viper and also hump-nosed viper (1). In snake bite, as our main objectve is to manage the envenomation, identification of the snake plays a critical role due to different types of venom in different snakes. From sepsis point of view as well, it is important as local effect of some venom e.g. venom of cobra, hump-nosed vipers, is well known to cause severe tissue necrosis and subsequent wound infection. In acute management of snake bite we almost always tend to overlook the possible sepsis even after identifying the snake. Hence, anticipation of such associations would be beneficial to prevent subsequent complications.

During my tenure as the consultant microbiologist in District General Hospital, Polonnaruwa, which is a major tertiary care centre in the north central province of Sri Lanka, I could help in management of several such cases of sepsis following snake bite and I will be reporting a few of the interesting cases. The patients were from different areas of the province and were ultimately transferred to DGH Polonnaruwa for specialized care. North central province is one of the seasonal dry zones of Sri Lanka in which many types of venomous snakes are found in the human vicinity as well as in the forest areas. Majority of dwellers of the region are farmers and naturally at a higher risk for snake bites and wild animal attacks than other more urban population.

Case report 1

There was a 68 year old previously healthy female from Hingurakgoda, who presented to DGH Polonnaruwa following humped nosed viper bite on the same day. She did not show any specific symptoms or signs of envenomation but complained of pain and swelling at the bite site on the left forearm. She was put on oral cloxacillin and intravenous penicillin, which was later changed to cefotaxime while in the ward. Her initial white blood cell count was 3300/mm^3 and platelets were within the normal range.

After about 36 hours of admission she developed respiratory distress and fever and admitted to intensive care unit. She was started on intravenous imipenem and vancomycin but within few hours she passed away. The following day her blood culture which was taken at the ward was flagged as positive for Gram negative bacilli by the automated blood culture system and the isolate was later identified as *Serratia marcescens* with 99% specification using the Api 20 E kit. The isolate was sensitive in-vitro to almost all antibiotics tested; however it must have been late to start on cefotaxime and later imipenem. The blood culture was drawn within 48 hours of admission and therefore healthcare associated infection is unlikely.

Case report 2

A 23 year old previously healthy female from Medirigiriya presented to DGH Polonnaruwa with cellulitis of the right leg following a viper bite. After managing the envenomation she was started on intravenous penicillin and cefotaxime while in the ward. She continued to have high spiking fever and blood culture taken within 24 hours of admission was positive for *Staphylococcus aureus*. The patient fully recovered after treatment with intravenous flucloxacillin for 14 days.

Case report 3

A previously healthy 19 year old boy was admitted to the local hospital Dehiattakandiya following hump nosed viper bite having developed extensive cellulitis leading to necrotizing fasciitis of the right leg up to the thigh within 24 hours of bite. He was drowsy and hypotensive and was in septic shock on admission. He was started on intravenous cefotaxime, coamoxiclav and penicillin at the local hospital and transferred to the DGH Polonnaruwa within 24 hours of bite. There the patient underwent extensive wound debridement and the tissues and blood were sent for culture before putting him on intravenous meropenem and clindamycin on the same day of admission.

The initial white blood cell count was 19000/mm^3 with thrombocytopenia and blood picture was suggestive of disseminated intravascular coagulation. Blood culture was negative but the tissue culture grew a pure growth of *Serratia marcescens* which was resistant to ampicillin and coamoxiclav but sensitive to all other antibiotics in vitro. As patient underwent wound debridement within 48 hours, and as it was a pure growth of the particular isolate, it is unlikely to be a health care associated infection. Considering the potential of developing resistance to cephalosporins in this organism we continued with meropenem and the patient fully recovered in 5 weeks.

Discussion

There are different types of both aerobic and anaerobic bacteria inhabiting the oral cavity of snakes including faecal Gram negative rods. The prey usually defecates while being ingested contaminating the snake mouth with its excreta (2). Studies have shown that cultures of fangs, fang sheaths, and venom of various snakes such as bothrops (pitvipers-subfamily crotaline which includes hump-nosed vipers), vipers, rattlesnakes and *Naja naja*, have shown heavy colonization with many bacteria such as members of Enterobacteriaceae including *Morganella* spp. and *Escherichia* coli, Group D streptococci, Staphylococci, *Aeromonas* spp., and anaerobes such as *Clostridium* spp (2,3).

Although it is not a widely discussed topic in the medical literature about sepsis or wound infection following snake bite, there are few well documented case reports and review articles on the subject. Most such reports are available from the Asian or African region including India, Nepal, Thailand, Taiwan, Malaysia, South Africa and Zimbabwe. A well illustrated study including follow up of cases of bacterial infections following snake bite has been published in northern Taiwan authored by Chun-Ming et al (4). They describe about the different bacterial species isolated in infections associated with snake bites including Staphylococci, Enterobacteriaceae and anaerobes, isolated mainly from the wound cultures (Table 1). The main types of snakes causing post-bite infections in that study were the pit vipers and cobras. Some review articles on animal bite wound infections, have also described the possible spectrum of organisms causing such infections including all described above (2,5).

Table 1. Bacterial isolates identified from snakebite wounds (4)

Organism	Number
Aerobic Gram positive bacteria	14
Enterococcus spp	12
Cogulase negative *Staphylococcus* spp	1
Bacillus spp	1
Aerobic Gram negative bacteria	39
Citrobacter amalonaticus	1
Citrobacter freundii	3
Escherichia coli	2
Klebsiella pneumoniae	1
Morganella morganii	14
Proteus mirabilis	1
Proteus vulgaris	4
Providencia rettgeri	3
Pseudomonas aeruginosa	5
Serratia liquefaciens	1
Serratia marcescens	1
Shewanella putrefaciens	3
Anaerobic bacteria	8
Bacteroides fragilis group	6
Peptostreptococcus species	2

There had been interesting case reports on *Serratia marcescens* infection following snake bites as well. Although *Serratia* cellulitis and related complications and sepsis was described following iguana bites (6), snake bites related wound infections caused by the organism is rarely reported. P. Subramani et al (7) from India have given a report similar to our 3rd case with tissue necrosis and cellulitis. Further, Sanjib K Sharma et al from Nepal (8) reported a case of endophthalmitis and necrotizing fasciitis following cobra bite with *Serratia marcescens* isolated from vitreous fluid aspirates. Some other case reports are available which describe of involevement of Enterococci (8) and Providencia (9) in wound infection and some have reported rare pathogens like *Shewanella* wound infection and sepsis (10) following different kinds of snake bite.

Routine use of prophylactic antibiotics following snake bite is controversial (11,12). It is documented that the snake venom itself has some antibiotic properties against some bacteria (2,12). Although venom is sterile, the necrosis caused by that will be a good ground for the faecal pathogens introduced through snake mouth during bite (5). A few review articles from India (3) and Zimbabwe (13) emphasizes the fact that if there is evidence of tissue necrosis, abscess formation and gangrene, it is better to start antibiotics with coverage of Staphylococci and enterobacteriaceae. As some snakes are known to cause tissue necrosis after bite, it is necessary to consider antibiotics if such snakes are involved. Tissue necrosis can be anticipated in blister formation or dark discoloration of skin at the bite site (12). According to our case reports if there is evidence of sepsis and wound infection following hump-nosed viper bites which contributes 22-77% snake bites (14) in Sri Lanka, it should be promptly attended with broad spectrum antibiotics. This suggestion is supported by several other articles from the Asian region especially for bite injuries by Malayan pit vipers, cobra and some other crotalids (4,12). However, almost all authors emphasize the fact that tissues and other necessary samples should be cultured and antibiotic treatment to be guided by the results (4,12).

Regarding the choice of the antibiotic, one study suggests a combination therapy of co-amoxiclav and ciprofloxacin as prophylaxis and another suggests ciprofloxacin as the sole agent (13,3). However importance of further research in this field should be emphasized when we consider developing local guidelines with knowledge of the spectrum of pathogens causing post snake bite infections and the antibiotic sensitivity patterns in the Sri Lankan set up.

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References

1. Karunarathne K. Snake bites. (Internet) Available from: http://www.gmoabuhorana.org/wp-content/uploads/2012/11/snake-bites.pdf
2. Fredrick M. Abrahamian, Ellie J. C. Goldstein. Microbiology of Animal Bite Wound Infections. *Clinical Microbiology Reviews*. 2011; **24**(2): 231-46.
3. Atul Garg, S. Sujatha, Jaya Garg, N. Srinivas Acharya, Subhash Chandra Parija. Wound infections secondary to snakebite. *J Infect Developing Countries* 2009; **3**(3): 221-3.
4. Chun-Ming Chen, Keh-Gong Wu, Chun-Jen Chen, Chuang-Ming Wang. Bacterial infection in association with snakebite: A 10-year experience in a northern Taiwan medical center. *Journal of Microbiology, Immunology and Infection*. 2011; **44**: 456-60.
5. Martin Rodriguez. Bite wound infections (internet) Available from: www2.massgeneral.org/id/hms/handouts20032004/martin4_04.pdf
6. Stephanie Hsieh, Franz E. Babl. Serratia marcescens Cellulitis Following an Iguana Bite. *Clinical Infectious Diseases*. 1999; **28**: 1182-3.
7. Parimala Subramani, Gokul Bindiganavile Narasimhamurthy, Bhaskaran Ashokan, Beena Prasavangada Madappa. Serratia marcescens: an unusual pathogen associated with snakebite cellulitis. *J Infect Dev Ctries* 2013; **7**(2): 152-4.
8. Sanjib K Sharma, S. Shrestha, B. Badhu, C. S. Agrawal, B. Khanal. Infectious Complications of Venomous Snakebite: 2 Cases from Eastern Nepal. *Int J Infect Dis*. 2010; 14.
9. CY Cheong, CK Lee, Z Zuki. A Rare Infection Following Snakebite. *Malaysian Orthopaedic Journal* 2010; **4**(1): 53-4.
10. Po-Yu Liu, Zhi-Yuan Shi, Chin-Fu Lin, Jin-An Huang, Jai-Wen Liu, et al. Shewanella infection of snake bites: a twelve-year retrospective study. *Clinics* 2012; **67**(5): 431-5.
11. Nicole Thomas, Itzhak Brook. Animal Bite-associated Infections; Microbiology and Treatment. *Expert Rev Anti Infect Ther*. 2011; **9**(2): 215-26.
12. R S Blaylock, Antibiotic use and infection in snakebite victims. *South African Medical Journal*. 1999; **89**: 874-6.
13. Dexter D Tagwireyi, Douglas E Ball, Charles FB Nhachi. Routine prophylactic antibiotic use in the management of snakebite. *BMC Clinical Pharmacology* 2001; **1**(4).
14. Kolitha H Sellahewa. Hump-nosed Pit Viper Bite in Sri Lanka-Unravelling an Enigma. *Journal of Tropical Diseases*. 2013; **1**(3).

A CASE REPORT AND REVIEW ON BRUCELLA ENDOCARDITIS

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Case Report

A 70 year old female presented with a history of intermittent fever, associated with chills, malaise, generalized body aches, anorexia and loss of weight of 10 weeks duration. During this period she had been admitted to hospital on three occasions and was investigated for PUO which including several blood cultures and a bone marrow biopsy. None of the investigations were conclusive. Her inflammatory markers (ESR and CRP) were persistently elevated. She was treated with several antibiotics during these admissions. She gave a history of recent travel to India three weeks prior to the onset of symptoms. A dietary history revealed that she had consumed fresh cow's milk during her stay in India.

On fourth admission she had persistent fever and appeared acutely ill, but not toxic. Cardiovascular system examination revealed a diastolic murmur. Abdominal, neurological and dermatological examination did not detect any abnormalities. Haematological tests revealed leucopenia and mild normochromic, normocytic anaemia and an elevated ESR and CRP. Initial trans-oesophageal echocardiogram (TOE) showed trivial aortic regurgitation but no vegetations. Subsequently, five blood cultures became positive for an oxidase positive, Gram negative, short bacillus which grew on chocolate, blood and MacConkey agar. The API 20NE system did not give a definitive identification. *Brucella* was suspected because of the typical Gram stain appearance and urease positivity and blood culture isolate was confirmed by PCR. TOE, done one month later, revealed trivial aortic regurgitation with an aortic valve vegetation of 0.36cm. Although initial *Brucella* antibodies were negative, subsequent tests done at 4 weeks and 6 weeks revealed 1/80 and 1/160titres for *B. abortus*, respectively. The patient was confirmed as having Brucella endocarditis and treated medically with no surgical intervention.

Prior to the diagnosis she was treated with IV ceftriaxone 2g daily and IV gentamicin 1mg/kg 8 hourly for one month. The antibiotic regimen was changed to a combination of oral rifampicin 450mg bd with doxycycline 100mg bd and trimethoprim-sulfamethoxazole 960mg bd, once the organism was identified. Since, she could not tolerate these antibiotics due to severe gastrointestinal symptoms, rifampicin and trimethoprim-sulfamethoxazole were discontinued after a week and the antibiotic regimen was modified to a combination of oral doxycycline 100mg bd and ciprofloxacin 500mg bd and continued for 4 months.

Her clinical parameters and renal and liver profile were monitored over 6 months and the therapeutic response assessed by serial CRP counts and *Brucella* serology. The CRP levels remained below 0.8mg/dl and two blood cultures and Brucella serology, repeated 6 weeks after initiating the modified therapy and were negative. A repeat 2D ECHO revealed healed AV vegetation and she recovered completely with no relapses.

Overview

Background

Brucellosis is a worldwide anthropozoonosis. It is caused by anaerobic intracellular Gram negative coccobacillus, belonging to the genus *Brucella*. The organism is localized in the reproductive organs of the host animal and is shed in large numbers in urine, milk, placental fluid and other fluids. Exposure to infected animals and animal products causes brucellosis in humans. Brucellosis was described more than 2000 years ago by the Romans. The bacterium was first isolated by Dr Bernhard Bang and named after Dr David Bruce. Interest in brucellosis has been increasing recently due to the potential for greater exposure due to increased international travel and also because of the possible use of *Brucella* as a biological weapon.

Classification of *Brucella* species is based largely on the preferred host. Of the 7 *Brucella* species, four cause disease in humans (*B abortus, B melitensis, B canis, B suis*). *B melitensis* is the most virulent species. Suppurative destructive lesions are associated with *B suis. B abortus* and *B canis* cause mild-to-moderate disease with rare complications. *B. abortus* is the most frequently isolated strain in humans, found in almost 99% of total cases (1).

Epidemiology

Brucellosis causes more than 500,000 infections worldwide, annually. The highest incidence is seen in the Middle East, Mediterranean region, China, India, Peru, and Mexico. Currently, the2 greatest increase in the number of cases is seen in Central and Southwest Asia (2).

Disease incidence and prevalence rates vary widely among nations. Incidence rates of 1.2-70 cases per

100,000 people are reported (2). *Brucella* endocarditis constitutes rare, but severe complications (3).The mortality rate of *Brucella* endocarditisis 80% (4).

Pathophysiology

The organisms enter through breached skin or through the mucous membrane of the respiratory tract during direct contact with an infected animal or animal product or through the gastrointestinal tracts by ingestion of infected food products especially unpasteurized milk. Brucellosis is an occupational disease affecting farmers, abattoir workers, food handlers, veterinarians, dairy-industry professionals, and laboratory personnel (5,6). A low concentration of organisms (10-100 bacteria) is sufficient to establish infection in humans. The incubation period varies from1-8 weeks.

Brucella species have the ability to invade both phagocytic and nonphagocytic cells and survive in the intracellular environment. This explains why brucellosis is a systemic disease and can involve almost every system. After ingestion by phagocytes, approximately 15-30% of organisms survive in the polymorphonuclear or mononuclear phagocytic cells using numerous mechanisms to avoid or suppress bactericidal killing. Susceptibility to intracellular killing differs among species. *B melitensis* is more resistant than *B. abortus*; explaining the differences in pathogenicity and clinical manifestations (7). They are transported into the lymphatic system and replicate locally in the kidney, liver, spleen, breast tissue, or joints causing both localized and systemic infection. After replication Brucellae are released with the help of hemolysins and induce cell necrosis.

Granulomas may accompany extracellular replication, especially in the liver and spleen. *B abortus* replicates in foetal tissue. *Brucella* species have relatively low virulence, toxicity, and pyrogenicity making them poor inducers of inflammatory cytokines such as tumor necrosis factor and interferon. Cardiac damage may be due to the direct effect of the microorganisms themselves or due to local deposit of immune complexes.

The primary mode of host defense is cell-mediated immunity rather than antibodies, although some immunity to re-infection is provided by serum immunoglobulins. Persistently elevated IgG titers or a delayed rise in IgG usually indicate chronic or relapsed infection.

Diagnosis

Familiarity with the clinical manifestations and the optimal laboratory diagnostic strategy is essential to recognize the disease. The primary diagnostic pitfall is failure to consider possible *Brucella* infection in a patient with history that suggests a possible source of infection (eg, a farmer, a traveler to an endemic region, or a veterinarian).

A history of exposure to potentially contaminated foodstuffs, travel to an endemic area and occupational exposure to infected animals or animal products would be the most supportive component in diagnosing brucellosis as symptoms are nonspecific. The patients will have systemic symptoms and almost any organ can be affected by *Brucella*. Onset may be acute or chronic. In case reviews, certain symptoms were noted to be more prevalent, with fever, malaise, myalgia, sweats, and arthralgia present in 90-95%, 80-95%, 40-70%, 40-90% and 20-40% of the patients respectively (2). In respiratory infections, a nonproductive cough and pleuritic chest pain predominate with a normal chest radiograph. Patients with prolonged illness often experience weight loss, fatigue, and anorexia. Physical examination in brucellosisis often nonspecific. In patients with signs of systemic toxicity and persistently elevated liver enzymes suspect a hepatic abscess which may serve as a source of bacteremia. Septic embolization is a common complication of endocarditis, while other cardiac complications, such as pulmonary oedema or dysrhythmias, are rare. The pre-existence of valvulopathy predisposes to valve involvement. The most commonly affected valve is the aortic (75%) followed by the mitral valve. Acute onset of aortic insufficiency is a poor prognostic factor (8).

Laboratory diagnosis

Definitive diagnosis of brucellosis is based on isolation of organism from blood cultures or tissues (eg, bone marrow or liver aspiration). Other Body fluid specimens (eg, synovial fluid, pleural fluid, or cerebrospinal fluid) can be cultured, but the yield is usually low. Any specimens potentially harboring *Brucella* spp. need to be handled in a bio-safety level 3 laboratories. Due to the slow growth of *Brucella* spp. and their requirement for a suitable culture medium, *Brucella* endocarditis is often associated with a high rate of negative blood culture. Blood cultures are positive in 10-90% of patients and blood culture sensitivity varies from 17-85%, depending on culture conditions, antibiotic treatment and the time lapse between the onset of symptoms and specimen collection. A *Brucella* positive blood culture is diagnostic of endocarditis, when predisposing heart lesions co-exist. Identification of organisms to a genus level is adequate for treatment; however, speciation is necessary for epidemiologic surveillance.

Serology testing is the most commonly used method of diagnosing brucellosis. Repeated serologic testing is recommended if the initial titer is low. The standard tube agglutination test (SAT), which tests for anti-O-polysaccharide antibody, is the most widely used serologic test for the confirmation of human brucellosis. The detection of seroconversion or high antibody titers (>or=1/160) are considered diagnostic when found together with a compatible clinical presentation. Titers higher than 1:320 are considered to be more specific, especially in endemic areas. Seroconversion and evolution of the titers can also be used for diagnosis. The 2-mercaptoethanol test detects immunoglobulin G (IgG),

and titers higher than 1:80 define active infection. A high IgG antibody titer or a titer that is higher after treatment suggests persistent infection or relapse. Other tests, such as tray agglutination (TAT) and modified TAT, are also popular. Each serological test has its own advantages and limitations and results require careful interpretation (9). Enzyme-linked immunosorbent assay (ELISA) typically uses the cytoplasmic proteins as antigens and measures specific IgM, IgG and IgA allowing a better interpretation and overcomes the problem of false negatives / positives, which may arise in the SAT (10). If the serum agglutination test result is equivocal, the ELISA test can give a definitive diagnosis (11). Because levels should decrease with effective treatment, ELISA is also helpful in follow-up.

Polymerase chain reaction (PCR) tests have been developed for the detection and rapid diagnosis of *Brucella* species in human Blood cultures and bone marrow aspirates (12). Two major genetic targets are the *Brucella* gene BCSP31 and the 16S-23S rRNA operon. Possible applications would include evaluating cases of relapse and monitoring response to therapy.

Treatment

A combination of pharmaceutical and surgical management is necessary in patients with *Brucella* endocarditis. Despite adequate antibiotic therapy, *Brucella* endocarditis needs surgical intervention in the majority of cases. There are only a few reports of treatment of *Brucella* endocarditis using antibiotics alone (13,14). Studies have shown that patients with short clinical disease duration of less than 2 months and mild cardiovascular involvement without left ventricular failure can be treated with antibiotic therapy only. The main indication for surgical treatment is the existence of significant insufficiency of the affected valve which deteriorates the haemodynamic functions.

The European Society of Clinical Microbiology and Infectious Diseases Guidelines recommend combination therapy with doxycycline, cotrimoxazole and rifampin or streptomycin for at least 3 months duration (15). Reguera et al. reported that the triple combination of doxycycline, cotrimoxazole and streptomycin for the first three weeks, followed by doxycycline and rifampicin for the next three months was successful and avoided surgical intervention (14). In *Brucella* endocarditis, addition of rifampicin has been advocated due to its excellent tissue distribution, high penetration into valvular vegetations, and to avoid streptomycin toxicity (2, 16, and 17). In areas where resistance to rifampacin is high, a combination of a fluoroquinolone and doxycycline can be used. Monotherapy is not recommended due to the high relapse rate (17). Relapses are also seen with short treatment courses (18). Most relapses occur within 3 to 6 months of stopping therapy (19).

Follow-up

A full course of antibiotic treatment is necessary to improve the prognosis. Most patients on appropriate antibiotic treatment recover completely without any complications. However, the relapse rate is approximately 10%, even with appropriate treatment. SAT titre can be used as a marker to follow up patients. Although treatment success is indicated by a *Brucella* antibody titre of < 1:60, this parameter alone cannot be used to determine complete cure.

Conclusion

Increased vigilance is required for the timely and accurate diagnosis of *Brucella* endocarditis. Surgical treatment, with replacement of the affected valve, combined with long term antibiotics is necessary for the successful treatment of *Brucella* endocarditis in most instances. Antibiotics should be continued until the *Brucella* antibody titre and CRP become negative along with clinical improvement.

References

1. Korea Center for Disease Control and Prevention. *CDWR*.2006; **52**:1-36.
2. Gerald E Maloney. A case of Brucella Endocarditis. CBRNE – Brucellosis.Medscape Education Emergency Medicine CME/CE: 10/28/2010.
3. Nikolaos K G, Konstantinos P M, Ioannis G, et al. Unusual Cardiovascular Complications of Brucellosis Presenting in Two Men. *J Med Case Reports* 2011; **5**(1).
4. Leandro J, Roberto H, Antunes M: Brucella endocarditis of the aortic valve. *Eur J Cardio-thoracic Surg* 1998; **13**: 95-7.
5. Pappas G, Akritidis N, Bosilkovski M, Tsianos E: Brucellosis. *N Engl J Med* 2005, **352**: 2325-36.
6. Tasbakan MI, Yamazhan T, Gokengin D, et al. Brucellosis: a retrospective evaluation. *Trop Doct* 2003, **33**: 151-3.
7. Brucellosis, Disease information, technical information. Division of Bacterial and MycoticDiseases.Centers for Disease Control and Prevention 2001; April 27.
8. Olaison L, G¨ostaPettersson G. Current best practices and guidelines: Indications for surgical intervention in infective endocarditis. *CardiolClin*. 2003; **21**: 235-51.
9. Araj GF. Update on laboratory diagnosis of human brucellosis. *Int J Antimicrob Agents* 2010; **36** Suppl 1: S12-7.
10. Alikan H, Bakent. The value of culture and serological ethods in the diagnosis of human brucellosis. *Mikrobiyol Bul* 2008; **42**(1): 185-95.
11. Ariza J. Brucellosis. *CurrOpin Infect Dis* 1996; **9**: 126-31.
12. Mitka S, Anetakis C, Souliou E, et al. Evaluation of different PCR assays for early detection of acute and relapsing brucellosis in humans in comparison with conventional methods. *J ClinMicrobiol* 2007; **45**(4): 1211-8.

13. Memish Z, Mah MW, Al Mahmoud S, et al. *Brucella-bacteraemia*: clinical and laboratory observations in 160 patients. *J Infect*. 2000; **40**: 59-63.

14. Reguera JM, Alarcón A, Miralles F, et al. Brucella endocarditis: clinical, diagnostic, and therapeutic approach. *Eur J ClinMicrobiol Infect Dis*. 2003; **22**: 647-50.

15. Guidelines on the prevention, diagnosis,and treatment of infective endocarditis (new version 2009) Gilbert Habib et al. The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and by the International Society of Chemotherapy (ISC) for Infection and Cancer. *European Heart Journal* 2009; **30**: 2369-413.

16. Baddour LM, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: American Heart Association: endorsed by the Infectious Diseases Society of America. *Circulation* 2005; **14**; 111(23): e394–434. *Circulation* 2007; **17**: 115(15): e408.

17. Brouqui P, Raoult D. Endocarditis Due to Rare and Fastidious Bacteria. *Clinical Microbiology Reviews*, Jan. 2001; 177-207.

18. Sanchez-Tamayo T, et al. Failure of short-term antimicrobial therapy in childhood brucellosis. *Pediatr Infect Dis J* 1997; **16**: 323-4.

19. Ariza J, et al. Characteristics of and risk factors for relapse of brucellosis in humans. *Clin Infect Dis* 1995; **20**:1241-9.

A THALASSAEMIA PATIENT WITH A LIVER ABSCESS

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Case report

A nine year old girl with a thalassaemia major underwent routine ultra sound scan of abdomen in April 2013 at the local hospital. It revealed an ill-defined hypoechoic mass in the liver with mild hepatosplenomegaly. Repeat scan confirmed the findings but as the lesion was non-progressive no intervention was done.

Three months later she presented with an intermittent central abdominal pain. There were no other symptoms or signs apart from mild hepatomegaly. CT scan of abdomen showed multiple, ill defined, hypo-dense areas in right lobe of liver and spleen which were suggestive of haemangiomas. She was referred to the Lady Ridgeway Hospital and the second CT scan confirmed the previous findings.Her routine investigations showed a WBC count of 13600 /mm^3 with 83% neutrophils and a CRP of 6.9 mg/dl.

Later she developed a rapidly enlarging subcutaneous lump over the hypochondriac region. Aspiration revealed frank pus. The subcutaneous abscess was recognized to be an extension of the liver abscess which initially was considered to be haemangiomas.

Gram stain of pus showed Gram negative bacilli with pus cells. Direct culture revealed no growth. Enrichment culture after 48hrs of incubation grew small colonies on blood agar which became dry and wrinkled on further incubation.Colonies were pinkish on MacConkey agar. Gram stain showed typical safety pin appearance. The isolate was identified as *Burkholderia pseudomallei* with biochemical tests and antibiotic sensitivity. Isolate was sensitive to ceftazidime, imipenem, meropenem, ciprofloxacin and cotrimoxazole. Titre of antibodies against *B. pseudomallei* was 1: 2800.

Patient was treated with IV imipenem and oral cotrimoxazole. Surgical drainage was done. As repeated drain fluid cultures were positive, IV ceftazidime infusion was added. Combination therapy was continued for one month. The CRP levels came down from 44 to 7.1 mg/dl after 4 weeks.Patient was discharged with oral cotrimoxazole 480mg twice daily for twelve weeks.

Discussion

Melioidosis is an infection caused by *Burkholderia pseudomallei*, a Gram negative, oxidase positive, motile non- fermenting bacilli. Organism is a saprophyte which exists in moist soil. Direct inoculation is the major mode of infection. Other modes are inhalation, ingestion and person to person transmission. The disease is endemic in South-East Asia and Australia (1,3).

Persons who are exposed to surface water and mud, especially those who work in rice paddies, at construction

sites and regular bathing in water tanks are at high risk. This child uses a water tank for bathing. Patient is a known thalassaemic and it is a risk factor for Melioidosis. Other predisposing factors are diabetes, alcohol excess, renal disease, chronic lung disease, malignancies, steroid therapy, iron overload, and tuberculosis (2).

The clinical spectrum of the disease varies from asymptomatic infection, localized skin ulcers or abscesses, pneumonia which resembles tuberculosis, septicaemia and septic shock with multiple abscesses in internal organs. Pneumonia is the commonest clinical presentation. Abscesses in internal organs, especially spleen, kidney, prostate, and liver are also frequent presentations (1).

Laboratory diagnosis requires isolation of *Burkholderia pseudomallei*. It grows in the basic culture media. Safety pin appearance in Gram stain, oxidase reaction, wrinkled pinkish non lactose fermenting colonies, and antibiotic sensitivity pattern with resistance to aminoglycosides and polymyxin and sensitivity to cotrimoxazole gives a presumptive clue for the identification.Both commercial API 20NE or 20E biochemical kit can be used in combination for further identification (4). Detection of antigen, antibody or DNA can be performed although they are not widely available. This patient's antibody levels were determined by an indirect haemagglutination test.
The treatment of this child consisted of four weeks of intensive treatment of IV imipenem, IV ceftazidime and oral cotrimoxazole followed by eradication therapy with oral cotrimoxazole. The longer duration of intensive therapy was given as the repeated samples taken from the drain became positive for the organism. Ceftazidime and imipenem are the drugs of choice for initial intensive therapy for melioidosis with cabapenems carrying the lowest MIC levels. These can be used in combination with cotrimoxazole as it has an excellent tissue penetration. Ceftazidime continuous infusions have been used successfully to shorten the hospital stay (2).

References

1. Dhodapkar R, Sujatha S, Sivasangeetha K, Prasanth G, Parija SC. *Burkholderia pseudomallei* infection in a patient with diabetes presenting with multiple splenic abscesses and abscess in the foot: A case report *Cases Journal* 2008; **1**: 224.
2. Wiersinga WJ, Currie BJ, Peacock SJ. Melioidosis. *The New England Journal of Medicine. 2012*; **367**: 1035-44.
3. Northfield J, Whitty CJM, MacPhee IAM. *Burkholderia pseudomallei* infection, or melioidosis, and nephrotic syndrome. *Nephrology, Dialysis and Transplantation.* 2002; **17**: 137-9.
4. Currie BJ. (2010). *Burkholderia pseudomallei* and *Burkholderia mallei*: Melioidosis and Glanders *Mandell, Douglas, and Bennett's Principles and Practice of I nfectious Diseases* (7th ed., Vol. 2, pp. 1399-1412). Philadelphia: Elsevier.

PHOTO QUIZ

A 9 month old female child from the North Central Province of Sri Lanka presented with a lump (1.5 cm x 1.5 cm) in the subcutaneous tissue of the abdominal wall. The mother had noticed a firm swelling for several days. The skin overlying the swelling was intact and showed no blister. No other subcutaneous lumps were present. There was no lymphadenopathy. Examination of the peripheral blood did not show eosinophilia. The clinical diagnosis was a lipoma. The lesion was excised and sent for histopathology. The specimen received was a pale grey nodular piece of tissue measuring 20 x 20 x 18 mm. Cut surface showed a central cavity containing a ball of worms. The specimen was routinely processed and 4-5 µm thick sections were obtained and stained with H and E.

Figure 1 shows the worms retrieved from the biopsy tissue and Figure 2 shows a section stained with H & E (x400).

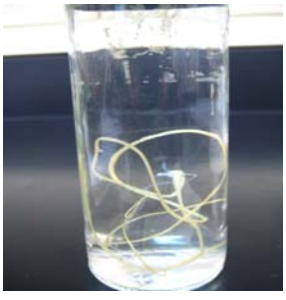

Figure 1.

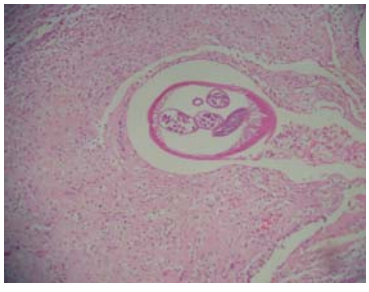

Figure 2.

Question 1. What is the possible diagnosis?

Question 2. How would you manage this patient?

Answers will be found on page 46

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FIRST ISOLATION OF *CRYPTOCOCCUS NEOFORMANS* IN BLOOD CULTURE IN SRI LANKA

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Introduction

Cryptococcus neoformans is a basidiomycetous yeast with a polysaccharide capsule. It has been isolated especially from soil contaminated with pigeon droppings, decaying wood in tree trunk hollows and fruit and vegetables (1).

The main portal of entry is the respiratory tract, and in most instances, the infection is subclinical and self-limiting. However, acute manifestations and life threatening infections are more common in immuno suppressed patients (AIDS, individuals receiving immunosuppressive therapy being treated for lymphoreticular malignancies) (2).

We describe a case of disseminated cryptococcosis in a renal transplant recipient manifesting as lower limb cellulitis and abscess formation, which was the only signet presentation.

A 36 year old woman – 16 years post-transplant – presented with fever and severe right knee pain of 3 days duration. She has been diagnosed with end-stage renal disease of uncertain etiology and had live-unrelated kidney transplantation in India in 1992. She was referred to our transplant clinic in 1996 by which time she was only on azothiaprine75 mg/day and prednisolone 10 mg as immune-suppressive medications. She has been on cyclosporine up to 2 years post-transplant and tapered off.In 2007 (15 years post-transplant), serum creatinine had risen up to 2 mg/dl with proteinuria of 1100 mg/24 hours. A clinical diagnosis of chronic allograft nephropathy was made and she was started on mycophenolate mofetil 750 mg/day after stopping azothiaprine continued for 3 months.

On examination she was febrile, tachypnoeic with a respiratory rate of 18/minute. A warm, tender and fluctuant lump was found on the posterior aspect of her right knee and she denied any injury. Respiratory and central nervous system examination was normal. Laboratory investigations revealed an ESR of 64 mm, WBC- 18,000, neutrophil count 80%, CRP- 16, serum creatinine-2.8 mg/dl, urea-142 mg/dl, slightly elevated liver transaminases and normal alkaline phosphatase and serum bilirubin. Chest radiograph was normal.

The lump was drained and pus was sent for microbiological work-up. Initially co-amoxiclav sensitive coliforms were isolated. However, her fever continued despite the antibiotics and on the third day of admission she noticed a new tender and warm lump on her right shoulder. This too was aspirated and *Cryptococcus neoformans* was isolated from both sites as well as from a blood culture taken after the appearance of secondary lesions. She refused to undergo a lumbar puncture for analysis of CSF.

She was started on fluconazole, 400mg on the 1st day followed by 200 mg per day for 3 weeks. Her fever subsided 6 days after commencement of anti-fungal therapy and her blood cultures were negative subsequently. The incision site needed prolonged surgical care including a skin graft. She was continued on a dose of 150mg/day of fluconazole as suppressive therapy.

Discussion

Cryptococcus neoformans has been shown to cause different cutaneous manifestations. Primary cutaneous cryptococcosis (PCC) is a distinct clinical entity where skin lesions are confined to a circumscribed body region with a positive cryptococcal culture with no evidence of dissemination. Primary cutaneous cryptococcosis (PCC) occur both in immunocompetent as well as immunocompromised individuals and preceding trauma is a commonly associated risk factor in both groups (2).

Cutaneous manifestations occur in upto 15% of patients with disseminated cryptococcosis and the lesions can be pustules, granulomata, abscesses, herpetiform or molluscum contagiosum-like lesions and cellulitis (3). Our patient presented with a solitary abscess with cellulitis in the lower limb which was initially treated with antibiotics. Secondary lesions developed subsequently on the upper limb.She may have been in an early phase of a disseminated disease at presentation since she was also systemically unwell. Skin lesions such as cellulitis have been described before other manifestations of systemic cryptococcosis (4).

On microscopic examination of the pus from the abscess as well as fine needle aspiration of secondary lesions, capsulated budding yeasts were seen. Features such as the shape of the yeast and the capsule prompted the probable diagnosis and a blood culture was performed to detect fungemia.To the best of our knowledge, this is the first time a *Cryptococcus spp*. has been isolated from a blood culture in Sri Lanka.

The yeast was later confirmed by culture and carbon assimilation tests to be *Cryptococcus neoformans,* serovar was not established.Recommended treatment of non meningeal cryptococcosis in non HIV patients is fluconazole for a less severe illness, and amphotericin B followed by fluconazole for a more severe illness (5). Our patient, even though fungemic responded well to fluconazole.

References

1. Lazera MS, Salmito Cavalcanti MA, Londero AT, Trilles L, Nishikawa MM, et al. Possible primary ecological niche of *Cryptococcus neoformans*. *Medical Mycology* 2000; **38**: 379-83.
2. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS–100 years after the discovery of *Cryptococcus neoformans*. *Clinical Microbiology Reviews* 1995; **8**: 515-48.
3. Gloster HM, Swerlick RA, Solomon AR. Cryptococcal cellulitis in a diabetic, kidney transplant patient. *Journal of the American Academy of Dermatology* 1994; **30**: 1025-26.
4. Schupbach CWCE, wheeler RA, Briggaman NA, Warner, Kanof EP. Cutaneous manifestations of Disseminated cryptococcosis. *Archives of Dermatology* 1976; **112**: 1734-40.
5. The Sanford Guide to Antimicrobial Therapy 39th Edition. 2009.

Answers to the photo quiz

The section shows a fragmented parasite surrounded by a thick laminated cuticle with fine external longitudinal ridges. Underneath the cuticle, a thick circumferential muscular layer interrupted by two lateral cords was seen. Some sections showed portions of the intestine as well as the uterine cavity. These features are consistent with ***Dirofilaria repens***. The parasite was in a cavity with a fibrous wall containing an inflammatory infiltrate comprising lymphocytes, plasma cells and eosinophils.

Patients usually present with single migratory nodule which may or may not be tender. Surgical removal of the worm or the lesion is the treatment of choice. Most cases are diagnosed retrospectively, when the histopathological sections of biopsy or excision material are viewed. There is no need for chemotherapy as microfilaraemia is extremely rare.

Note from the editorial board

The titles of articles, names and affiliations of authors are published as it has been submitted to the Sri Lanka College of Microbiologists by the principal or corresponding authors. Editorial board is not responsible for the typographical or any other errors.

ERRATUM

The names of authors of the following research article published in the 2013 *Bulletin of the Sri Lanka College of Microbiologists* (Volume 11 Issue 1 July 2013 ISSN 1391-930X, page 44) should be read as follows.

Prevalence, risk factors and clinical outcome of bacteremia caused by extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae in a District General Hospital in Sri Lanka

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Editor regrets any inconvenience caused by this error.

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