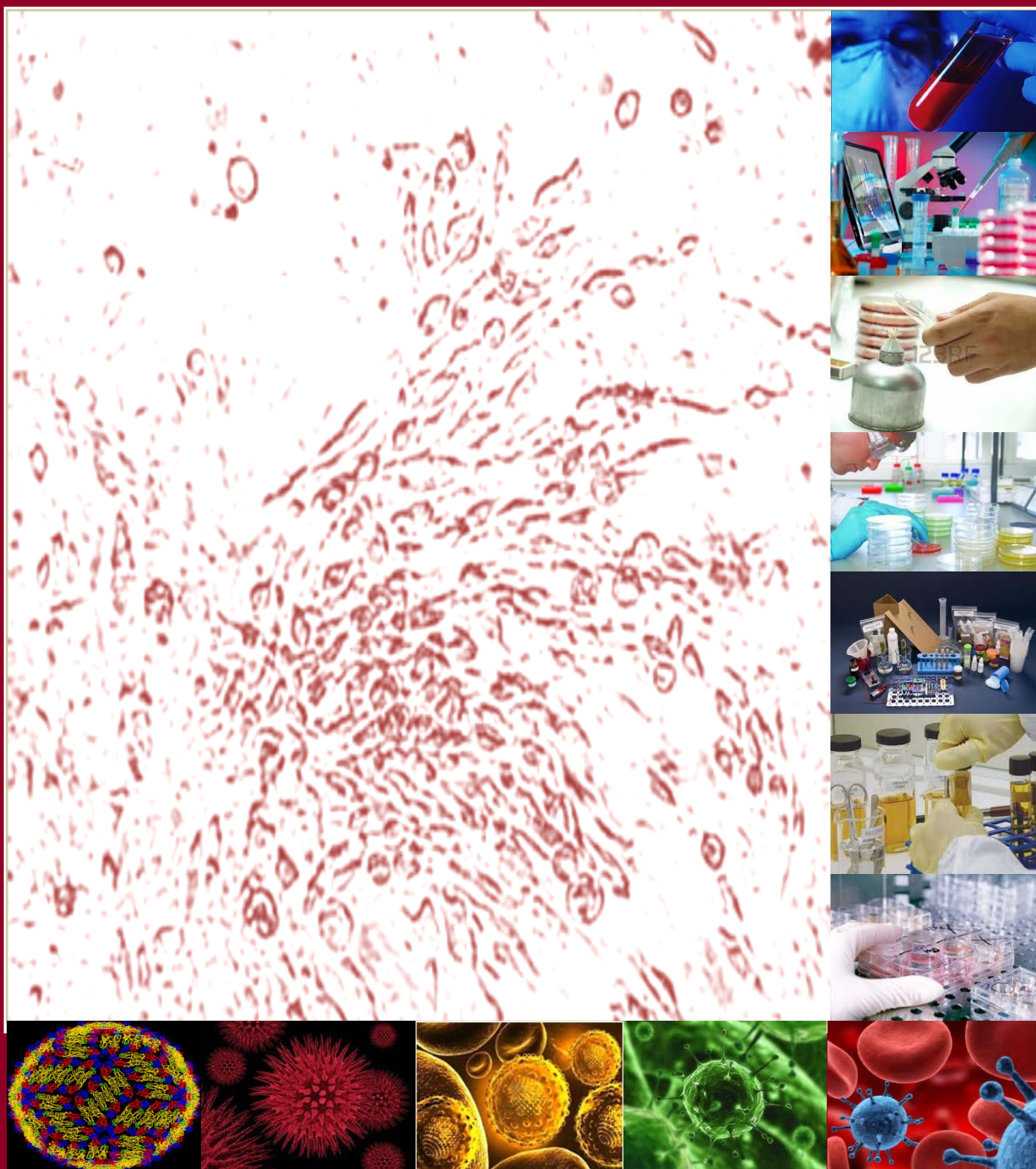




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The Sri Lanka College of Microbiologists

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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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22nd Annual Scientific Sessions



The Sri Lanka College of Microbiologists

Inauguration

24th July 2013 at 6.15 pm

Sri Lanka Foundation

Colombo 7

Pre-Congress Workshop

“Rational use of Antibiotics - Need of the hour”

24th July 2013

Scientific Programme

“Emerging infections - meeting the challenges”

25th & 26th July 2013

Aldo Castellani Auditorium

Medical Research Institute, Colombo 8

MESSAGE FROM THE PRESIDENT



It is with great pleasure I forward this message at the event of Annual Scientific Sessions of the Sri Lanka College of Microbiologists.

From the inception, Sri Lanka College of Microbiologists has contributed immensely towards the enhancement of the microbiological services in Sri Lanka and become an important contributor to the health care of this country. Revision of Laboratory manual, training of health personnel, development of antibiotic guidelines with collaboration of other professional colleges are some of the recent activities carried out for the betterment of health care in this country.

In keeping with the current situation of emerging and re-emerging infectious diseases the college has chosen "Facing the Challenges of an Era of Emerging Infectious Diseases" as the theme for this academic year, which will be the theme for the Scientific Sessions also. With participation of foreign and local delegates I am sure that the sessions will be interesting and informative.

I extend my best wishes for the 22nd Annual Scientific Sessions of the Sri Lanka College of Microbiologists.

Dr. Sunethra Gunasena

President

Sri Lanka College of Microbiologists

MESSAGE FROM SECRETARIES

Dear Colleagues,

It gives us great pleasure to welcome you to the 22nd Annual Scientific Sessions of the Sri Lanka College of Microbiologists. This year's conference consists of a Pre Congress Workshop and a two day scientific session. The Scientific Session is focused on the timely theme of "Emerging infections; meeting the challenges". This conference has a wide array of subject areas which is relevant to the evolving field of microbiology. Experts from both the local and overseas faculties will share their knowledge and experience at this conference. The sessions will help you to gather new knowledge in the field of clinical microbiology. Furthermore it gives the budding microbiologists a platform to showcase their research interests.

Emerging infections are a constant challenge to the global population threatening our survival. While we are increasingly facing a broader spectrum of new killer infections, old microbes are evolving into more resistant strains. "Super bugs" are now rapidly invading our local hospitals and becoming endemic. The antibiotics which are available to combat these multi resistant microbes are limited in number. Hence it is our responsibility to strengthen the preventive measures while saving the antibiotics for the future.

We take this opportunity to express our heartfelt gratitude to the organizing committee and the college office secretary for their tireless efforts to make this event a success.

Finally we wish all participants a constructive and memorable scientific session.

Hony. Joint Secretaries



Dr. Malika Karunaratne



Dr. Bhagya Piyasiri

MESSAGE FROM THE CHIEF GUEST



I am indeed privileged to send a message as the Director General of Health Services on the occasion of the 22nd Annual Scientific Sessions of the Sri Lanka College of Microbiologists.

Since its inception the Sri Lanka College of Microbiologists has contributed immensely towards the strengthening of the microbiological services in Sri Lanka. Public health sector in particular.

I am pleased to note that the specialists in different disciplines of microbiology have taken many initiatives to introduce new technological advancements to Sri Lanka.

I understand that the College has chosen the theme “Emerging Infections - Meeting the Challenges” for the 22nd Annual Scientific Sessions. In the backdrop of outbreaks of influenza, Middle Eastern Respiratory Syndrome Corona virus and emergence of multi-drug resistance in bacteria, I think the theme chosen is extremely appropriate.

I believe it is very important that not only the clinicians but also the policy makers, administrators, epidemiologists, microbiologists, virologists and immunologists should also be ready to meet challenges in the diagnosis, management, prevention and control of infectious diseases.

I admire the President and the Council of the College for taking timely efforts to enlighten all these stakeholders on this global health challenge. I wish the 22nd Annual Scientific Sessions of the Sri Lanka College of Microbiologists every success and would like to pledge my fullest support for the future activities of the College.

Dr. P G Mahipala

Director General of Health Services

Ministry of Health

Sri Lanka

INAUGURATION PROGRAMME

24 - 07 - 2013 – Sri Lanka Foundation, Colombo 7

- 6.15 pm Invitees take their seats
- 6.30 pm Arrival of the Chief Guest
Introduction of Members of the Council
- 6.35 pm Ceremonial Procession
- 6.40 pm National Anthem
- 6.45 pm Traditional lighting of the Lamp
- 6.50 pm Welcome Address
Dr. Malika Karunaratne
Hony. Joint Secretary
- 6.55 pm Address by the Chief Guest
Dr. P. G. Maheepala
Director General of Health Services, Ministry of Health
- 7.15 pm Address by the President
Dr. Sunethra Gunasena
Consultant Virologist, MRI
- 7.45 pm Introduction of the Orator
Siri Wickremesinghe Memorial Oration 2013
Dr. Sunethra Gunasena, President, SLCM
- 7.50 pm Siri Wickremesinghe Memorial Oration 2013
Dr. Geethani Wickramasinghe
Consultant Virologist
- 8.20 pm Vote of Thanks
Dr. Bhagya Piyasiri
Hony. Joint Secretary
- 8.25 pm Ceremonial Procession leaves
- 8.30 pm Reception

PRE-CONGRESS WORKSHOP PROGRAMME

22nd Annual Scientific Sessions of The Sri Lanka College of Microbiologists

- Theme -

“Rational use of Antibiotics – Need of the hour”

24th July 2013

Pre congress workshop – programme

9.00 - 9.30 am	Registration
9.30 - 10.15 am	Rational use of antibiotics; Challenges in developing countries Dr. Rathna Rao <i>Consultant Microbiologist, Apollo Hospital, India</i>
10.15 - 10.45 am	National antibiotic policy and guidelines: Where are we now? Dr. Kumudu Karunaratne <i>Consultant Microbiologist, Lady Ridgeway Hospital, Colombo 8</i>
10.45 - 11.00 am	Tea
11.00 - 11.45 am	Emerging antibiotic resistance: The global perspective Dr. Andrew Swann <i>Consultant Microbiologist, University Hospitals of Leicester NHS Trust, United Kingdom</i>
11.45-12.15 pm	Antibiotic resistance surveillance project of Sri Lanka Dr. Jayanthi Elwitigala <i>Consultant Microbiologist, National STD and Aids Control Programme, Colombo</i>
12.15 - 12.30 pm	Discussion Chairpersons: Prof. Nelun De Silva and Prof. Jennifer Perera



**22nd Annual Scientific Sessions of
The Sri Lanka College of Microbiologists**

- Theme -

“Emerging infections - meeting the challenges”

25th & 26th July 2013

Scientific Programme - Day 1 - 25th July 2013

8.30 - 9.00 am **Registration**

9.00 - 9.30 am **Free paper session - 1**

Chairpersons: Dr. Samanmalee Gunasekara and Dr. Dhammika Vidanagama

OP 1 A study of residual viable bio burden in reprocessed side-view endoscopes used for Endoscopic Retrograde Cholangiopancreatography (ERCP) in a clinical setting
Ubhayawardana DLNL¹, Kottahachchi J¹, Weerasekera MM¹, Wanigasooriya IWMP³, Damayanthi KWN¹, De Silva M², Fernando SSN¹
¹Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, ²Department of Surgery, Faculty of Medical Sciences, University of Sri Jayewardenepura, ³Endo-therapy Unit, Colombo South Teaching Hospital

OP 2 Comparison of clinical criteria and laboratory criteria used for the diagnosis of bacterial vaginosis
Mendis KHC¹, Dassanayake KMMP², Kasthurirathne A³, Ginige S⁴
¹Postgraduate Institute of Medicine, Colombo, ²North Colombo Teaching Hospital, Ragama, ³Faculty of Medicine, Ragama, ⁴Epidemiology Unit, Colombo

9.30 - 10.15 am **Plenary 1 - Viral encephalitis - An update**

Prof. Ravi Vasanthapuram

Professor of Neurovirology, National Institute of Mental Health and Neuro Sciences, India

Chairperson: Dr. Omala Wimalaratne

10.15 - 10.30 am **Tea**

10.30 - 11.30 am **Free paper session - 2**

Chairpersons: Dr. Ajith Nagahawatta and Dr. Mahen Kothalawala

OP 3 Surgical site infections and *Staphylococcus aureus* colonization in renal transplant patients
Karunanayake L¹, Hapuarachchi CT², Harischandra P K¹, Rambukwella IWUB¹, Tshokey¹
¹Teaching Hospital, Kandy, ²Medical Research Institute, Colombo 08

SCIENTIFIC PROGRAMME

- OP 4 Study of prevalence and risk factors for MRSA colonization in patients presenting to the orthopaedic unit in Teaching Hospital, Peradeniya
Yapa TLG, Ekanayake A, Thevanesam V
Department of Microbiology, Faculty of Medicine, University of Peradeniya
- OP 5 Detecting bacterial pathogens causing infections in neonates admitted to the Neonatal Intensive Care Unit (NICU) at a tertiary care hospital in Southern province of Sri Lanka
Weerasinghe NP¹, Vidanagama DS¹, Perera B²
¹Department of Microbiology, Teaching Hospital, Karapitiya, Galle, ²Department of Community Medicine, Faculty of Medicine, Karapitiya, Galle
- OP 6 Bacterial pathogens in infections of bone complicated by trauma or chronic wounds
Jayawardhana JMDD¹, Herath HMC¹, Chandrasiri NS², Ransimali LGHN¹, Sakey Ali MTM¹
¹General Hospital, Ampara, ²Colombo South Teaching Hospital, Kalubowila.
- 11.30 - 12.30 pm **Symposium 1 - Infections in Trauma**
Moderators: Dr. Malka Dassanayake and Dr. Shirani Chandrasiri

Critical care management - Role of the anaesthetist
Dr. Shirani Hapuarachchi
Consultant Anaesthetist, National Hospital of Sri Lanka, Colombo 10

Microbiological nightmare
Dr. Geethika Patabendige
Consultant Microbiologist, National Hospital of Sri Lanka, Colombo 10
- 12.30 - 1.30 pm **Lunch**
- 1.30 - 2.15 pm **Plenary 2**
Atypical pathogens in pneumonia: Challenges in management
Dr. Andrew Swann
Consultant Microbiologist, University Hospitals of Leicester NHS Trust, UK

Chairperson: Dr. Philomena Chandrasiri
- 2.15 - 3.00 pm **Free paper session - 3**
Chairpersons: Dr. Geethika Patabendige and Dr. Lilani Karunanayake
- OP 7 The incidence of hospital acquired infections among ICU patients at a tertiary care hospital in Southern province of Sri Lanka
Lewkebandara RH¹, Vidanagama DS¹, Nagahawatta A²
¹Teaching Hospital, Karapitiya, Galle, ²Faculty of Medicine, Karapitiya, Galle
- OP 8 Incidence, antibiotic susceptibility pattern and factors associated with ventilator associated pneumonia in patients in Intensive Care Units in Ratnapura district
Wimalaratne KBD¹, Nanayakkara GM¹, Illangasinghe TDB²
¹Provincial General Hospital Ratnapura, ²Teaching Hospital Anuradhapura
- OP 9 Retrospective study of blood culture positives in a special care baby unit (SCBU) in a General Hospital in Sri Lanka
Piyasiri DLB, Edirisooriya R, Kulathilaka HRAK, Wijesundara WMSK, Bodhipaksha BRS
General Hospital Polonnaruwa

SCIENTIFIC PROGRAMME

- 3.00 - 3.45 pm **Plenary 3 - Leishmaniasis in tropical regions**
Prof. Nadeera Karunaweera
 Professor of Parasitology, Faculty of Medicine,
 University of Colombo
 Chairperson: Prof. Deepika Fernando
- 3.45 - 4.15 pm **Free paper session - 4**
 Chairpersons: Dr. Geetha Nanayakkara and Dr. Sanath Senanayake
- OP 10 Randomized placebo-controlled trial of the efficacy of mebendazole polymorphs in the treatment of hookworm infections
Gunawardena NK, Kumarendran B, Manamperi NH, Senarathna BP, Silva M, Pathmeswaran A, De Silva NR
 Departments of Parasitology and Public Health, Faculty of Medicine, University of Kelaniya
- OP 11 Prevalence of *Helicobacter pylori* infection in a sample of patients with dyspeptic symptoms and gastric antral inflammation
Buharideen SM¹, Kotakadeniya HMSRB², Galketiya KB², Samarasinghe AKBBTB², Peiris SPM², Dharmapala A², Noordeen F³, Tennakoon TRDSK¹, Wickramasingha SM¹, Wijetunge S¹
¹Department of Pathology, Faculty of Medicine, University of Peradeniya, ²Department of Surgery, Faculty of Medicine, University of Peradeniya, ³Department of Microbiology, Faculty of Medicine, University of Peradeniya
- 4.15 pm **Tea**

Scientific Programme - Day 2 - 26th July 2013

- 9.15 - 10.00 am **Free paper session - 5**
 Chairpersons: Dr. Geethani Galagoda and Dr. Primali Jayasekara
- OP 12 Proportion of fungal foot infections in patients with type 2 diabetes at a tertiary care hospital
Wijesuriya TM¹, Weerasekara MM¹, Kottahachchi J¹, Dissanayake MSS¹, Prathapan S¹, Gunasekera TDCP¹, Nagahawatte A³, Guruge LD¹, Bulugahapitiya U², Fernando SSN¹
¹Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, ²Diabetic Clinic, Colombo South Teaching Hospital, Kalubowila, ³Department of Microbiology, Faculty of Medicine, University of Ruhuna, Karapitiya.
- OP 13 Development of recombinant protein antigens using a bacterial expression system for the detection of anti-Chikungunya (CHIK) antibodies
Athapaththu AMMH¹, Khanna N², Inouye S³, Gunasena S⁴, Abeyewickreme W¹, Hapugoda M¹
¹Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, ²International Centre for Genetic Engineering and Biotechnology, New Delhi, India, ³WHO Collaborative Centre for Viral Reference and Research, Institute of Tropical Medicine, Nagasaki University, Japan, ⁴Department of Virology, Medical Research Institute, Colombo 8.

SCIENTIFIC PROGRAMME

- OP 14 Molecular evidence of hantavirus infection among clinically suspected patients with haemorrhagic fever with renal syndrome (HFRS).
Muthugala, MARV¹, Manamperi AAPs², Gunasena S³, Hapugoda MD², Göran Butch⁴
¹Post Graduate Institute of Medicine, University of Colombo, ²Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Ragama, ³Department of Virology, Medical Research Institute, Colombo 08, ⁴Swedish Defence Research Agency, CBRN Defence and Security, SE-901 82, Umeå, Sweden.
- 10.00 - 10.45 am **Plenary 4**
Emerging and re-emerging flaviviruses and their effect on dengue virus immunology
Dr. Neelika Malavige
Senior Lecturer and Immunologist, Director, Centre for Dengue Research, Dept. of Microbiology, Faculty of Medical Sciences, University of Sri Jayawardanapura

Chairperson: Dr. Sunethra Gunasena
- 10.45 - 11.00 am **Tea**
- 11.00 - 11.30 am **Free paper session - 6**

Chairpersons: Dr. Jayanthi Elwitigala and Dr. Janani Kottahachchi
- OP 15 Enhancing the sensitivity of methicillin-resistant *Staphylococcus aureus* (MRSA) to oxacillin by tea catechins and proanthocyanidins
Mediawake SS², Bandara BMR¹, Thevanesam V², Ekanayake A²
¹Department of Chemistry, Faculty of Science, University of Peradeniya, ²Department of Microbiology, Faculty of Medicine, University of Peradeniya
- OP 16 The prevalence of urinary tract infections in post renal transplant patients within the first six months of the post-transplant period
Karunanayake L¹, Harischandra P K¹, Hapuarachchi CT², Tshokey¹, Rambukwella IWUB¹
¹Teaching Hospital, Kandy, ²Medical Research Institute, Colombo 08
- 11.30 - 12.45 pm **Symposium 2 - Management of renal transplant patients**

Moderators: Prof. Vasanthi Thevanesam and Prof. N. P. Sunil-Chandra

Infective complications in renal transplant
Dr. Chula Herath
Consultant Nephrologist, General Hospital Sri Jayawardhenapura, Nugegoda

Diagnosis of opportunistic infections in post renal transplant patients
Dr. Rathna Rao
Consultant Microbiologist, Apollo Hospital, India

SCIENTIFIC PROGRAMME

	Infection prevention in renal transplantation <i>Dr. Kushlani Jayathilleke</i> Consultant Microbiologist, General Hospital Sri Jayawardhenapura, Nugegoda
12.45 - 1.45 pm	Lunch
1.45 - 2.30 pm	Symposium 3 - Quality Assurance in Microbiology: A Global Requirement Moderators: Dr. Pranitha Somaratne and Dr. Kushlani Jayathilleke Standards in Quality Assurance <i>Dr. Vio Peiris</i> Consultant Microbiologist and Clinical Director for Pathology, Hull & East Yorkshire Hospitals NHS Trust, UK Limitations in practice <i>Dr. Lilani Karunanayake</i> Consultant Microbiologist, Medical Research Institute, Colombo
2.30 - 3.15 pm	Plenary 5 - Patients at high risk of invasive fungal infections: When and How to treat? <i>Dr. Preethi Perera</i> Consultant Mycologist Chairperson: Dr. Maya Atapattu
3.15 - 3.45 pm	Interactive session - Laboratory safety <i>Dr. Sunethra Gunasena</i> Consultant Virologist, Medical Research Institute, Colombo <i>Dr. Geethani Galagoda</i> Consultant Virologist, Medical Research Institute, Colombo
3.45 - 4.15 pm	Award ceremony
4.15 pm	Tea

LIST OF GUEST SPEAKERS

Dr. Andrew Swann

Consultant Microbiologist, University Hospitals of Leicester NHS Trust, UK

Dr. Viv Peiris

Consultant Microbiologist and Clinical Director for Pathology, Hull & East Yorkshire Hospitals NHS Trust, UK

Prof. Ravi Vasanthapuram

Professor of Neurovirology, National Institute of Mental Health and Neuro Sciences, India

Dr. Rathna Rao

Consultant Microbiologist, Apollo Hospital, India

Dr. Chula Herath

Consultant Nephrologist, General Hospital Sri Jayawardenapura, Nugegoda

Dr. Shirani Hapuarachchi

Consultant Anaesthetist, National Hospital of Sri Lanka, Colombo

Dr. Neelika Malavige

Senior Lecturer and Immunologist, Director, Centre for Dengue Research, Dept. of Microbiology,
Faculty of Medical Sciences, University of Sri Jayawardanapura

Dr. Preethi Perera

Consultant Mycologist

Prof. Nadeera Karunaweera

Professor of Parasitology, Faculty of Medicine, University of Colombo

Dr. Kumudu Karunaratne

Consultant Microbiologist, Lady Ridgeway Hospital, Colombo

Dr. Sunethra Gunasena

Consultant Virologist, Medical Research Institute, Colombo

Dr. Geethani Galagoda

Consultant Virologist, Medical Research Institute, Colombo

Dr. Geethika Patabendige

Consultant Microbiologist, National Hospital of Sri Lanka, Colombo

Dr. Kushlani Jayathilleke

Consultant Microbiologist, General Hospital Sri Jayawardenapura, Nugegoda

Dr. Jayanthi Elwitigala

Consultant Microbiologist, National STD and Aids Control Programme, Colombo

Dr. Lilani Karunanayake

Consultant Microbiologist, Medical Research Institute, Colombo

Diagnosis of opportunistic infections in post renal transplant patients

Dr. Ratna Rao

Convenor, Infection Prevention and Control Committee, Sr.Consultant, Head of Department, Microbiology, Apollo Hospital, Hyderabad, India



In the era of advancing medical facility, the span of life has increased so has the number of patients with chronic disease leading to end organ failure. Renal transplant has become a valid therapeutic option in the management of end-stage renal failure. Opportunistic infections are a frequent life-threatening complication of transplantation. Opportunistic infections – bacterial, viral, fungal, parasitic have grown in clinical importance as major cause of infections in immunosuppressed renal transplant recipients, forcing clinicians to reconsider diagnostic and management strategies. With the advent of molecular technique, upgradation of microbiology laboratory and better understanding of the aetiological agents the diagnostic armamentarium has improved drastically over the years.

Rational use of antibiotics – Challenges in developing countries?

Dr. Ratna Rao

Convenor, Infection Prevention and Control Committee, Sr.Consultant, Head of Department, Microbiology, Apollo Hospital, Hyderabad, India

Rational use of antibiotics refers to the correct, proper and appropriate use of the drug. Rational use requires that patients receive the appropriate medicine, in the right dose, for an adequate period of time. More than 50% of all antibiotics are prescribed, dispensed or sold inappropriately, and half of all patients fail to take it correctly. The overuse (too many combination at a time, injections when oral drug choice available), underuse (inappropriate dose, miss one dose, self medication) or misuse (antibiotic for viral sore throat, viral diarrhoea) of antibiotics has resulted in antibiotic resistance organisms.

More than 50% of all countries do not implement basic policies to promote rational use of antibiotics. Multiple factors such as lack of central health agency directive of mandatory reporting of resistance pattern, antibiotic prescribing policy, surveillance data collection expertise, upgradation of pharmacology knowledge of doctors have

contributed to the development of multi drug resistant bacteria.

In developing countries, less than 40% of patients in the public sector and 30% in the private sector are treated according to clinical guidelines. A combination of an active central health agency, surveillance network, mandatory reporting of healthcare associated infections, health-care provider education and supervision, consumer education will be effective in improving use while any of these interventions alone will have limited impact.

National antibiotic guidelines – Where are we now?

Dr. Kumudu Karunaratne

Consultant Microbiologist, Lady Ridgeway Hospital, Colombo 8



Prudent antibiotic prescribing is required to provide effective treatment and prophylaxis, avoid toxicity, avoid emergence of resistance of organisms and to keep the costs down. Under the Health sector development project of the Ministry of Health, several years ago some of the professional colleges developed guidelines on management of infections in which antimicrobial therapy too was included.

Presently at many forums of medical professionals, the need of antibiotic guidelines for therapy is being highlighted. The alarming pattern of antibiotic resistance observed in local settings further supplemented the need for availability of practice guidelines leading to development of an antibiotic policy.

Therefore developing of national guidelines for antimicrobial therapy with the final goal of implementing a policy was recognized by the Council of Sri Lanka College of Microbiologists under the presidency of Dr. Philomena Chandrasiri in year 2011/2012. A decision was taken to develop guidelines for empirical therapy. To achieve this goal the participation and contribution of all relevant professional colleges need appreciation.

The topics were confirmed at a meeting where all relevant professional colleges were invited. The draft guidelines developed and discussed by SLCM members were subsequently discussed with the nominees from relevant professional colleges. Currently we are at the stage of sending these draft documents developed by relevant professional colleges/associations to the professional colleges for comments by other members. Each finalized guideline will be circulated by the Ministry of Health and final document is expected to be printed as a booklet.

Emerging antibiotic resistance: the global perspective

Dr. Andrew Swann

Consultant Microbiologist,
University Hospitals of Leicester
NHS Trust, United Kingdom



For some time the inexorable rise in antibiotic resistance has concerned scientists, clinicians and the pharmaceutical industry. More recently we have seen individual countries, their governments and international organisations call for concerted action to try and turn back the tide. This presentation will review the current situation around the world with regard to resistance patterns focussing principally on bacterial pathogens. Particular concerns are the emergence of multi- and pan-resistant Gram-negative rods including *Escherichia coli*, *Klebsiella* spp., *Acinetobacter* spp. and pseudomonads. Transmissible mechanisms include the extended-spectrum and metallo- β -lactamases, carbapenemases. Increasing resistance in salmonella, shigella, meningococcal and gonococcal infections is also of concern. On the Gram-positive front resistance to newer agents has added to the difficulties in managing infections caused by meticillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. Various national and international action plans targeting basic science (including drug development), surveillance, infection control, antibiotic usage (human and veterinary) and regulation will be summarised.

Atypical pathogens in pneumonia: challenges in management

Dr. Andrew Swann

Consultant Microbiologist, University Hospitals of Leicester NHS Trust, United Kingdom

The term 'atypical pneumonia' has traditionally been applied to a clinical presentation distinct from that associated with the common pathogens such as *Streptococcus pneumoniae*. However some authorities (including the British Thoracic Society) now consider that such a distinction is clinically unhelpful and prefer the term 'atypical pathogens' to include *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci* and *Coxiella burnetii*. In their community-acquired pneumonia guidelines the BTS did not include *Legionella* spp. in the atypical pathogens group as these can be both community and hospital-acquired. Although not strictly atypical pathogens it should be remembered that respiratory viruses (including influenza and coronaviruses) may have an atypical presentation. Unfortunately the clinical history and the chest radiograph cannot confidently predict the pathogen. Thus the laboratory has

a key role in diagnosis and some of the newer techniques will be reviewed.

With the exception of *Legionella* species culture is generally not available in clinical laboratories. Historically most laboratories have relied on serological techniques to detect a rising antibody response to infection. However this is unsatisfactory both in specificity and time-liness of diagnosis. The introduction of antigen-based techniques (as in pneumococcal and legionella diagnosis) and the emerging role of molecular diagnostics for atypical pathogens may lead to a more targeted approach to management. Many community-acquired pneumonia cases are treated empirically for both typical and atypical pathogens. A more timely and specific diagnosis would allow refinement of antimicrobial regimens – an issue of considerable importance in the era of increasing antimicrobial resistance. Identification of the specific pathogen would also greatly improve our understanding of the epidemiology of these conditions. Treatment guidelines generally categorise patients according to the severity of their condition and whether they are hospitalised or treated in the ICU. Traditionally tetracyclines and macrolides have been the mainstay of treatment of atypical pathogens. However macrolide-resistant *Mycoplasma pneumoniae* has been reported in Japan, especially in paediatric patients. The fluoroquinolones, especially the newer agents with enhanced activity against respiratory pathogens such as levofloxacin and moxifloxacin are now recommended for many atypical agents. Indeed levofloxacin is now considered to be the first choice agent for legionella pneumonia. Current recommendations and future challenges for treatment will be presented.

Infections in trauma – Microbiological nightmare

Dr. Geethika Patabendige

Consultant Clinical Microbiologist,
National Hospital of Sri Lanka,
Colombo 10



Traumatic injuries are a significant cause of death with 5.8 million deaths per year globally. Infection continues to be a common complication after trauma and is associated with an increase in morbidity and mortality, longer hospital stay with high economic burden. Severe infections result in poorer cosmetic outcomes.

Reported patterns of nosocomial infections in trauma patients vary widely among institutions and over time. Severity of injury has a profound influence on their patterns. Multiple factors increase the trauma patient at risk for infections namely interruption of tissue integrity, haemorrhage and tissue hypoperfusion, increased frequency of invasive procedures, impaired host defense

and initial interventions performed at the site of trauma. In scoring systems for quantitative characterization of injuries, incidence of infections increases with the increasing severity of injury.

The infections frequently encountered in trauma patients are ventilator associated and other pneumonias, empyema, central nervous system infections, surgical site infections, urinary tract infections, catheter related blood stream infections, sinusitis, gastrointestinal infections and severe sepsis. Both Gram negative and positive organisms are encountered namely *Acinetobacter* spp., *Pseudomonas* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter cloacae*, *Proteus mirabilis*, *Staphylococcus aureus*, coagulase negative staphylococci and *Enterococcus* spp. There is convincing evidence that antibiotic pressure increases the incidence of MRSA, VRE, multiply resistant Gram negative rods including Extended Spectrum β -Lactamase (ESBL) producing coliforms. With the exception of catheter related UTI, fungal infections are still relatively rare in this population when antibiotics are used selectively.

Diagnosis of infections in trauma is not an easy task. The clinical signs of patients may not allow accurate evaluation of the clinical condition of the patient. The hallmarks of infection elevated white cell count, fever, hyperdynamic state are often manifestations of acute trauma and do not always signify an infection. Evaluation of infections is limited by immobility, ventilation and devices used in patient care. The CRP is the most widely used marker in clinical practice but it cannot distinguish between inflammation and infection. Procalcitonin does not point to be an indicator or marker that is valid for prediction of bacterial infections including sepsis during the early period of trauma. From all available cytokines, IL-6 seems to be the most reliable marker for systemic inflammation and Lipopolysaccharide Binding Protein (LBP) appears to be an accurate and early marker of infection. But they are not freely available to be useful clinically. The use of these two markers together may offer the ability to detect the onset of SIRS (systemic inflammatory response syndrome) and allow early intervention to prevent MODS (multiple organ dysfunction syndrome), to distinguish between inflammation and infection and to monitor the response to standard and innovative therapies.

Isolation of organisms from a patient can easily be done but deciding when these organisms cause infections is often difficult. Antibiotic use is a double-edged sword. Timely use benefits the individual. Inappropriate or excessive use can increase the incidence of resistant organisms, unnecessary cost and increased adverse reactions. The use of appropriate prophylactic antibiotics in the setting of trauma is a generally accepted practice in specific situations. Research studies have proven that use of them in thoracic and abdominal injuries and open fractures is beneficial.

Effective infection control is essential in minimizing infections and preventing transmission in order to reduce morbidity and mortality. There are challenges for effective infection control due to poor hand hygienic compliance, lack of awareness, no continuous supply of resources for cleaning, disinfection and sterilization and disposable items, problems related to infrastructure facilities due to poor designing of buildings, lack of staff and poor dedication and commitment and attitudes of some of the staff members.

Healthcare worker (HCW) protection too is essential because HCWs are at high risk of getting exposed to several blood borne pathogens. There is urgent need for the implementation of trauma quality improvement system initiated by the World Health Organization and also trauma nurse co-ordination system for better management of trauma victims thus reducing the infection risk too in them. There are issues and challenges in relation to rehabilitation of trauma patients as some of them continue to have risk for infections.

New enteral feeding solutions with immune enhancing supplements, new solvent- detergent- treated plasma products transfusions and cell free haemoglobin products are some of the new innovative therapies.

Leishmaniasis in the tropics

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Leishmaniasis is an intracellular protozoan infection caused by a parasite that belongs to the genus *Leishmania* and transmitted by phlebotomine sandflies. It is an important public health problem in the tropics, particularly in countries like India, Bangladesh, Nepal, Brazil and Sudan, where it causes high morbidity and mortality. There are 3 main clinical entities seen in humans; viz. cutaneous, mucosal and visceral. Visceral leishmaniasis is targeted for elimination from the Indian subcontinent by the year 2020.

Sri Lanka is a new focus of human leishmaniasis caused by a genetically distinct variant of *Leishmaniadonovani*, which is the species more widely known to cause visceral leishmaniasis (VL) in other endemic countries, particularly in the neighbouring country, India. The clinical entity that is most frequently observed in Sri Lanka is cutaneous leishmaniasis (CL), though a few cases of mucocutaneous (MCL) and VL have also been reported in the recent past. In the local setting, papules, nodules, ulcerating nodules and ulcers occur either as single or multiple lesions on exposed areas of the body in CL.

patients who belong to a wide age range with both males and females being affected. Studies indicate that the local parasites are essentially dermotropic, without any evidence of an induced humoral immune response, as shown by a negative rK39 rapid dipstick assay (a qualitative test for the presence antibodies against rK39, a *L. donovani*-specific antigen) given by almost all CL patients when their sera were tested. Early evidence also indicates that the dermo-tropic variant of *L. donovani* has the ability to protect against visceralization of the infection in a rodent model, with interesting implications for vaccine development, an area rigorously pursued by many research groups.

The sandfly *Phlebotomus argentipes*, the known vector of *L. donovani*, is a widely prevalent insect in almost all parts of Sri Lanka, and is commonly referred to as 'welimassa' or 'hohaputuwa' in the local language. Vector status of this insect species was recently confirmed with field studies indicating *Phargentipesto* be the commonest sandfly species, which demonstrates behavioral habits that largely favour zoophilic and nocturnal feeding preferences [Senanayake *et al.*, submitted]. *L. donovani* parasites are transmitted between this vector and the human, the only host for this parasite species, as generally accepted, though lively debates continue within the scientific community regarding its so called 'strict' anthroponotic status. Likely presence of zoonotic reservoirs for *L. donovani* has been strengthened by recent reports demonstrating *Leishmania* parasites and anti-*L. donovani* antibodies in sera of dogs. Similar findings have also been made in the local setting, providing evidence for the existence of potential animal reservoirs in Sri Lanka. Interestingly, field-based risk factor studies have shown the existence of peri-domestic transmission, which favours anthroponotic nature of the parasite in certain areas, where as zoonotic/outdoor transmission cycles in some other parts of the country. Therefore, varying vector habits or behaviours might exist, which influence the pattern of transmission of leishmaniasis in different parts of Sri Lanka with important implications for the use of targeted vector control measures.

At present patients are detected mainly through passive means with only a proportion of these patients proceeding for pre-treatment laboratory confirmation of diagnosis due to the limited facilities and expertise available for diagnosis. CL is usually treated with either local application of liquid nitrogen or intralesional injections of sodium stibogluconate. None of the recognized anti-leishmanial drugs are registered locally. However, though leishmaniasis is a disease with a relatively short history in Sri Lanka; the country has successfully initiated many steps towards its control. Leishmaniasis was made a notifiable disease in Sri Lanka in 2008. Action plan for its control was drawn up with the involvement of all local stake

holders, including the Ministry of Health and academic community with the participation of recognized international experts in in year 2008. The second international colloquium on leishmaniasis was held more recently in order to update the 'action plan' for control, to assess the successes in its implementation and to identify the road blocks. Preventive and control activities are required to be put in place sooner rather than later. Enhanced case detection and active treatment are of prime value in controlling *L. donovani* infections. Availability and use of cost-effective and field-friendly diagnostic services in a decentralized manner, timely case management and vector control using appropriate protocols based on scientific evidence are pressing needs. Scientific information already available continues to be useful in the development of feasible and practical strategies for effective control of this disease. However, further research is required to fill in the gaps, particularly with regard to entomological and epidemiological aspects of this newly emerged disease, which has the potential to grow in to epidemic proportions with emergence and spread of more virulent forms that could create a huge burden on the national health system.

Emerging and re-emerging flaviviruses and their effect on dengue virus immunology

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Dengue is a major public health problem and it is estimated that around 390 million dengue infections occur annually resulting in 96 million clinically apparent infections. Many dengue vaccines are currently being developed and are undergoing clinical trials. However, much to the disappointment of all, one of the dengue vaccines which appeared very promising in the early clinical phases was found to fall short of providing protection against all four dengue virus (DV) serotypes. The main hurdle in developing a safe and effective dengue vaccine appears to be the lack of knowledge regarding the constituents of a dengue specific protective immune response. In addition, as many flavivirus infections co-exist in the same geographical regions, there appears to be the added complexity of modulation of dengue specific immune responses by immune responses to other flaviviruses such as the Japanese encephalitis virus (JEV) and the West Nile virus (WNV).

It has been suggested that vaccination or natural infection with other flaviviruses such as JEV and WNV has a potential of modulating the immune response to subsequent DV infection. Many children living in JEV and DV endemic countries receive the JEV vaccine and immune responses to the vaccine could potentially modulate immune responses to subsequent dengue infection. The WNV was also recently identified in Sri Lanka and infection with this virus adds to the complexity of immune responses to the dengue virus.

In our ongoing study, we found that 28% of individuals had antibodies to the JEV despite only 13.4% of them receiving the JEV vaccine. Interestingly, individuals who had a past episode of dengue haemorrhagic spp. fever (DHF) were more likely to have JEV-specific IgG antibodies and also significantly higher JEV ELISA IgG units. Therefore, our data suggests that either the presence of JEV antibodies could predispose to occurrence of apparent dengue infections / DHF or it is possible that more cross reactive forms of antibodies associate with severe dengue. Due to the highly cross reactive nature of the antibody responses to DV, JEV and WNV, it is difficult to differentiate true past infection with JEV from the presence of JEV specific cross reaction antibodies. However, if previous infection with JEV (or vaccination) does modulate the clinical outcome of dengue infection this would have significant implications for JEV and dengue vaccine design and management of individuals and public health.

Infection control in a renal transplant unit

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Infection is a much bigger issue for transplant recipients than for the general population due to the immunosuppression state. Therefore infection control is more important in renal transplant unit than in any other unit. Infections in the transplant recipients are of two types. In the initial period after surgery they are prone to get conventional nosocomial infections such as ventilated associated pneumonia, catheter associated urinary tract infections, surgical site infections and intravascular catheter infections. To prevent these usual infection control practices in the theatre, wards and the ICU will be important.

Later they become prone to get opportunistic infections due to immunosuppression and thus more stringent targeted preventive measures should be practiced.

All potential transplant recipients should be tested for prior exposure to viral infections such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella zoster virus (VZV), hepatitis B and C and human immunodeficiency virus (HIV). Depending on the results of these tests certain measures need to be taken before transplantation. Immunization should be offered to all hepatitis B (if not already immunised) and VZ virus antibody negative patients before transplantation. They should have HBsAb levels rechecked annually and revaccination carried out if antibody titres fall below 10mIU/ml and should not receive live attenuated vaccines. They should receive pneumococcal vaccine and one booster every five years. The renal transplant recipients should be vaccinated with inactivated viruses as per the normal population except for HBV and annual influenza vaccination should be given unless contraindicated.

Prophylaxis with antibiotic and antiviral agents is common. Low-dose trimethoprim-sulfamethoxazole, for example, is used to prevent PCP and UTIs, and the agent is well tolerated. Prophylactic antivirals are recommended for sero-negative recipients of kidneys from CMV positive donors.

Patients who are awaiting renal transplant usually undergo dialysis. This procedure makes them vulnerable to infections such as intra-vascular catheter associated infections and blood borne viral infections. Therefore these patients should be immunized against Hepatitis B and the antibody levels should be monitored. Also strict guidelines should be in place to prevent intra-vascular catheter associated infections and to minimize exposure to blood borne infections.

Standards in quality assurance

Dr. Viv Peiris

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Quality is not easy to define yet we find it relatively easy to spot when it fails to reach our expectation.

This talk will consider how microbiology laboratories should reflect on what quality means as far as the service they provide and how it can be measured, managed and delivered.

Quality assurance in microbiology – limitations in practice

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The National External Quality Assessment Scheme (NEQAS) for Bacteriology in Sri Lanka is conducted by the Quality Control Laboratory of the Department of Bacteriology, Medical Research Institute. This programme commenced in 1997, with 14 voluntary participating laboratories under the supervision of the late Dr. R. S. B. Wickramasinghe, Consultant Bacteriologist, Department of Bacteriology.

The main objective of this programme is to standardize the performance of bacteriological techniques and antibiotic susceptibility testing in clinical bacteriology laboratories in the island. This will help to generate reliable, timely bacteriology reports to the clinicians for the optimum management of their patients.

Furthermore, it will promote Internal Quality Control (IQC) in the laboratories with improvements in good laboratory practices. Also, it will help the participating laboratories to identify the competency of their laboratories. It identifies areas for improvements and facilitates liaison with other laboratories for expert advice to improve performance.

There are many limitations and drawbacks in the programme. Limited resources, difficulty in sending the QC samples on time to reach the laboratories, lack of knowledge in processing QC samples and reporting, poor resource settings in participating laboratories and no follow-up of laboratories are some of these limitations.

Patients at high risk of fungal infections: when and how to treat

Dr. Preethi Perera

Consultant Mycologist



Over the past few years, major advances in healthcare have led to an unwelcome increase in the number of life-threatening infections due to true pathogenic and opportunistic fungi. These infections have increased in numbers largely because of the increasing size of the population at risk, use of novel and more intensive regimes resulting in more profound levels of immunosuppression and the use of invasive monitoring and aggressive therapeutic technologies in intensive care units resulting in improved survival of individuals with life threatening illnesses.

The two most prevalent invasive fungal infections are invasive candidiasis and invasive aspergillosis. It is imperative that early treatment is initiated in patients with invasive fungal infections as it has a major impact on mortality rates. Reliable diagnostic measures that are lacking is a major draw back. This has led to the parallel use of different treatment strategies. Prophylaxis, empirical, pre-emptive and targeted treatment with a definitive diagnosis.

Extensive research has been carried out to facilitate the best possible treatment methods. With the antifungals in use the investigative arm should be strengthened for early diagnosis of invasive fungal infections which is imperative for treatment success.

Infection in trauma critical care – the role of the Anaesthetist

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Victims of trauma are highly susceptible to infections due to contamination of wounds at the time of accident. Patients who are admitted to the intensive care unit following trauma are the patients with severe life threatening injuries. They either need system support due to failure of any one of the systems which may necessitate ventilator care in head injury or chest injury patients, extensive surgery in the event of abdominal, brain or limb injuries resulting in opening into abdominal cavity, brain and the use of prosthesis in bone. In addition extensive monitoring of the critically injured will necessitate intra vascular catheters, peripheral cannulae, and urinary catheters thereby breaching the body's defenses to microorganisms.

The commonest cause of infection in the ICU setting is nosocomial infection. The EPIC study shows an incidence of 20.6% in Europe. In Sri Lanka our Neuro-trauma ICU showed an incidence of 54%. The commonest type is ventilator associated pneumonia (VAP). The incidence in the west is 10-60% while ours in Sri Lanka is 37%. Of the bacteremias catheter related blood stream infection (CRBSI) is the commonest. Our statistics show an incidence of 8%. The other infections are wound sepsis (3%), urinary sepsis (3%) and meningitis (3%) following external ventricular drains used to monitor ICP and for the removal of CSF in the event of intra cranial hypertension.

The causes, management and preventive strategies for VAP, CRBSI, wound sepsis or surgical site infection will be looked at. The NICE guidelines for prevention of wound sepsis and the latest recommendations of the surviving sepsis campaign will be reviewed.

OP 1

A study of residual viable bio burden in reprocessed side-view endoscopes used for Endoscopic Retrograde Cholangiopancreatography (ERCP) in a clinical setting

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Introduction

High level disinfection of side-view endoscopes used for ERCP is recommended since accessories such as biopsy forceps, polypectomy snares, guide wires come in contact with sterile sites. Procedural errors in cleaning and disinfection of endoscopes have been documented. To the best of our knowledge, this is the first study carried out in Sri Lanka to objectively analyze the sterility concerning therapeutic side-view endoscopes.

Objective

Aim of this study was to evaluate the efficacy of reprocessing of side-view endoscopes in tertiary reference endo-therapy unit of Colombo South Teaching Hospital.

Methods

Over a period of seven months hundred and two samples obtained from two different flexible side-view endoscopes were tested for microbial growth. Three samples were collected before and after the reprocessing procedure; one swab from the tip before reprocessing, another after manual reprocessing with 1.62% peracetic acid and a normal saline sample after flushing through the working channel on completion of reprocessing. Cultures were done according to European Society of Gastrointestinal Endoscopy (ESGE) and the European Society of Gastroenterology Endoscopy Nurses and Associates (ESGENA) protocol.

Results

After reprocessing, tip and working channel of the side-view endoscope were positive for microorganisms 20% and 10% respectively. Multiple organisms were found in 4% of swabs from the tips and 2% the working channels. *Klebsiella* species were found to be the commonest in the tip and *Candida* species were found to be the commonest in the working channel of the reprocessed side-view endoscopes.

Conclusion

Current manual reprocessing procedure is not sufficient for inactivation and removal of bio-burden from the side-view endoscopes in spite of strict adherence to the protocol describes for manual reprocessing. Microbiological monitoring of reprocessed side-view endoscopes is valuable to rectify reprocessing method to prevent transmission of infection secondary to ERCP.

OP 2

Comparison of clinical criteria and laboratory criteria used for the diagnosis of bacterial vaginosis

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Bacterial vaginosis (BV) is a common cause of vaginal discharge affecting millions of women annually.

It is caused by an imbalance of naturally occurring bacterial flora resulting loss of vaginal lactobacilli and concomitant overgrowth of mixed bacterial flora. BV is associated with adverse gynecological and pregnancy outcomes and with an increased risk of acquisition of HIV and other sexually transmitted diseases.

There are no studies carried out in Sri Lanka to assess the validity of the methods used to diagnose BV at present.

Objectives

- To determine the prevalence of BV among women who present with vaginal discharge.
- To determine the usefulness of Amsel's clinical criteria to diagnose BV by comparing it with the Nugent criteria, which is the gold standard.

Methodology

300 patients who presented with vaginal discharge to the sexually transmitted diseases (STD) clinic, gynecology clinics and gynecology wards at North Colombo Teaching Hospital, Ragama and STD clinic - Colombo, between 1st January 2011 to 30th April 2011 were included in the study.

Four high vaginal swabs were collected during the speculum examination and examined according to the Amsel's and Nugent's criteria. Appearance of the vaginal discharge was observed.

Results

The prevalence of BV among women who presented with vaginal discharge was 25.3% (76/300) by the Nugent's method. Among the women with vaginal discharge BV was diagnosed more in STD group which is 33.8% (52/154) compared to the non STD group which is 16.4% (24/146). The sensitivity, specificity, positive and negative predictive values were calculated to assess the validity of the Amsel's method considering the Nugent's criteria as the gold standard. Diagnosis of BV by performing Amsel's method exhibit a low sensitivity (55.3%) and high proportion of false positives (positive predictive value-59.2%) when compared against Nugent's method.

Conclusions

The Amsel's method is not a satisfactory method to be used as a diagnostic test for BV. At present BV is diagnosed by examining the nature of vaginal discharge or by using some of the Amsel's criteria. As we have found that Amsel's criteria cannot diagnose BV satisfactorily, we have to establish Nugent's method to diagnose BV in Sri Lanka.

OP 3

Surgical site infections and *Staphylococcus aureus* colonization in renal transplant patients

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Introduction

Surgical Site Infections (SSIs) are a common post-operative complication in renal transplantation. Its incidence differs within study populations (3%-11%). Whilst some centers screen all electively admitted patients for methicillin-resistant *Staphylococcus aureus* (MRSA), there is no clear consensus regarding renal transplant recipients.

Objectives

1. To determine the prevalence of SSIs and colonization of *S.aureus* in renal transplant patients at TH Kandy.
2. To assess the importance of pre-operative screening for *S.aureus* in renal transplantation.

Design, Setting and Methods

A prospective, descriptive study was carried out from September 2009 for six months duration. Thirty five patients undergoing renal transplant at the Renal Transplant Unit, Kandy were included after informed consent. Ethical clearance was obtained from the Ethical Committee, Teaching Hospital Kandy.

Patients were screened pre-operatively for *Staphylococcus aureus* colonization using nasal, throat, axillary and perineal swabs. Decolonization was not done following a positive MRSA report.

Following transplant, daily inspections were done to exclude SSI and swabs/pus was taken from infected areas. Standard methods were used for the isolation and identification of organisms in the microbiology laboratory.

Results

At pre-operative screening, all patients were positive for *S.aureus* in at least one screening swab.

Staphylococcus aureus was isolated in 85.71% of the axillary swabs, 82.86% of the nasal swabs and 80% of the perineal swabs were positive. Only 54.29% of throat swabs had *Staphylococcus aureus*. All the patients were positive for *S.aureus* in either axillary or nasal swabs whilst 97.14% were positive in either nasal or perineal swabs.

Twenty two patients (62.86%) were positive for MRSA colonization on one or more screening swabs.

Only 3 (8.57%) SSIs were identified. *Staphylococcus aureus* was identified on pus culture in one and the other two were culture negative.

Conclusion

According to this study, the high carriage of *S.aureus* prior to transplantation and the low prevalence of SSIs in post-renal transplant patients may render pre-operative screening for MRSA carriage in renal transplantation unnecessary. Further studies should be done to confirm this result.

Optimum *S.aureus* carriage was detected by screening all four sites or by combined axillary and nasal swabbing. Combined two site swabbing with either nasal and axillary or nasal and perineal swabs may prove more cost effective.

OP 4

Study of prevalence and risk factors for MRSA colonization in patients presenting to the orthopaedic unit in Teaching Hospital, Peradeniya

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Introduction

Methicillin resistant *Staphylococcus aureus* is a major problem in orthopaedic units as infection of prosthesis has many dreaded complications including loss of

prosthesis, increased morbidity and increased cost to the health care system. Colonization with MRSA on admission is a known factor associated with increased rate of subsequent infections. Identification of patients colonized with MRSA is needed to prevent infections and also to reduce the burden of MRSA within a unit. Target screening is a cost effective method in a resource poor country to identify colonized patients. Prevalence and risk factors for colonization with MRSA have to be known to employ target screening. Hence it is not suitable if the prevalence of MRSA is high in patients without any health care associated risk factors.

Methods

In this study prevalence and risk factors for colonization with MRSA on admission was studied in patients presenting to the orthopaedic ward, Teaching Hospital Peradeniya. Prevalence of MRSA in patients without any health care associated risk factors was also studied.

A prospective cohort study was carried out in 276 patients admitted to the Orthopaedic ward, Teaching Hospital Peradeniya from 01st January to 30th April 2011. Nares, throat and groin were sampled within 36 hrs of admission to the unit with moistened swabs. Swabs were pooled and enriched in 7% NaCl overnight. Broth was inoculated into mannitol salt agar and *S. aureus* like colonies were further identified using slide coagulase, tube coagulase and DNase tests. Methicillin resistance was detected using agar dilution method. Information on risk factors for colonization was gathered through a validated questionnaire.

Results and Conclusion

Prevalence of MRSA on admission was 3.3% in the study population. History of surgery (odds ratio 4.25 90% CI 1.30 to 13.90), history of nursing home admissions (odds ratio 75.71, 90% CI- 9.17 to 624.97), and history of hospital admissions (odds ratio 3.71, 90% CI- 0.98 to 14.11), within the previous 12 months were associated as significant risk factors for colonization with MRSA on admission. None of the patients without any health care associated risk factors carried MRSA. Therefore targeting only the selected high risk groups for an MRSA surveillance and control program will be cost effective in this unit.

OP 5

Detecting bacterial pathogens causing infections in neonates admitted to the Neonatal Intensive Care Unit (NICU) at a tertiary care hospital in Southern province of Sri Lanka

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Introduction

A prospective study was carried out in the Neonatal Intensive Care Unit (NICU) at Teaching Hospital Karapitiya from January to April 2011 to detect bacterial pathogens causing neonatal infections.

Objectives

To find the incidence of culture proven episodes of infections, to identify bacterial pathogens and their sensitivity patterns, to determine the most appropriate empirical antibiotics for the current use in the NICU, to find the rate of colonization with possible pathogens among neonates on admission and to assess the usefulness of screening tests in predicting the causative organisms of sepsis.

Method

Fifty consecutive neonates admitted to NICU (< 28 days of age) were screened on admission and weekly during the NICU stay to detect colonization with possible pathogens. Appropriate specimens were cultured when sepsis was suspected clinically. Antibiotic sensitivity tests (ABST) were performed on all isolates.

Results

Out of 55 clinically suspected episodes of sepsis, 1/3 (17 episodes) became culture positive with incidence of 340 per 1000 admissions. Early onset sepsis (EOS) was 4% and late onset sepsis (LOS) rate was 30%.

Nosocomial infection rate was 13.61 episodes per 1000 patient days. Thirteen (76%) of clinically significant isolates were coliforms. 84.61% (11 isolates) of them were extended spectrum beta-lactamase (ESBL) producers. According to sensitivity results imipenem, meropenem, amikacin and netilmicin were the appropriate antibiotics for the empirical treatment of LOS in this NICU.

Thirty five (70%) neonates were colonized with possible pathogens on admission. The commonest colonizer was coagulase negative Staphylococcus (CoNS) (50%). Twenty (40%) neonates were colonized with coliforms on admission including 8% with ESBL producers.

From the 17 culture positive episodes, 7 episodes (41%) had the same organism isolated in surveillance samples before clinical culture positivity.

Conclusions

There was a high degree of clinical suspicion of sepsis among neonates and 1/3 was proven by cultures. Coliforms were the predominating pathogens and had a high level of resistance to commonly used antibiotics. Carbapenems and aminoglycosides were the most appropriate empirical antibiotic therapy for LOS in this NICU. Presence of multidrug resistant isolates on admission was a problem.

OP 6

Bacterial pathogens in bone infections complicated by trauma or chronic wounds

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Introduction

Osteomyelitis results in considerable morbidity and increase health cost. Studies using bone samples to find out the causative agents of chronic osteomyelitis resulting from trauma or chronic wounds are rare. In this situation blood cultures are sterile and wound swabs are considered as unsuitable samples.

Objective

To find out pathogens and their sensitivities in chronic bone infections to use as a guide for treatment

Method

Between March 2012 to February 2013, a descriptive study was carried out at General Hospital, Ampara. Pus, tissue and bone samples were collected from diabetic foot bones and traumatic bones of patients clinically diagnosed to have chronic osteomyelitis. All isolates were identified using standard methods and antibiotic sensitivity testing was done using Stokes method.

Results

Of 16 bone samples, 14 samples yielded a growth. Seven samples yielded 2 organisms while remaining seven grew only one. In those with a single organism *Staphylococcus aureus* was isolated in four while *pseudomonas* spp., coliform and coagulase negative *staphylococcus* spp. were isolated from the other three. In the seven mixed cultures, there were three bone specimens having both *Staphylococcus aureus* (*S.aureus*) and coliform spp. Two samples had coliform spp. in each with either *pseudomonas* spp. or *streptococcus* spp. Of the remaining two samples, *pseudomonas* and *streptococcus* were isolated in one while Group A *streptococcus* and coagulase negative *staphylococcus* were isolated in the other of *staphylococcus aureus* isolate 85.7% (6/7) were MRSA. All *pseudomonas* isolates were sensitive to anti-pseudomonal antibiotic tested including ceftazidime. All coliforms were sensitive to amikacin and resistance to ampicillin and coamoxiclav while 50% (3/6) were sensitive to cefotaxime and ciprofloxacin.

Conclusion

S. aureus was the commonest organism causing osteomyelitis due to a single pathogen while in mixed infections coliforms are the commonest isolates associated with other organisms. Methicillin resistant *S. aureus* (MRSA) and resistance coliforms should be considered when treating osteomyelitis following trauma and chronic diabetic foot infections.

Recommendation

Though this study generates evidence on pathogens involved and their sensitivity patterns in chronic bone infections, a further study with a larger sample size is needed to determine the statistical significance of these occurrences.

OP 7

The incidence of Hospital Acquired Infections among ICU patients at a Tertiary Care Hospital in Southern Province of Sri Lanka

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Introduction

Hospital Acquired Infections (HAI) are frequent complications in the healthcare facilities throughout the world. This study describes the HAIs among critically ill patients managed at Teaching Hospital, Karapitiya which is the main tertiary care hospital in Southern Sri Lanka.

Objectives

General Objective

To study the HAIs among patients admitted to the general ICU at Teaching Hospital, Karapitiya.

Specific Objectives

1. To determine the incidence of different types of HAIs.
2. To determine device utilization ratio.
3. To study the antibiotic sensitivity pattern of the bacterial isolates.
4. To determine the rate of colonization of patients with multi-resistant organisms.

Methods

All patients admitted to and staying for more than 48 hours in the general ICU at Teaching Hospital, Karapitiya were followed up. Infections were diagnosed using clinical parameters, and microbiological and other investigations.

Results

There were 655 'patient days in the ICU' during the study period. There were 616 mechanical ventilator days. Almost all the patients were catheterized and 644 urinary catheter (UC) days were noted. The number of Central Venous Catheter (CVC) days was 396.

During our study period, we detected 12 device associated infections (DAI) and 14 HAIs with overall DAI and HAI rates of 15.78% and 18.42% respectively among 76 patients.

Ten patients 13.15% developed ventilator associated pneumonia (VAP), 2.63% (2/76) developed catheter associated urinary tract infections (CAUTI) and 2.63% (2/76) developed blood stream infections (BSI) which were not CVC related BSI (CRBSI). Percentage distribution of different infections acquired in the ICU in this study were as follows; VAP 71.42%, CAUTI 14.28% and BSI 14.28. VAP and CAUTI rates per 1000 device days were 16.23 and 3.1 respectively.

Mechanical ventilator utilization ratio was 0.94 and urinary catheter utilization ratio was 0.98 but CVC utilization ratio was 0.6.

Most of *Acinetobacter* species (72%) were carbapenem resistant and many of these isolates (39%) were also pan drug resistant.

Most patients (>65%) were colonized with nosocomial microorganisms at the end of the second week.

Conclusions

HAI and DAI were significant problems in this ICU.

Carbapenem resistance was the worst problem encountered when managing infections in the ICU. Risk of respiratory tract colonization increased with the duration of the ICU stay. Colonization with drug resistant *Acinetobacter* species, Coliform species and *Staphylococcus aureus* increased when patients were in the ICU.

OP 8

Incidence, antibiotic susceptibility pattern and factors associated with ventilator associated pneumonia in patients in Intensive Care Units in Rathnapura district

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Introduction

Ventilator associated pneumonia (VAP) has emerged as an important challenge in the intensive care units (ICU) and it remains as an important cause of morbidity and mortality despite advances in antimicrobial therapy, better supportive care modalities and the use of wide range of preventive measures.

Since ICUs are functioning with minimal infrastructure facilities it favours the transmission of infections. So it is important to assess the aetiological agents, antibiotic sensitivity and associated factors for the development of VAP.

Objectives

1. To determine the incidence, causative organisms and antibiotic susceptibility pattern (ABST) of VAP in ICU patients.
2. To assess any associated factors for the development of VAP.

Methodology

A prospective cohort study was conducted at ICU in PGH Rathnapura and BH Embilipitiya from 1st of January to 30th of April 2012. All patients who were ventilated for more than 48 hours were included in this study except children less than 12 years of age and those who are having pneumonia.

Associated factors assessed were chronic lung diseases, obesity, immunosuppression, preexisting sinusitis, re intubation, self-extubation, position of the patient, enteral feeding, use of sedation, gastric ulcer prophylaxis, modulation of colonization, recent antibiotic therapy, residence in health care facilities, recent hospitalization and number of suction till development of VAP.

VAP was diagnosed by modified Clinical Pulmonary Infection Score. Etiological agents were isolated up to species level by API methods. ABST of isolates were done according to CLSI method.

Results

Fifty nine patients were studied for 470 ventilator days and 589 ICU days. There were 16 cases of VAP with overall incidence of 27.12% and 34.04 VAP per 1000 ventilator days. Out of 16 cases 5 cases (31.25%) were polymicrobial origin. The commonest organism isolated was *Acinetobacter baumannii* (38.09%, 8/21) and others were *Acinetobacter lwoffii* (9.52%, 2/21), *Pseudomonas aeruginosa* (28.57%, 6/21), *Aeromonas hydrophila* (4.76%, 1/21), *Klebsiella pneumoniae* (9.52%, 2/21), *Escherichia coli* (4.76%, 1/21) and Methicillin Resistant *Staphylococcus aureus* (4.76%, 1/21).

Resistance to Ciprofloxacin and Carbapenems were 87.5% (7/8) and 62.5% (5/8) respectively for *Acinetobacter baumannii*. *Acinetobacter lwoffii* were 100% (2/2) resistance to carbapenems and cephalosporins.

Ciprofloxacin sensitivity to *Pseudomonas aeruginosa* was 83.33% (5/6). *Klebsiella pneumoniae* were sensitive to all antibiotics.

Among the associated factors tested, number of suction was significantly associated with the development of VAP ($P < 0.05$).

Conclusion

Majority of organisms were multidrug resistant and pan drug resistant. Frequent suctioning of endotracheal tubes has significantly contributed for development of VAP.

Acknowledgement

Financial assistance by Ministry of Health Sri Lanka is acknowledged.

OP 9

Retrospective study of blood culture positives in a special care baby unit (SCBU) in a General Hospital in Sri Lanka

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Introduction

Neonates are prone to develop infections due to multiple reasons such as immature immune system, contamination during child birth, prematurity; aspiration of amniotic fluid, non-adherence to infection control practices, etc.

Global data shows a reduction of Group B Streptococci and an increase of Gram negative bacteraemia. The data from Sri Lanka in this field is scarce. Extensive analysis of spectrum of organisms will be valuable in deciding of empirical antibiotics in neonatal sepsis.

Objectives

- To study the risk factors for bacteraemia among neonates.
- To study the spectrum of organisms causing bacteraemia in neonates.

Methodology

Retrospective and prospective analysis of positive blood cultures in SCBU at GH Polonnaruwa was done from August 2012 to March 2013. During that period, all positive blood culture results from SCBU were taken in to the study. Risk factors were analyzed according to the history.

Results

There were 41 blood culture positive neonates during the study period. Twenty one cultures were confirmed as clinically significant. Those isolates included 8 coliform spp., 5 *Staphylococcus aureus*, 4 Group B *Streptococcus*, etc. Fifteen positive cultures were reported as probable contaminants and significance of 5 coagulase negative *Staphylococcus* spp. (CoNS) was questionable.

Most of the organisms were fully sensitive to the tested antibiotics. Only one Extended Spectrum Beta Lactamase producing coliform was isolated from a 6 day old baby with multiple anomalies.

Eight neonates with significantly positive blood cultures were having identifiable risk factors such as prematurity, obstructed labour, and premature rupture of membranes.

All babies with coliform and *Pseudomonas* bacteraemia had risk factors leading to longer hospital stay and may have acquired nosocomial infection. Except 5 babies who later had positive culture with CoNS, which could be contaminants, all others were given empirical antibiotics. In 13 patients, initial antibiotic regime had to be adjusted according to the culture and clinical response. Of 41 patients, only 3 died while all others recovered without residual effects.

Conclusion

Most common isolate among neonatal blood cultures was CoNS but its significance was questionable. Coliform spp. seems to be the most significant isolate in this patient group. Group B *Streptococcus* isolation rate is lower than the coliforms and *Staphylococcus aureus* and the reason may be empirical antibiotic treatment.

OP 10

Randomized placebo-controlled trial of the efficacy of mebendazole polymorphs in the treatment of hookworm infections

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Introduction

Mebendazole has three polymorphic forms, identified as A, B and C. Animal studies and one previous study in humans have suggested that unlike polymorph C, polymorph A is ineffective in the treatment of hookworm and whipworm infections.

Objectives

A randomized double-blind, placebo-controlled trial was carried out to compare the efficacy of single dose 500 mg tablets of pure mebendazole polymorph C with those containing a 1:1 mixture of polymorphs A and C, for the treatment of hookworm infections.

Design, Setting and Methods

All eligible individuals living in 219 households in 8 estate divisions in Ratnapura District known to have a high prevalence of hookworm, were recruited after obtaining written, informed consent. A single faecal sample was obtained and examined the same day, using the Kato-Katz technique for quantification of intestinal nematode infections. Those who were found infected with hookworms were randomized to one of three treatment arms and requested to provide a second faecal sample 10 - 14 days after treatment. This was examined in the same manner as the first.

Results

A total of 892 individuals were recruited; 601 provided faecal samples; 214 were found positive for hookworm infection; 70, 74 and 70 individuals were randomized to treatment arms A (mixture of polymorphs A and C), B (pure polymorph C) and C (placebo) respectively. Follow-up faecal samples were provided by 53, 48 and 49 persons respectively in each treatment arm. The cure rates in the three treatment arms were 28.3%, 18.8% and 16.3% respectively; they were not significantly different from one another. Comparison of faecal egg count reductions (FECR) in the 3 treatment arms (86.1%, 84.5% and -6.6% in arms A, B and C respectively) showed that both mebendazole formulations performed significantly better than placebo, but there was no statistically significant difference between FECR with the two drug formulations.

Conclusions

A single 500mg dose of mebendazole, either as Polymorph C alone, or as a mixture of Polymorphs A and C, has little efficacy in curing hookworm infections. However, both formulations are significantly better than placebo in reducing the intensity of infection, with no statistically significant difference between the two formulations.

OP 11

Prevalence of *Helicobacter pylori* infection in a sample of patients with dyspeptic symptoms and gastric antral inflammation

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Introduction

Gastric *H. pylori* infection is considered a common cause of dyspeptic symptoms and is always associated with gastric mucosal inflammation, commonly the antrum. This study was conducted to determine the prevalence of *H. pylori* infection in gastric antral biopsies with chronic inflammation in a sample of patients with dyspeptic symptoms, using different diagnostic methods, in Teaching Hospital, Peradeniya.

Design

A cohort of 103 patients referred for upper gastrointestinal endoscopy due to dyspeptic symptoms with endoscopically visible gastric antral inflammation was included

for the study. All patients underwent endoscopic gastric biopsy: four from the antrum and one each from incisura angularis and body. The latter two sites were biopsied because it is recognized that *H. pylori* organisms migrate to body in patients on proton pump inhibitors. One minute urease test was performed on one antral biopsy. The rest were stained with Haematoxylin and eosin (H & E), toluidine blue and immunohistochemistry. Prevalence of organisms with each mentioned test was assessed. Histologically, chronic inflammation and *H. pylori* organisms were assessed and graded using Sydney system.

Results

Thirty (30/103; 29.1%) cases had chronic inflammation histologically; 20, 9 and 1 had Sydney grade 1, 2 and 3 chronic inflammation respectively. With both H&E and toluidine blue stains *H. pylori* infection was present in 4/30 (13.3%) cases. Prevalence of *H. pylori* infection in Sydney grade 1, 2 and 3 chronic inflammation cases were 0, 33.3% and 100%, respectively. Immunohistochemistry was positive in only two cases (6.7%) which were also positive with other methods. One minute rapid urease test was positive in 9 cases (30%), 4 of which were also positive with H & E and toluidine blue. The 73/103 (70.9%) cases without chronic inflammation had no evidence of *H. pylori* infection by any method.

Conclusion

In the cases with chronic inflammation, prevalence of *H. pylori* infection with H & E, toluidine blue, immunohistochemistry and one minute rapid urease test are 13.3%, 13.3%, 6.7% and 30% respectively. Histologically, Sydney grade 1 chronic inflammation appears to be not associated with *H. pylori* infection. Only 40% of the cases with Sydney grade 2 or 3 chronic inflammation were associated with *H. pylori* infection and the likelihood of detection of *H. pylori* appears to increase with increased severity of chronic inflammation. Sensitivity and specificity of each method tested should be assessed by using a gold standard test.

Acknowledgement

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OP 12

Proportion of fungal foot infections in patients with type 2 diabetes at a tertiary care hospital

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Introduction

Superficial fungal foot infection (SFFI) in diabetic patients increases the risk of developing diabetic foot syndrome. Sixteen percent of urban population is suffering from diabetes in Sri Lanka. Higher percentage (85%) of patients with long term diabetes is suffering from SFFI. As the diabetic patients are more prone to get fungal foot infections, early intervention is advisable owing to the progressive nature of the infection and to prevent from recurrent SFFIs which could even lead to diabetic foot syndrome. There is no documented data on the prevalence of fungal foot infections in diabetic patients in Sri Lanka.

Objective

To determine the etiological agents causing SFFI in patients with type 2 diabetes.

Methods

This descriptive cross sectional study included 385 out patients from the diabetic clinic at Colombo South Teaching Hospital. Nail clippings and swabs were collected from the infected sites using the standard protocol. Laboratory identification was done and pathogens were identified to the species level by direct microscopy, culture and biochemical tests. Statistical analysis was performed with the software SPSS version 15, using odds ratio and Chi-square tests.

Results

Clinically 295 patients (77%) showed SFFI, of which 255 (86%) were mycologically confirmed for nail infection and 46 (16%) were confirmed for both skin and nail infection. Out of 236 direct microscopy (KOH) positives, 227 (96%) were culture positive. Two hundred fifty one patients (98%) with SFFI had diabetes for more than 10 years. Of the patients with SFFIs 92% had >100 mg/dl FBS and 81% had >140mg/dl PPBS levels and 80% had both elevated FBS and PPBS levels. Non-dermatophyte fungal species were the commonest pathogens followed by Yeast and dermatophytes.

Conclusion

Aspergillus niger was the commonest cause of SFFIs in diabetes patients in the study setting followed by *Candida albicans*. Occurrence of SFFI was significant with the increasing age, gender, duration of diabetes and with less glycaemic control. Diabetic patients require regular foot examination and education on preventive care practices, as they are at high risk of developing SFFI.

OP 13

Development of recombinant protein antigens using a bacterial expression system for the detection of anti-Chikungunya (CHIK) antibodies

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Introduction and objective

Laboratory confirmation of Chikungunya (CHIK) virus is very useful as clinical symptoms of CHIK can overlap with other diseases. Chikungunya virus specific antigen, which shows high specificity, sensitivity and low cross reactivity with other related diseases, is required for laboratory confirmation. Objective of this study was to develop and compare two recombinant protein antigens for detection of anti-CHIK antibodies.

Design, setting and methods

Recombinant CHIK protein antigens were prepared using Envelope (E1 and E2) regions of the CHIK virus. The genes were custom designed and chemically synthesized with a 6X His tag. Bacterial expression systems [BL21 (DE3)] were used to clone and express the recombinant proteins. The recombinant proteins were purified with >95% of purity per liter of culture using Ni-NTA columns under denature conditions. In this study, two antigens were evaluated for detection of anti-CHIK antibody by using novel optimized in-house IgM and IgG ELISAs, using a panel of well characterized serum samples obtained from the Dept. of Virology (WHO Reference Center for Viral Reference and Research) Institute of Tropical Medicine, Nagasaki University, Japan.

Results

A total of 55 serum samples confirmed as positives and 186 confirmed as negatives by HAI test, IgM capture ELISA and indirect IgG ELISA using the purified CHIK antigen were used to evaluate the antigens using novel IgM ELISA. A total of 78 serum samples confirmed as positives and 148 (E1) or 227 (E2) (148 + extra 79) confirmed as negatives were used to evaluate the antigens using novel IgG ELISA. The E1 recombinant protein showed 5% (3/55) sensitivity and 99% (184/186) specificity for IgM ELISA and 60% (47/78) sensitivity and 63% (94/148) specificity for IgG ELISA. The E2 recombinant protein showed 65% (36/55) sensitivity and 70% (131/186) specificity for IgM ELISA and 83% (65/78) sensitivity and 86% (195/227) specificity for IgG ELISA.

Conclusion

Recombinant CHIK-E2 protein antigen showed higher specificity and sensitivity in detection of both IgM and IgG antibodies. Thus the E2 recombinant protein antigen used in this study could be expressed in an eukaryotic expression system to achieve much higher results.

Acknowledgment

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OP 14

Molecular evidence of hantavirus infection among clinically suspected patients with haemorrhagic fever with renal syndrome (HFRS)

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Introduction

Hantavirus disease is an emerging zoonotic viral infection with high fatality. Transmission is by inhalation of aerosols generated from virus contaminated rodent excreta. There are two major clinical forms, haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Clinical features of HFRS, often mimic leptospirosis. Large number of cases of leptospirosis like illness has been reported in Sri Lanka annually. Although there were serological evidence of different types of hantavirus infection in Sri Lanka, diagnosis of hantavirus is not routinely performed. Due to the genetic and antigenic diversity, an assay that could detect a wide range of hantaviruses need to be established.

Objectives

To establish, evaluate and validate a genus specific hantavirus RT-PCR assay.

To diagnose hantavirus infection among clinically suspected HFRS patients in three selected hospitals. To describe clinical manifestations of hantavirus infections in the study population.

Methodology

Genus specific conventional RT-PCR assay was established using panhanta primers and evaluated, optimized and validated using synthetic genes of 12 known

hantavirus species as reference samples. Assay was able to detect a wide range of hantaviruses at minimum detection limit of 70 copies/ reaction.

Molecular diagnosis of hantavirus infection was carried out in three hospitals in Colombo and Gampaha districts. Study was conducted from 01st of January 2011 to 31st of April 2011 and 61 adult patients were recruited to this study.

Hantavirus RT-PCR was performed on all collected samples after extraction of RNA by TRIzol[®] method.

Results

Of 61 tested samples, 05 were positive for hantavirus genome. These results were confirmed at reference laboratory as well and species identification result is pending.

Of 58 tested samples, 06 samples were positive for hantavirus IgM by in-house ELISA. All PCR positive samples were positive for hanta virus IgM.

All patients with hantavirus infection had clinical and biochemical features of liver involvement in addition to fever, thrombocytopenia and renal involvement.

Conclusion

Established RT-PCR assay was able to detect a wide range of hantaviruses and by using it molecular evidence of hantavirus infection was demonstrated in humans in Sri Lanka.

Further studies are required to describe the disease epidemiology and to identify natural hosts in the country.

OP 15

Enhancing the sensitivity of methicillin-resistant *Staphylococcus aureus* (MRSA) to oxacillin by tea catechins and proanthocyanidins

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Introduction

The emergence of MRSA has become a disturbing clinical problem. New antimicrobials and novel strategies for treatment are urgently required to combat drug-resistance. The tea plant (*Camellia sinensis* L.) is a potential source of non-toxic and low-cost antimicrobials.

Objectives

To evaluate the antibacterial activity of catechins and proanthocyanidins extracted from green tea - against MRSA, and to determine the minimum inhibitory concentration (MIC) of oxacillin against MRSA incubated with catechins and proanthocyanidins.

Methods

Green tea was separately extracted into 70% aqueous methanol and 70% aqueous acetone. Solvent fractionation of the methanol extract with dichloromethane and ethyl acetate followed by concentration of the ethyl acetate extract yielded catechins. The acetone extract, after washing with hexane, was concentrated to obtain proanthocyanidins. The antibacterial activity of catechins and proanthocyanidins was tested using agar cut well method against *S. aureus* NCTC 6571, 9 strains of MRSA and 2 strains of methicillin-sensitive *S. aureus* (MSSA) isolated from the clinical samples received in the laboratory. The MIC of oxacillin was determined, by agar dilution method, for each MRSA strain after incubation with different concentrations (1, 10, 100, and 1000 ppm) of catechins and proanthocyanidins and varying incubation periods (0, 2 and 4 hours).

Results

Proanthocyanidins inhibited all the *S. aureus* strains (inhibition zone, 22-30 mm) at 5000 ppm. Catechins inhibited all (inhibition zone, 18-28 mm) except 2 MRSA strains. The MIC of oxacillin decreased from 32-128 ppm to 1-8 ppm and 0.12- 4 ppm upon incubation of MRSA with catechins (1000 ppm) and proanthocyanidins (1000 ppm) for 4 h, respectively. The enhancement of sensitivity of MRSA to oxacillin increased with increasing concentration of catechins and proanthocyanidins (1 to 1000 ppm) and with increasing incubation time (0 to 4 h).

Conclusion

Tea catechins and proanthocyanidins showed antibacterial activity against MRSA and MSSA. The incubation of MRSA with catechins or proanthocyanidins reduced the MIC of oxacillin to MRSA in time- and dose-dependent manner. Further studies with more isolates are being carried out to confirm the results.

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OP 16

The prevalence of urinary tract infections in post renal transplant patients within the first six months of the post-transplant period

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Introduction

Urinary tract infections (UTIs) are the commonest infectious complication following renal transplantation and may be associated with increased mortality and graft failure in transplant recipients. The prevalence of post renal transplant UTIs differed widely in previous studies. This study was the first to assess the prevalence and bacteriological causes of post renal transplant UTIs in Sri Lanka.

Objective

To determine the prevalence of UTIs and their causative organisms in post-renal transplant patients admitted to TH Kandy.

Design, Setting and Methods

A prospective, descriptive study was carried out from September 2009 for six months duration at the Transplant Unit, TH Kandy. Patients undergoing renal transplantation during this period were included after informed consent. Ethical clearance was obtained from the Ethical Committee, Teaching Hospital, Kandy.

Urine full reports and cultures were taken pre-operatively and post-operatively on days 1, 3, 7 and monthly for six months following transplant. Patients were assessed for features suggestive of UTIs.

Un-centrifuged urine was examined microscopically to determine the number of pus cells and red cells per high power field. It was plated on cysteine lactose electrolyte deficient agar (CLED) and the colony counts examined after 18 hours incubation at 37°C. Those giving a pure growth of more than 105 CFU/ml of urine were considered significant UTIs and the organisms were identified by their colony morphology and biochemical tests.

Results

Of the 39 patients included in the study, 2 (5.13%) had pre-operative culture positive UTIs with > 105 CFU/ml of coliform spp. They were treated pre operatively and did not develop UTI after transplantation within the first six months.

4 patients (10.26%) had culture positive UTIs within the six month follow up period. Of these 3 (7.69%) were within the first month following transplantation. Majority of the post-transplant UTIs were due to coliform spp whilst *Staphylococcus aureus* and *Pseudomonas* spp were 25% each.

Conclusion

The prevalence of UTIs in renal transplant recipients within the first 6 months was found to be 10.26% and majority of the infections occurred in the first month. Gram negative bacilli were the commonest bacteriological cause.

PP 1

Immunogenicity study following reduced dose (4 doses) intradermal vaccination for anti-rabies post exposure therapy

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Introduction

Rabies is a zoonotic 100% fatal viral encephalitis, but preventable by prompt application of post exposure prophylaxis. However due to the high cost of vaccines and long duration of vaccine regimens, variety of affordable and practical empirical schedules, vaccine doses and routes of vaccination have been recommended over time, based on immunogenicity, clinical experience and epidemiology as modern biologics have improved and scientific knowledge has grown dramatically during the last few decades. Historically the total number of rabies vaccine doses administered for human prophylaxis has decreased and this not only reduces the number of clinic visits but also increases patient compliance.

Objectives

To study the immunogenicity of WHO approved reduced dose (4 doses) 2-2-2-0-2 on D0, D3, D7, and D30 intradermal (ID) vaccination for anti-rabies post exposure therapy (PET) in healthy adults following exposure to suspected rabid animals.

Evaluate the degree of protective antibody levels in patients following anti-rabies post exposure vaccination on day 14 and day 90.

Methodology

This cross sectional prospective study was carried out from October 2011 to April 2012. Ethical clearance was obtained from scientific and ethical review committee, Medical Research Institute, Colombo. Eighty healthy adults presenting to the ARU (Anti Rabies Unit) at NCTH (North Colombo Teaching Hospital) Ragama with minor exposures to suspected rabid animals were recruited in the study after informed written consent. 3 ml of blood from each subject was drawn on days 0, 14 and 90. Antirabies neutralizing antibodies were assessed by RFFIT (Rapid Fluorescent Focus Inhibition Test). The titer of virus suspension was calculated using the method of Reed and Muench.

Results

All subjects had NAT (Neutralizing antibody titres) above 0.5 IU/ml, WHO approved minimum protective titres on day 14 (11.44 IU/ml) and day 90 (5.375 IU/ml). Five who had initial antibody titres, developed very high NATs due to anamnestic reaction.

Conclusion

The new modified 4 dose ID antirabies post exposure therapy recommended by WHO is effective and produces adequate neutralizing antibody levels against rabies virus infection.

PP 2

Mobile Malaria Clinics: an important aspect of extended malaria surveillance

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Introduction and Objectives

With the incidence of malaria in Sri Lanka declining, parasitological surveillance has been identified as a key component to achieve elimination by 2014. Tropical and Environmental Diseases and Health Associates Private Limited, carries out active case detection among high risk groups by mobile malaria clinics (MMC) in Mannar, Trincomalee, Batticaloa and Ampara districts. A study was carried out in one district to evaluate the outcomes of MMCs and assess participant satisfaction and acceptance towards the MMCs.

Design, setting and methods

The number of MMCs carried out and the number of malaria positive patients diagnosed in the Mannar district in 2010 and 2011 were recorded. A cross sectional study was carried out in July 2011 to grade the satisfaction of participants above 12 years of age with regard to conduction of the MMCs. Participants attending two of the nine MMCs carried out during that month, one in a school and one in a school which also enabled the community to attend were recruited consecutively. Satisfaction with regard to the MMCs was determined using a scale ranging from 0-5. Interviews were conducted by investigators assisted by trained interpreters.

Results

A total of 213 MMCs have been carried out in the Mannar district over the two years screening 17,823 persons for asymptomatic malaria infections, including armed forces personnel, communities and schools. Seven positive cases, all *Plasmodium vivax* infections were detected.

Of a total of 188 participants above 12 years of age who participated in the MMCs, 142 (78.9% from schools and 21.1% from the community) gave consent for interview. Approximately half (50.73%) of the participants were females and majority were students (69.72%). The median age was 16.0 years. Significant factors influencing the participants overall satisfaction with the service included satisfaction with the blood taking procedure, cleanliness, duration of time taken to give the result and knowing the reason for conducting the MMC ($p=0.026$, 0.005, 0.04 and <0.001 respectively). Complete satisfaction with cleanliness was seen in all participants of indoor MMC while only 55.76% participants were satisfied with the cleanliness of outdoor MMCs. Knowing the reason for conducting an MMC was associated with perception of malaria as a serious illness, prior education regarding malaria and awareness of symptomatology of the disease.

Conclusions

MMC are a valuable method of intensive parasitological surveillance in the process of achieving malaria elimination state. Participant satisfaction should be considered, when organizing and carrying out a MMC which includes organizing it in an indoor area. Improving community awareness will increase the acceptance of MMCs as a method of active malaria surveillance.

Acknowledgements

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PP 3

Study on *Acinetobacter* isolates in Intensive Care Unit (ICU) Base Hospital Angoda (IDH)

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Objectives

To calculate the prevalence of *Acinetobacter spp* and their antibiotic resistance rates in an ICU setting

Design, setting and methods

A descriptive study was carried out on all patients who were in the Intensive Care Unit of Base Hospital Angoda from 01/01/2012 to 01/01/2013. Retrospective analysis of clinical data, microbiological culture results and antibiotic sensitivity test (ABST) patterns were carried out.

Results

The total number of patients managed at the ICU within the time period was 256. Out of which, 32 patients had positive bacterial culture reports. Thirteen out of the 32 patients (40.6%) had at least one positive culture of *Acinetobacter spp*. A total of 27 isolates of *Acinetobacter spp* were detected from sputum, urine, blood, cannula tip, CVP line tip, endotracheal and tracheostomy tube secretions. Endotracheal tube secretions accounted for 16 (59.2%) of these positive cultures. Eleven out of the 13 were intubated for mechanical ventilation. The average number of days taken to detect the first *Acinetobacter spp* positive culture following ICU admission was 8.6, ranging from 2 to 15 days. Nine out of the 13 patients were transfers from the NHSL, while 4 were first admitted to B. H. Angoda. Six of the 13 patients died while in the ICU. The resistance pattern of the *Acinetobacter spp* isolates were as follows; Amikacin 77.3%, Gentamicin 84.6%, Ceftazidime 91.6%, Ciprofloxacin – 88.0%, Imipenem 84.0%, Meropenem 94.1%, Piperacillin/Tazobactam 93.7%, and Ticarcillin/Clavulanate 82.3%.

Conclusions

The rate of *Acinetobacter* infection/colonization reported from around the world is said to be about 10-30%. The current study shows a rate of 5.07%. A major concern is that most of the *Acinetobacter spp* isolates detected in the ICU, BH Angoda were multi-drug resistant. A prominent feature was the high rate of detection seen in patients who were intubated and ventilated. It is highly recommended that further studies be conducted on this nosocomial pathogen.

PP 4

A case of brain abscess diagnosed following positive blood culture

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Introduction

In a substantial number of cases, the cause of brain abscess is unknown. No primary focus of infection can be identified in 10% to over 60% of cases.

Case report

A 45 years old female patient, presented to General Hospital Polonnaruwa, with headache for 3 weeks and fever with vomiting for 3 days. She did not give a history of significant past medical conditions but was diagnosed as having type 2 diabetes after admission. She was treated with IV cefotaxime on the clinical diagnosis of meningitis but after 24 hours of admission she developed drowsiness and altered behavior as well. Cerebrospinal fluid (CSF) and blood cultures were done on admission. CT brain was not performed initially.

Her white cell count was 14800/mm³ with neutrophil leukocytosis. CSF full report was highly suggestive of meningitis having polymorphs 3600/μl, and protein 286mg/dl. She seemed to be responding to the initial treatment. Her blood culture became positive for extended spectrum beta lactamase (ESBL) producing coliform spp and the antibiotic treatment was changed to IV Meropenem. Her CSF and urine cultures were negative. We concentrated on her presenting symptoms and suggested a CT brain which clearly showed a well-defined cerebral abscess in the frontal lobe with local mass effects.

Neurosurgical opinion was to manage with antibiotics alone and she responded very well. Retrospectively she was diagnosed as having atrial septal defect (ASD) with severe pulmonary hypertension with possible right to left shunting which can be a risk factor for spreading a septic embolus to the brain.

She is still being followed up in the clinic with repeat CT scans and C-reactive protein levels and she is on antiepileptic and other supportive treatment. No evidence of any sequelae noted yet.

Conclusion

With correct investigations and high index of suspicion brain abscesses can be detected early and be successfully treated with or without surgical drainage. Though the primary source of ESBL producing coliform could not be found, diabetes and ASD could have been the risk factors. Optimal assessment of the likely pathogenesis of the lesion and causative pathogens is essential for a favorable outcome.

PP 5

What oral antibiotics available for paediatric UTI? Oral antibiotic sensitivity pattern of Enterobacteriaceae

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Introduction

In children, appropriate treatment of urinary tract infections (UTI) prevents development of pyelo-nephritis, urosepsis and renal scarring. Children with acute UTI are started with parenteral and then oral antibiotics are needed to complete the treatment. Antibiotic resistance, especially of Extended Spectrum Beta Lactamases (ESBL) is on the rise and it varies between hospitals. This study was conducted to determine the oral antibiotic sensitivity pattern of urinary Enterobacteriaceae in children.

Method

This descriptive cross-sectional study on all urine cultures done of patients below 12 years of age was carried out from January 2011 to December 2012 in a district general hospital in Sri Lanka. Standard isolation and identification was carried out. Antibiotic susceptibility test was done by Stokes disk diffusion method. Disk approximation test using clavulanic acid was done to identify ESBL production. Descriptive statistics were used in the analysis.

Results

Of 1657 cultures, 260 were positive and 90% (232) of them were Enterobacteriaceae. Among Enterobacteriaceae, 44% (103) were from children below 1 year of age while 35% (82) were between 1 to 5 years and 20% (47) were over 5 years of age. Ampicillin, cephalixin, cotrimoxazole and nalidixic acid resistance was 80%, 63%, 55% and 45% in all age groups respectively. However cefuroxime and co-amoxiclav resistance was about 47% and 50% below 1 year and over 5 year children but it was 50% and 45% in 1-5 year children. Nitrofurantoin sensitivity is very good (>77%) in all age groups. Although norfloxacin and ciprofloxacin sensitivity was over 70% below 5 years, it was 57% and 72% respectively in children over 5 years. ESBL rate was 10% in 2011 but 22% in 2012. ESBL rate is high in children below 5 years when compared to those over 5 years.

Conclusion

Nitrofurantoin showed a very good susceptibility. Cephalixin, cotrimoxazole and nalidixic acid used in prophylaxis showed a high resistance. Therefore breakthrough infections can occur. Co-amoxiclav is a common antibiotic used in the treatment of paediatric UTI, but the susceptibility is only 50% in our hospital. ESBL rate more than doubled in one year. Local antibiotic sensitivity data and ESBL prevalence should be considered to prevent treatment failures in children.

PP 6

An audit of compliance with hand hygiene using alcohol hand rub in a District General Hospital

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Introduction and Objectives

Effective Hand Hygiene (HH) is the single most important strategy in preventing health care associated infections and preventing the spread of antimicrobial resistance. However HH practices have been universally poor among health care workers (HCW). Limited physical resources and heavy workloads have been identified as barriers to compliance. Improved compliance has been reported following education, introduction of alcohol hand rubs

(AHR) and local promotion activities. AHR is indicated for situations when hands are not visibly soiled. We conducted this audit to determine the AHR compliance rate by before and after events and by the HCW type.

Method

HCWs attached to surgical, medical and obstetric units were included in the audit. All HCWs were observed by an observer during their working shift using a check list for “5 before” and “5 after events”. After pre audit results, AHR availability was increased and hand hygiene posters were displayed in the units. Post interventional audit was carried out using the same check list.

Results

56 HCWs (32 nurses 20 doctors, 4 minor staff) participated in the audit. Over all alcohol hand rub compliance rate for “before events” was 37% and “after events” was 48%. Nurses, doctors and minor staff AHR compliance in all “before events” was 43%, 41%, 33% respectively and for “after events” 48%, 54% 41% respectively. Use of AHR among nurses before giving parenteral medication was poor in surgical and medical units. AHR compliance after removal of gloves and after touching the patient was good but before was very poor in all categories.

Discussion

Over all HH compliance rate was poor. Increasing the availability of AHR bottles or reminder posters made little or no impact on the compliance of HH among HCWs in our hospital. Therefore, continuous education is necessary in order to change attitudes and to emphasize the importance of correct practice of HH.

Conclusion

Regular audits are essential to understand the actual rate of HH compliance.

PP 7

Surveillance of occupational blood and body fluid exposures among health care workers at the National Cancer Institute of Sri Lanka (NCISL) 2000 – 2010 A prospective study

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Introduction

Accidental needle stick injuries and other high risk exposures are occupational hazards for healthcare

workers. They are at high risk of contracting blood borne infections in their daily work through these accidents.

Objectives

1. To review epidemiology of needle stick injuries (NSIs) and other high risk exposures
2. To identify trends in accidental exposures in relation to new protocols, new recruitments and educational activities

Methodology

A prospective descriptive study was carried out from 2000 to 2010. All NSIs and other high risk exposures reported to the infection control unit through an accident reporting register and discussion followed with victims were recorded.

Results

There were 329 reported needle stick injuries and mucus membrane exposures with increasing numbers since 2006. The highest reported numbers were in 2006 among all categories of staff except in paramedical staff members, with awareness programmes on management of NSIs. The highest numbers were among nursing followed by medical staff. Among assistant staff incidents were during cleaning instruments or needles in the sharps bin during its removal. All exposures were due to non-adherence to standard precautions. Mucus membrane exposures were very low. There was a recognized increase in number of exposures per 1000 respective health care workers only among nurses and paramedics especially Laboratory Technologists in 2010.

Awareness on hepatitis B in 2002 and end of 2005 led to an increase in number of staff vaccinated. Reviewed policies for vaccination by end of 2005 and in 2006 and continued awareness through 2007 onwards led to the increasing coverage. But the victims with known antibody level had been low throughout regardless of the available facility onsite.

Conclusions

Importance of adherence to standard precautions has to be further emphasized. Although a high percentage of HCW have been fully vaccinated against hepatitis B, efforts must be made to improve the coverage and testing of hepatitis B antibody status in them has to be encouraged. Safety awareness to minimize NSIs including the exposures through sharp bins has to be carried out periodically.

This has been presented at the 5th International Congress of the Asia Pacific Society of Infection Control in 2011 as a poster.

PP 8

A case of *Arcanobacterium haemolyticum* associated with abscess formation and cellulitis

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Case report

A 65-year-old female with a history of uncontrolled diabetes mellitus presented with a lower leg cellulitis and an abscess. Following incision and drainage we received a specimen of aspirated pus.

Pus culture yielded a heavy growth of small beta-hemolytic colonies on blood agar with no MacConkey growth. It was Gram positive bacilli, catalase-negative and non-motile. Stokes disk diffusion antibiotic susceptibility test showed susceptibility to penicillin, cephalaxin, clindamycin, erythromycin, ciprofloxacin, gentamicin and vancomycin but resistant to trimethoprim-sulfamethoxazole.

Then we followed the identification tests suggested in the literature. It showed a 12 mm zone of inhibition with bacitracin (0.04 U) susceptibility test. Furthermore, it showed cross-agglutination with all Lancefield antisera for groups A, B, C, D, F & G. There was no growth on Tellurite medium.

It was confirmed as *Arcanobacterium haemolyticum* with the CAMP test and reverse CAMP test using *Streptococcus agalactiae* and *Staphylococcus aureus* ATCC 25923, respectively. Cellulitis subsided with penicillin and the patient was discharged home after 7 days of penicillin.

Discussion

A. haemolyticum could be misidentified as *Streptococcus* or *Corynebacterium* species. Microscopic morphology differentiates *A. haemolyticum* from *Streptococcus* species and by absence of catalase from *Corynebacterium* species.

Arcanobacterium haemolyticum was first described by MacLean and others in 1946 as a pathogen in cases of exudative pharyngitis and soft-tissue infections. Currently, there are four identified species within this genus: *A. haemolyticum*, *A. pyogenes*, *A. phocae*, and *A. bernardiae*.

A. haemolyticum has been implicated as a causative agent of non-streptococcal pharyngitis in adolescents and skin and soft-tissue infections in older, immunocompromised patients and also in osteomyelitis, pneumonia, endocarditis, sepsis, and central nervous system infections.

Conclusion

A Gram-positive rod that exhibits β -hemolysis on human blood agar, that is catalase negative, and gives a positive result with the CAMP test and reverse CAMP test using *S. agalactiae* and *S. aureus* respectively, may be identified as *A. haemolyticum*.

A. haemolyticum as a pathogen is probably under-reported; therefore, the microbiological identification of this organism is emphasized.

PP 9

Dairy products spoilage

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Introduction

Microorganisms can “spoil” food items by increasing their numbers. They can render our food unfit for consumption. Milk is an excellent culture medium for microorganisms, being high in moisture, neutral PH and rich in microbial food.

Milk and other dairy products are preserved in a number of different ways, which involve killing of part of microorganisms and inhibition of the growth of the remainder. Therefore they have a limited keeping time and may spoil if the methods of preservation are inadequate.

A study was carried out to find out how satisfactory the available milk and other dairy products are for consumption in the open market.

Objective

To determine the microbiological contamination of milk and milk products.

Method

A retrospective study was done on all the milk and milk products received at the Food Microbiology Laboratory at National Institute of Health Sciences, Kalutara; between 01st January and 31st December 2012. Samples were brought by Public Health Inspectors when the new product is introduced to the market, if there is a suspicion or a complaint by the customers and also randomly.

Tested food items were sweetened full cream condensed milk, pasteurized milk, sterilized milk, milk powder, fermented milk (curd), ice cream, butter, cheese, yoghurt, chocolate and milk added drinks.

Food samples were processed (test methods) and reported according to Sri Lanka standards approved by the Sri Lanka Standards Institute (SLSI).

Results

Total number of dairy samples processed during the study period was 152 and 47.3% (72/152) of dairy samples were unsatisfactory as the tested parameters were not within the specified bacteriological limits.

51% of samples were satisfactory.

1.7% of samples were acceptable. [Acceptable - Level of microbiological contamination falls between satisfactory and unsatisfactory (according to SLS) hence need interventions to improve hygienic conditions and repeat the test].

Sixty eight of total dairy samples were yoghurts of which 58% (40/68) unsatisfactory. Majority (37/40) of the yoghurt samples were contaminated with yeast and moulds. Other 3 samples were contaminated with *Escherichia coli*.

Conclusion

Milk and milk products spoil during processing, improper or unsatisfactory preservation or storage. Implementing an educational program on, method of spoilage, preservation and storage of dairy products for manufacturers and handlers is important. Regular monitoring and supervision of manufacturing process and storage facilities will be necessary.

Surveillance of dairy products needs to be done to assess their microbiological quality and to ensure the consumer safety.

PP 10

Analysis of dried blood spots of children with sensorineural hearing loss due to possible congenital cytomegalovirus infection: A preliminary study

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Introduction

Dried blood spots (heel prick blood, spotted on special filter paper) are routinely obtained from newborns to screen for metabolic disorders in the state of New South Wales, Australia. Literature shows that it has been used to screen for congenital infections. Congenital cytomegalovirus infection is the leading cause of sensorineural hearing loss (SNHL) in the developed world. Analysis of DBS may provide vital information to make policy decisions on screening of congenital infections.

Objectives

To determine the incidence of congenital CMV infection in infants identified with sensorineural hearing loss by analyzing DBS.

Design, setting and methods

Children who presented to hearing impairment clinics of four hospitals between January 2011 to August 2011 were recruited for the study. Children with acquired hearing loss were excluded. Informed written consent was obtained from parents using the special state request form of retrieving DBS. Clinical features of children were obtained from medical records. Punches of a diameter of 3 mm from DBSs with a single-hole paper puncher was used for extraction. Extraction of DNA from DBS was performed manually by using QIAamp spin column (Qiagen, Germany) according to a previously published protocol. CMV DNA was detected by realtime PCR Lightcycler 450 platform (Roche, Germany). A positive result for CMV DNA was repeated.

Results

Only 57 DBS cards were available for testing from 65 children. Mean age at presentation for DBS testing was 7.4 months (range 1 to 60 months). Seven out of 57 DBS were repeatedly positive for CMV DNA. Incidence of CMV viraemia was 12.28. Children with positive DBS, gave histories of mild hearing loss (3) profound hearing loss after rapid deterioration (2) and global development delay (1). History of the other child is not available.

Conclusions / Recommendation

Further studies are recommended to assess the usefulness of DBS as a screening tool for congenital cytomegalovirus infection.

PP 11

Incidence of congenital rubella syndrome / congenital rubella infection and characterization of rubella virus from samples presented to MRI

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Introduction

Congenitally transmitted rubella virus infection can cause severe birth defects. Congenital rubella infection (CRI) when associated with severe defects is defined as congenital rubella syndrome (CRS). CRS was greatly reduced globally, following introduction of rubella vaccination. CRS is confirmed by detecting virus specific IgM antibodies. Virus genotyping provides important epidemiologic information.

Objectives

To describe the incidence of CRS between 2010-2012 among specimens received at MRI.

To characterize circulating rubella virus in Sri Lanka by virus isolation, PCR and sequencing.

Design, setting and methods

Serum samples received at MRI between January 2010 to December 2012 from infants suspected with congenital rubella infection, were tested for rubella IgM using a commercial kit (*Siemens – Dade Behring*). Available clinical history was analysed for clinical features of CRS. Virus culture and PCR were performed in nasal and throat swabs from 5 infants. PCR products from three of these were sent to the Regional Reference Laboratory in Thailand for sequencing.

Results

Thirty samples out of 3305 tested during this period 30 were positive for rubella IgM. Eight (1037), 4 (907) and 18 (1366) samples were positive in 2010, 2011 and 2012 respectively. Out of the 3 samples sent to Thailand, 2 were type able (genotype 2B). Clinical history was available only in 16 cases of IgM positives. The common congenital defects were congenital heart disease (8/16), hepatomegaly (5/16) IUGR/ low birth weight (4/16), failure to thrive (3/16) corneal opacities, microphthalmia and chorioretinitis (1/16).

Incidence of CRS reported at MRI from 2010 to 2012 is 2.06, 1.32 and 4.77 per 100,000 live births respectively. The incidence of CRS reported at MRI from 2006 to 2009 is 0.26, 1.05, 1.05 and 0.79 per 100,000 live births respectively.

Conclusions

A high incidence of CRI/CRS occurred in 2010-2012. The increased number of cases in 2012 could be due to several outbreaks of rubella infection in 2011.

Genotype 2B has been the circulating genotype of rubella virus in the past few years. The present outbreak is not due to a new imported virus.

Recommendation

There should be an uninterrupted, closely monitored vaccination programme to prevent similar episodes.

PP 12

The prevalence of carbapenemase producing in *Enterobacteriaceae* isolated in two selected teaching hospitals in Sri Lanka

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Introduction

Carbapenemase producing *Enterobacteriaceae* are now emerging worldwide and challenging to the current infection control and antibiotic therapy due to its high drug resistance and exceptional ability of rapid dissemination. Among those Carbapenemases KPC-type is a clinically common enzyme which can be detected by Modified Hodge Test (MHT) with $\geq 90\%$ sensitivity and specificity.

The general objective of this study

To determine the prevalence and associated risk factors for carbapenemase mediated carbapenem resistance in *Enterobacteriaceae* in two teaching hospitals in Sri Lanka.

Design, setting and methods

Enterobacteriaceae isolated from blood cultures and respiratory tract specimens from National Hospital of Sri Lanka (NHSL) and North Colombo Teaching Hospital (NCTH) from January 2012 to April 2012 were included in this cross sectional analytical study (total of 590 isolates, 558 from NHSL and 32 from NCTH). All isolated *Enterobacteriaceae* were screened with ertapenem disk diffusion test for carbapenem nonsusceptibility and positive isolates were subjected to MHT to detect carbapenemase.

Results

The prevalence of carbapenemase producers among *Enterobacteriaceae* isolated in NHSL is 7.9% and in NCTH is 0%. Of the NHSL isolates 100 gave positive results in screening test. Out of these isolates 44 gave positive results in MHT. Therefore, prevalence of carbapenemase producers among ertapenem non-susceptible isolates in the NHSL is 44.0%. None of the NCTH isolates gave the positive screening test results. The identified risk factors which are significantly associated with the prevalence of carbapenemase production among *Enterobacteriaceae* are prolonged hospital stay of > 5 days ($P < 0.001$), admission to special care units such as ICUs ($P < 0.001$), use of third generation cephalosporins and carbapenems ($P < 0.001$), previous admission to health care institutions ($P < 0.001$) and use of invasive devices like central venous catheters, femoral lines, endotracheal tubes and tracheostomy tubes ($P < 0.001$).

Conclusions

The carbapenemases are responsible for a significant proportion of carbapenem resistance in *Enterobacteriaceae* in NHSL. Appropriate measures should be taken to avoid exposure to risk factors as far as possible to prevent the acquisition of bacteria producing carbapenemases in health care facilities. The screening test with Epm disk diffusion and the confirmatory test with MHT for the detection of carbapenemase producing

Enterobacteriaceae should be established in routine clinical laboratories which will be useful for appropriate antibiotic therapy and infection control purposes.

Financial assistance by E. T. and R unit, Ministry of Health for research grant is acknowledged.

PP 13

Seroprevalence of varicella zoster antibodies in patients with chronic renal failure

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Introduction

Chronic kidney disease (CKD) is a major public health problem in Sri Lanka and the number of renal transplant surgeries performed has increased dramatically during recent years.

Chickenpox can lead to devastating consequences such as severe skin disease, visceral involvement, DIC in transplant recipients. Mortality is around 34%. Currently there is no screening or vaccine policy for varicella zoster virus (VZV) in CKD patients in Sri Lanka.

General objective

To ascertain the seroprevalence of VZV antibodies in patients with chronic renal failure (CRF) attending nephrology clinics in Colombo district Sri Lanka.

Specific objectives

1. To ascertain the seroprevalence of VZV antibodies in patients with CRF
2. To determine the association between a positive history of chickenpox and protective immunity to VZV in patients with CRF in the study population

3. To determine the seropositivity of VZV antibodies in patients with CRF who have received VZV vaccine

Methodology

Patients with end stage CKD were selected from four hospitals in Colombo District. Blood samples and data were taken following informed written consent from 196 participants. VZV specific IgG antibodies were detected using a whole VZV infected cell lysate antigen containing commercial ELISA kit.

Results

Estimated seroprevalence of VZV antibodies was 71.43%.

65.12% of the study population were awaiting renal transplant. Out of them 28.35% lacked VZV specific IgG. Predictive value of positive past history of chickenpox is 96.26% and predictive value of negative past history is 85.45%. Chi-squared independence test confirmed that at 5% significance level there is a strong association between history of chickenpox and VZV specific IgG status.

Only 5 had completed vaccination. They were positive for antibodies.

Conclusions

A significant number of CRF patients remains susceptible to VZV infection. As there is a strong association between history of chickenpox and VZV specific IgG status, vaccination of all history negative potential transplant recipients should be considered in the absence of facilities for serology.

Limitations

Efficacy of VZV vaccination could not be assessed due to the limited number of vaccine recipients.

Hospital acquired infections, antibiotic resistance and patient safety



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It gives me great pleasure and privilege to address you at the inauguration ceremony of the 21st Annual Scientific Sessions of Sri Lanka College of Microbiologists.

I would like to start my address with a quote by Florence Nightingale in 1823. I quote “It may seem a strange principle to enunciate as the very first requirement in a hospital that it should do the sick no harm” unquote.

A Hungarian physician Ignaz Philipp Semmelweis in 1847 was devastated that his First Clinic had a much higher mortality rate due to puerperal fever than the second clinic. The women were begging him not to admit them to the first clinic. He said “It made me so miserable that life seemed worthless”. The two clinics used almost the same techniques, and Semmelweis started a meticulous process of eliminating all possible differences, including even religious practices. The only major difference was the individuals who worked there. The First Clinic was the teaching service for medical students, while the second clinic had been selected in 1841 for the instruction of midwives only. He observed that women examined by medical students who had not washed their hands after leaving the autopsy room had very high death rates. This observation made him realize that hand decontamination can prevent puerperal fever. He ordered all his students to wash hands with chlorinated lime before examining patients. With this intervention he was able to bring down the maternal death rate from 12% to 1% in 2 years. Even though he was able to do this by enforcing hand decontamination he was unable to convince the medical community. His theory was ignored by other clinicians then and is still ignored by clinicians today. Unable to convince the medical community he spent his later life in an asylum.

Almost two centuries after the existence of Nightingale and Semmelweis, the extraordinary advancements in

medical knowledge and with access to modern technologies, we are still unable to ensure the safety of the sick who come to us for treatment.

During my speech today I am going to talk about the health and economic burden of hospital acquired infection and antimicrobial resistance globally and with specific reference to data from the National Hospital of Sri Lanka; and how these factors affect the safety of our patients. This is a subject which is dear to my heart, as it has been a focal point of my work over many years.

The burden of Hospital acquired infection is rising worldwide. Every year 1.4 million HAI occur throughout the world. Although most available data on HAI come from developed countries, the burden is likely to be greater in developing countries. The rates vary from 5% -10% in developed countries. According to 2011 Lancet article; pooled data showed the prevalence of HAI in developing countries to be 15.5 per 100 patients and the incidence in adult intensive care units as 47.9 per 1000 patient-days. Methicillin resistance was found in more than 50% of *S. aureus* isolates. In the United States, health care-associated infections are implicated in more than 99 000 deaths per year and this exceeds the number of deaths caused by HIV. The estimated cost associated with HAI in the US is around 7 billion dollars - 33 billion dollars / year. Although many countries are spending millions of dollars to treat these infections, the mortality remains high among those who are affected.

World Health Organization has recognized this as a critical issue. The High 5s Project was launched by the WHO in 2006 to address continuing major concerns about patient safety around the world. The major components of the High 5s Project include the development and implementation of problem-specific Standardized Operating Protocols. Addressing Health Care-Associated Infections – hand hygiene being one of the main elements.

Antibiotic resistance

The first antibiotic penicillin was discovered by Alexander Fleming in 1927 but antibiotics against infectious agents were introduced during World War 2. Infectious diseases like meningitis, tuberculosis and pneumonia declined due to subsequent introduction of penicillins, aminoglycosides and many other antibiotics.

With the introduction of antibiotics, bacteria started reacting to them and developed resistance. Resistance mechanisms have been described for all known antibiotics currently available for clinical use. Today, antibiotic resistance has become a major threat to human beings. The prevalence of multidrug resistant microorganisms both in the hospitals and the community is increasing worldwide; thus driving us back to the pre antibiotic era. The resistant organisms evolve, survive, spread and cause infections within health-care facilities. The emergence and rapid spread of resistance is a common occurrence in developing countries with uncontrolled antibiotic usage and overcrowding of health care facilities. A crisis has been building up over decades and today; common yet life-threatening infections are becoming difficult, or even impossible, to treat. It is time to take much stronger action worldwide to avert this ever-increasing health and economic burden.

Antimicrobial resistance among bacteria has been demonstrated even before the discovery of antibiotics by Fleming. Bacteria find a way to survive in adverse conditions by various means. They produce enzymes that will inactivate the antibiotics or they make their cell wall impermeable to antibiotics by changing the binding sites or target molecules; or they effectively pump out the antibiotics by efflux mechanism. Overuse and irrational use of antibiotics help the bacteria to develop resistance and also to select resistant mutants effectively.

The spread of resistant bacteria is facilitated by the transfer of patients between wards within a hospital, between different hospitals and more widely by travel. When Lancet reported a new drug resistant Gram negative bacteria NDM1 strain in 2010, the medical community worldwide were alarmed and, media reports blamed medical tourism for its spread.

While infectious agents are becoming more and more resistant to the antibiotics that are currently in use, not enough drugs are being developed to combat them

Over the years the number of new antibiotics coming to the market has decreased gradually. There are only a few antibiotics in the pipeline for the next five years or so.

The health and economic burden due to antibiotic resistant infections in hospitals varies between different countries and regions. In Europe, the death rate from hospital acquired infections due multi drug resistant bacteria is more than 25 000 per year. Infections due to multidrug-

resistant bacteria in the European Union are estimated to result in extra health-care costs of 1.5 billion • each year.

Hospital environments harbor multidrug resistant strains. These organisms will colonize the patient's skin and also can transfer them via the hands of healthcare workers and by other means. Touch surfaces in hospital rooms can serve as sources, or reservoirs, for the spread of bacteria from the hands of healthcare workers and visitors to patients. Although it is not the only way, hand hygiene is the most effective way to prevent infection. Improving hand hygiene alone can bring down the hospital acquired infection rates by 20-30%. Hospitals throughout the world display posters and banners to remind healthcare workers about the importance of hand hygiene and to change their attitudes.

These are some of the posters displayed at the National Hospital. This leaflet was given to each hospital worker to mark the world hand washing day in 2011. Alcohol hand rub has been provided to each bed in the ICUs. We were able to remove common towels for hand drying and provide the wards with single use hand towels. Even with all of these measures, the hand washing compliance is poor among the healthcare workers; especially among the doctors.

We carried out an audit to assess adherence to hand washing steps and knowledge about hand washing among healthcare workers in our hospital. 67% of nurses and 100% of doctors did not follow all the recommended steps for hand washing. Steps 6 and 7 were missed by all the doctors and knowledge about hand washing steps and indication was poor among doctors than nurses. Following this study we designed a poster campaign targeting the medical officers. But an ongoing audit shows hand washing compliance among medical officers is still around 28.8%. As clinicians ignored Semellweis in 1840, clinicians today are also ignoring our plea to wash their hands.

Respiratory tract infections and vascular catheter associated blood stream infection are the commonest health care associated infections among the ICU patients. These infections will increase the patients ICU stay, mortality and the cost associated with ICU care. Methicillin resistant *Staphylococcus aureus*, multidrug resistant *Acinetobacter* and *Pseudomonas* species are mostly responsible for these infections. Drug resistance causes increased and prolonged illness, a greater risk of complications and higher death rates. Crude mortality rates of 30% - 75% have been reported for hospital acquired pneumonia due to *Acinetobacter* species with the highest mortality among ventilated patients.

I would like to illustrate the magnitude of this problem with some presentations and papers that have been presented by my unit and through a few case scenarios. An outbreak of *Aspergillus fumigatus* meningitis occurred in five women following spinal anaesthesia for caesarian

section in 2005. They were admitted to the National Hospital for management. Three young mothers lost their lives due to this. Two of them survived with some neurological sequelae. Those who survived spent 60-62 days in the ICU. The source of these infections was traced to the syringes used for spinal anaesthesia which had been stored under highly unsatisfactory conditions in the government stores. The stores were full of regular medical supplies and donations after the Tsunami which occurred in 2004. Withdrawal and incineration of all unused syringes controlled the outbreak. Even though the outbreak was controlled, the damage caused to those babies who lost their mothers is immeasurable. Who was ultimately responsible for this unfortunate outbreak? Are we to blame the Tsunami? Are we to blame the government for accepting donations without adequate storage facilities? Are we to blame the administrators for not establishing the right policies? Unfortunately there was no one to take the responsibility or accountability for these lives.

A 45 years old male patient was admitted with a history of 44% of accidental burns. Two of his blood cultures became positive for MRSA two weeks after admission. By this time most of his burn wounds were healing. In spite of effective treatment with vancomycin, his fever continued and the third blood culture became positive for MRSA while on vancomycin. At this point his ECHO cardiogram revealed tricuspid valve vegetation. Right sided endocarditis occurs in patients with catheter associated blood stream infections. Since he did not respond to standard medical treatment, he had to undergo valvotomy 3 months after his admission. He spent 79 days in the hospital, including 10 ICU days due to this hospital acquired infection.

2006-2007

An analysis of central venous catheter tip culture results at the National Hospital of Sri Lanka

In a study conducted to analyse the results of central venous catheter tip cultures over a period of six months, it was found that 53% of catheters were colonized with possible pathogens. *Staphylococcus aureus* was isolated from 50% of catheter tips more than 70% of them were methicillin resistant.

Similar analysis done over 2 year period from 2008-2010 showed 56% of catheter colonization with 10.2% incidence of catheter related blood stream infections. 43% of the isolates were due to *Staphylococcus aureus* with >70% of them were due to MRSA. Out of the Gram negatives *Pseudomonas* species and *Acinetobacter* species were the commonest.

(A study of catheter related blood stream infections due to intravascular catheter colonization presented at the 12th Western Pacific Conference in Chemotherapy and Infectious Diseases in Singapore 2010).

How common is MRSA? A study to determine the prevalence of MRSA in the National Hospital of Sri Lanka.

We conducted a study to identify the prevalence of MRSA in NHSL over a 12 month period in 2007 and 2008. The study revealed that out of the total *Staphylococcus aureus* isolates 62% were MRSA.

VISA or VRSA? A study to detect the prevalence of VISA and VRSA in the National Hospital of Sri Lanka

A study was conducted in NHSL to detect the prevalence of Vancomycin intermediate and vancomycin resistant *Staphylococcus aureus*, where we tested 100 MRSA isolates by vancomycin agar screening test and found no VISA or VRSA. Even though we did not detect VISA or VRSA in this study, a subsequent study done in another hospital has demonstrated a gradual increase in vancomycin MIC.

Inducible clindamycin resistance in clinical isolates of *Staphylococci*

We conducted a research to detect the prevalence of inducible clindamycin resistance in *Staphylococci* and found 68% of isolates were MRSA and 45.8% of isolates showed inducible clindamycin resistance due to MLSB phenotype.

***Staphylococcus aureus* carrier states among patients awaiting cardiac surgery and the antibiotic susceptibility pattern of the isolates**

A study was carried out in 2006 and 2007 to determine the MRSA carrier rate among patients awaiting cardiac surgeries. Out of the 524 patients 23% were colonized with MRSA by the time they were ready for surgery. This shows the importance of minimization of pre operative stay of these patients

Preliminary study on aetiology and outcome of infections associated with VP shunts and EVD at NHSL

A preliminary study on aetiology and outcome of infections associated with VP shunts and EVD showed that the most common pathogen in EVD and VP shunt associated infection was due to methicillin resistant *Staphylococcus aureus* followed by *Pseudomonas* species.

Analysis of tracheal aspirate isolates in ICUs at the National Hospital of Sri Lanka

An analysis of tracheal aspirate culture of ICU patients from 2009 to 2010 showed a predominance of gram negatives. 26.5% were *Pseudomonas* species and 20.8% were *Acinetobacter* species. 40% - 70% of these isolates were multidrug resistant.

Presented at Annual Academic Sessions of Sri Lanka College of Microbiologists - 2010.

Clinical and laboratory parameters of *Salmonella paratyphi* infection in Colombo

A study conducted on clinical and laboratory parameters of *Salmonella paratyphi* infection in Colombo showed that the ciprofloxacin resistance was around 98%. Uncontrolled use of ciprofloxacin in the community has led to quinolone resistance making ciprofloxacin ineffective in the treatment of enteric fever 30th Anniversary Academic sessions; Post Graduate Institute of Medicine Colombo; 2011

Unpublished data

Surveillance on Hospital Acquired Infections (HAI) and antibiotic resistance pattern in Critical Care Units at NHSL – 2011

In a surveillance on HAI and antibiotic resistance pattern in critical care units at NHSL, showed 24% incidence of HAI and 31% of device associated infection rate in 2011. Ventilator associated pneumonia was the commonest type of infection.

MRSA was found in 48% of *Staphylococcus aureus* isolates and 34% of gram negatives were multidrug resistant.

This study shows that the prevalence of MRSA and multidrug resistant gram negatives has decreased at the National Hospital when compared to previous years statistics. This may be due to improvement of Infection control practices, introduction of various guidelines and awareness programs that has been carried out by us.

A study to determine the prevalence of KPC carbapenamase producing coliforms at NHSL showed a prevalence of 8.06%. These organisms are resistant to carbapenem antibiotics such as imipenem and meropenem.

A study to determine the prevalence of vancomycin resistant *enterococci* colonization among ICU patients.

According to this study, the prevalence of vancomycin resistant *enterococci* was 6.4%, as opposed to a similar study done at National Hospital in 2001 which showed none.

Ladies and gentlemen, I hope that my presentation has convinced you of the very real and significant issue the health care profession is faced with today. Multi model and multi disciplinary interventions are necessary to bring a system change in the country, individual institute and in health care worker behavior. Unless we all get together, combating this situation would not be possible.

Health education has been identified as an effective method to bring down infection rates in hospitals. During the last 4 years I was able to conduct 6 refresher programs for infection control nurses with financial assistance from WHO. 200 Infection control nurses working throughout the country participated in these workshops. Through them we will be able to impart knowledge to the staff in their respective hospitals. Physiotherapists plays a major role in managing ICU patients. Therefore during this year our College conducted workshops for the paramedical staff, including physiotherapists working throughout the country.

The College was able to conduct workshops in different provinces for medical officers regarding the rational use of antibiotics. We plan to conduct a few more workshops before the end of this year.

Good laboratory surveillance is essential to detect drug resistance strains accurately. Addressing this issue, our College has initiated antimicrobial resistance surveillance project and conducted many workshops for the medical laboratory technologists during the last two years in different provinces. I was able to organize all these workshops and seminars due to the untiring dedication of our membership.

We need strong administrative support to minimize hospital acquired infection rates and the incidence of drug resistant organisms in our hospitals. An antibiotic policy and stewardship program with strict implementation is necessary to overcome this threat. Introduction of bundle care in the management of patients and adherence to aseptic procedures by all categories of staff is essential to bring down HAI rates; especially ventilator associated pneumonia and catheter related blood stream infections, thereby reducing the mortality in ICU patients. Strengthening of infection control committees and allocation of funds for infection control activities should be a priority. This will in turn reduce cost and improve the quality of patient care.

Ladies and gentlemen, let us join hands to ensure the safety of our patients by helping to improve and strengthen infection control and by using antibiotics rationally to prevent the emergence and spread of multidrug organisms within our hospitals and the community. Let us save the antibiotics for our future generation.

I would like to thank all of you for your patience in listening to my address and for gracing this occasion today.

Gonococcus: The story of the old villain



Dr. Sujatha Mananwatte

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Colombo 10*

Thank you Madam President for that kind introduction and good evening ladies and gentlemen. I would like to thank the Sri Lanka College of Microbiologists for giving me this great opportunity to pay my gratitude to a wonderful person whom I was fortunate to have been taught by.

Dr. Philonena Chandrasiri, President of the Sri Lanka College of Microbiologists, Mrs. Ranganie Wickremasinghe, family members and friends of late Dr. Wickremasinghe, chief guest Prof. Sriyal Peiris, members of the council, members of the Sri Lanka College of Microbiologists and distinguished invitees. We are here to cherish the memory of a distinguished personality and an eminent microbiologist this country has produced.

I am honored to be invited to deliver the 2012 Siri Wickremesinghe oration.

Late Dr. Rakkitha Sirimal Bandara Wickremesinghe, or Dr. Siri Wickremesinghe as we called him, whose life we remember today, was born on 28th November 1937 to Dr. Artie and Helen Wickremesinghe. He obtained his education at Royal College Colombo and his MBBS in 1963 from the Faculty of Medicine, Colombo.

He started his medical career in the dermatology unit at Kandy and thereafter joined the Medical Research Institute (MRI) to work in the Department of Microbiology. 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. He continued to work at MRI as the Consultant Microbiologist until he left to Australia with his family. Having worked as a successful microbiologist in the Fairfield Hospital in Melbourne, he came back to the MRI and continued as the consultant microbiologist in charge of the bacteriology division until his retirement. He was the

Director of MRI from 1996 to 1998. After retirement he worked as the Resident Pathologist and Laboratory Manager at Durdans Hospital, Colombo.

He was a past President of the Sri Lanka College of Microbiologists and the Secretary to the Board of Study in Microbiology at the Postgraduate Institute of Medicine at the very early stages, during a difficult time when the board was established.

Apart from teaching and his professional commitments, his interests were many. He was a walking encyclopedia. We know of people who read widely. They know something about everything. But Dr. Wickremesinghe, I thought was exceptional. It was as if he knew everything about everything, whether it was sports, wild life, history or even religion. He could talk on any topic with equal ease. Be it food poisoning by coleslaw, history of Muthurajawela, dusra of Muralitharan or even the four noble qualities of a person according to Buddhist scriptures, Metta, Muditha, Karuna, Upeksha. His knowledge was profound and abundant. He was also a voracious reader and that was something I admired in him.

Ladies and gentlemen I do not think I have sufficient time to talk about all his academic and professional achievements, and his wide interests, to day. It will require a lot of time to capture all his achievements and the contributions he made to the betterment of microbiology in Sri Lanka. As you will all agree, that cannot be done in a short time like this. But let me take this occasion to reflect upon some of the key things I could remember about him during the short period I was fortunate to know him.

I first met Dr. Wickremasinghe at the old MRI when I enrolled for postgraduate training for the Diploma in Microbiology. At that time we were not fully released to the PGIM but had to continue in our respective places of

work during the week and follow the lectures during the weekends. Since I was not getting sufficient exposure in general bacteriology at the VD Clinic I was asked to work under Dr. Wickremesinghe for some time. His first question to me was 'What experience do you have at the bacteriology bench'. My answer was 'none'. From the expression on his face I knew he was very disappointed. I knew that was not a very good start. So I went back to the VD Clinic and told my superior Dr. Nihal Perera that I had done a big mistake in selecting Microbiology because I did not know anything what Dr. W was talking about. He knew the size of every bacterium. He knew the colour, the shape, the features and even the smell of every organism. I was certain that I wouldn't be able to remember all that. Later, I realized that all these came in the package called 'Microbiology' and I had to learn them. However, it all ended very well and he even agreed to be the supervisor for my MD dissertation. Although he was very serious when teaching, he had a unique sense of humor. In the very early stages of the HIV epidemic when we wanted to screen the beach boys in the southern coast for HIV, we faced difficulties in getting to them. They all went underground when we approached them. I was discussing this with him and he promptly said "I can pose as a middle aged rich German and when I walk on the beach there will be enough and more boys coming behind me". He was the perfect fit for this purpose with his silver hair and fair complexion and the stature. He was serious about it.

He was a perfect teacher and role model to most of us who were fortunate to be his students. He hardly spoke about anything other than the subject, when he was teaching. He never inquired about our personal lives or about our families even when we were absent from work when our children were sick. His style was in sharp contrast to that of my superior Dr. Nihal Perera who always inquired about my family and advised me about the future of my young children and my future plans. Only when I went for my post MD training to Fairfield Hospital in Melbourne that I realized how concerned he was about his students.

When I was leaving for Australia, he very casually gave me the contact details of his family in Melbourne. He did not even tell me to contact them in case I needed some help. But to my surprise the day after I landed in Melbourne I had a visitor to my quarters. Mrs. Rangani Wickremesinghe. I was even more surprised to hear what she said. She said 'Siri wanted me to come and see you and find out how you were getting on'. This is the kind of person he was. He was ever willing to help his students in whatever way possible. Not only Rangani but his son Rakkitha too helped me to make my stay away from home as easy as possible. Even now that friendship is very dear to me.

There is so much I could recollect about him, but I will stop for now.

Ladies and gentlemen, in a situation like this it is customary for the speaker to select a topic close to his/

her heart. With my background everyone expected me to talk about HIV. But my first love was not HIV but the Gonococcus. HIV came some time later. Hence, my topic for today is, Gonococcus. The story of the old villain.

There are two reasons for selecting this topic for today.

Firstly, Dr. Siri Wickremasinghe was the first to introduce the testing of susceptibility of *Neisseria gonorrhoeae* to antibiotics, in Sri Lanka. That was the beta lactamase test which detects the plasmid mediated penicillinase production. The work I am going to present to you today could be in a way, called the continuation of that initiative.

Secondly, this work was undertaken by me initially as my MD dissertation and I have continued to work on it up to the end of my career. Dr. Wickremasinghe was my supervisor for the dissertation and I thought this would be an appropriate way to show my gratitude. I was greatly privileged to be able to have had him supervise my work and, therefore I dedicate this work to him.

I also think it is timely to talk about an acute problem that the world is facing now, in the management of gonococcal infection. Over the years gonococcus has developed resistance to many classes of antibiotics. It has led to a situation that the WHO is warning that we may run out of treatment options unless urgent action is taken. The WHO is urging governments and doctors to step up surveillance of antibiotic resistant gonorrhoea. Many countries are reporting cases of resistance to cephalosporin antibiotics, the last treatment option against gonorrhoea.

Gonococcus or more precisely *Neisseria gonorrhoeae* which was discovered by Neisser in 1879 is a very fastidious bacterium which causes the sexually transmitted infection, gonorrhoea; urethritis in men and cervicitis in women. It is a strict parasite and dies in 1-2 hours outside the body. Gonorrhoea is an exclusively human disease and transmission is almost exclusively sexual.

The name gonorrhoea (meaning 'flow of seed') was first employed in 130AD. Before that gonorrhea was referred to as 'clap'. There are various theories as to why this name was given. The most interesting is the one which claims that the name for the infection came from French brothels, which were known as 'les clapiers', and that the men who visited these brothels invariably ended up with the infection.

This is an article published in the California State Journal of Medicine in 1914, on the Treatment of gonorrhoea in the female. It says.....

- No routine treatment should be attempted.
- The bowels should be kept freely open, food should be of the simplest character, reduced in some cases to a milk diet, rest; preferably in bed, there should be a daily bath.

- Urethritis to be treated with irrigation using silver preparations.
- Endocervitis, with vaginal irrigation and mechanical cauterization.

The most radical treatment of an intractable infection is **the amputation of the cervix**.

The search for specific anti-bacterial drugs against gonorrhoea began in the 1890s. At the beginning most were metallic: compounds of arsenic, antimony, bismuth, gold, and mercury. This is an old patent medicine advertisement. It says an unequalled remedy for all unnatural discharges allays inflammation and cures gonorrhoe and gleet. Quickly relieves an irritated condition of the bladder and will not cause stricture.

With the advent of antibiotics the first reports of the effect of sulfanilamide on gonorrhoea appeared in 1937. Publications from Johns Hopkins University and from London showed that sulfanilamide cases responded much faster. The duration of hospitalization decreased from 50 days to 22 days. Complications reduced to 6% from 28%.

However year by year, increased amounts of sulfonamides were required to cure gonorrhoea. The efficacy of sulfanilamide was short lived. The initial euphoria that was created was diminished.

By this time Alexander Fleming had discovered penicillin. Immediately the efficacy against *N. gonorrhoea* was established. In the face of increased resistance to sulphonamides, penicillin started to be widely used for patients with gonorrhoea. At that time all gonococci were susceptible to a very small dose of less than 0.03 units of penicillin. Penicillin became the recommended antimicrobial regimen for gonorrhoea for the next 40 years. The response to penicillin was dramatic. Gonorrhoea could be cured in 4 hours. But this was not to be. A step by step resistance to penicillin developed. In 1946 four cases of gonorrhoea were reported which were resistant to even large amounts of penicillin. The number of strains which were resistant to penicillin increased gradually.

The effective dose that was sufficient at the beginning required serial increases. The 50,000 units that were initially effective to kill 96% of the gonococci were not useful anymore. The dosage was increased gradually. I can remember when I first joined the VD clinic in Colombo in the mid 80's gonorrhoea was treated with 4.8 million units of penicillin. This amounted to twelve vials of liquid; 6 vials into each buttock. Gonococci were building up resistance to penicillin.

This was the beginning of the long drawn struggle which continues to date. Attempts to find an antibiotic alternative to penicillin for the treatment of gonococcal infections were begun. After penicillin, tetracycline was found to be effective. This was also short lasting. Spectinomycin,

introduced in 1967, and then the aminoglycosides replaced tetracycline as the alternative to penicillin in the 1970s. Then came the macrolides; erythromycin and azithromycin. These were followed by the quinolones: ciprofloxacin and ofloxacin. In 1989 the 3rd generation cephalosporine, ceftriaxone was recommended as the primary antgonococcal treatment.

Over the past 70 years *N. gonorrhoeae* has developed resistance to multiple classes of antimicrobials. The graph shows the historical perspective in the United States. Arrows show a change in the treatment protocol with the development of resistance. Starting with sulfonamides in 1936 and ending with ceftriaxone at present.

It has not been very different in other countries. The picture had been the same in the United Kingdom. Sulphonamides were introduced in the mid 30s, ending with quinolones and 3rd generation cephalosporines.

The Sri Lankan scenario is not very different. We have followed the same pattern. We have opted for oral cephalosporines and the recommended choice now is cefuroxime axetil 1g in a single oral dose. This regimen meets less stringent efficacy criteria than cefixime which is the only oral regimen recommended for treatment of gonorrhoea. At the time the decision was made to switchover to oral cephalosporines, cefixime was not available in Sri Lanka.

We are now seeing a repetition of the situation observed with penicillin in the 1940s and 50s.

– Two cases of failed ceftriaxone treatment in pharyngeal gonorrhoea verified by molecular microbiological method. John Tapsall et al. Journal of Medical Microbiology (2009) 58: 683-687.

– Two cases of verified clinical failures using internationally recommended first-line cefixime for gonorrhoea treatment, Norway. Magnus Unemo et al. Euro Surveill. 2010; 15(47): pii=19721.

– Ceftriaxone treatment failure of pharyngeal gonorrhoea verified by international recommendations, Sweden. Magnus Unemo et al. Euro Surveill. 2011; 16 (6): pii= 19792.

Warnings such as these have become more frequent in the recent past. Gonococci with reduced susceptibility to cephalosporins are spreading not only in the United States and Europe but in the Asia Pacific and other regions of the world.

Because cephalosporins are the only currently recommended class of antimicrobials, it is critical that susceptibility to these drugs be actively monitored.

The words multidrug resistant and extensively drug resistant *Neisseria gonorrhoeae* (MDR GC and XDR GC) have become common topics of discussion.

This is a publication by the WHO expert team in 2009.

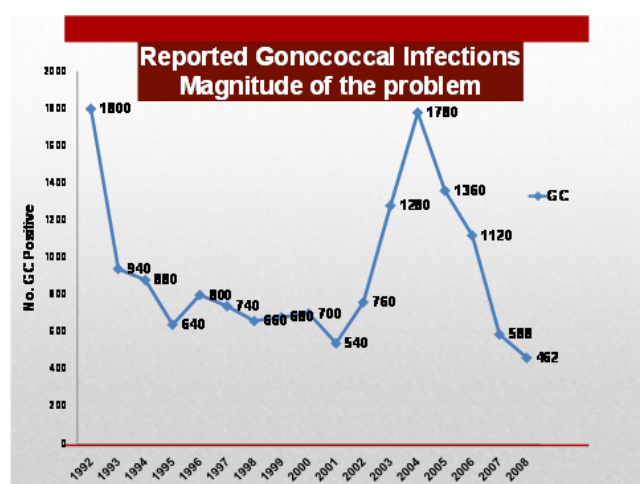
- Meeting the public health challenge of multidrug- and extensively drug-resistant *Neisseria gonorrhoeae*. John W Tapsall, Francis Ndowa, David A Lewis, Magnus Unemo. Expert Rev. Anti Infect. Ther. 7(7), 821- 834 (2009)

The WHO is making a plea to all governments and doctors to be vigilant.

How have we responded to this situation? What has been Sri Lanka's contribution to overcome this crisis?

Gonococcal Antimicrobial Resistance in Sri Lanka

The NSACP in Sri Lanka has around 30 STD clinics distributed throughout the country, of which six have facilities for gonococcal culture testing.



These are the absolute numbers of the reported gonococcal infections from these STD clinics over a period of about 20 years. All these infections have not been confirmed by culture. The culture confirmed reported cases which is equal to the number of isolates, are much lower than the reported infections.

Like in other countries we too had to change the treatment protocols with the development of resistance. In the 1960s procaine penicillin 600,000 IU was given as a single dose to treat gonorrhoea. Over the years the dose was gradually increased and by 1980 it was increased up to 4.8 million IU. The only basis for increasing the dose was clinical failure. No laboratory based studies were available to justify these changes.

These were the findings and the recommendations of the first laboratory based study on antibiotic susceptibility of Gonococci in Sri Lanka (Susceptibility to penicillin of *Neisseria gonorrhoea* isolated in Sri Lanka, S Mananwatte, 1992)

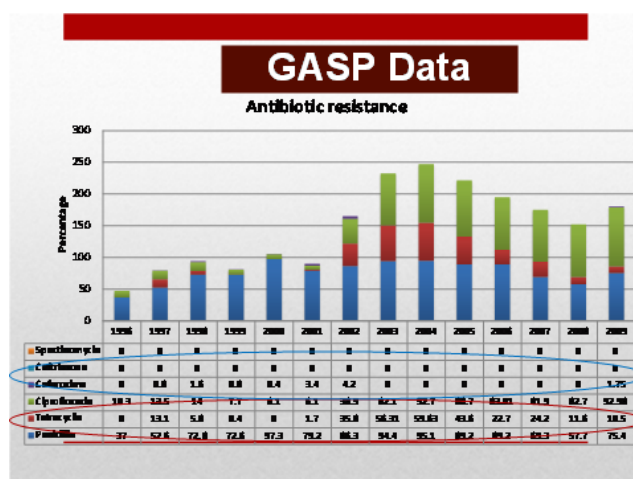
- 36% of the gonococci were resistant to penicillin study recommended fluoroquinolones as first line Rx
- spectinomycin /cefuroxime in pregnancy and in children

Based on these recommendations in 1993 ciprofloxacin was introduced as the 1st line treatment of gonorrhoea in Sri Lanka. This could not be continued for long. As you may see here, by 1996/1997 more than 10% of the gonococci in the country developed resistance to ciprofloxacin. What it meant was, every 10th patient who was treated with the recommended 1st line antibiotic did not get cured. This was not acceptable in the management of the disease as well as controlling the spread of infection. We had to move on to another class of antibiotics.

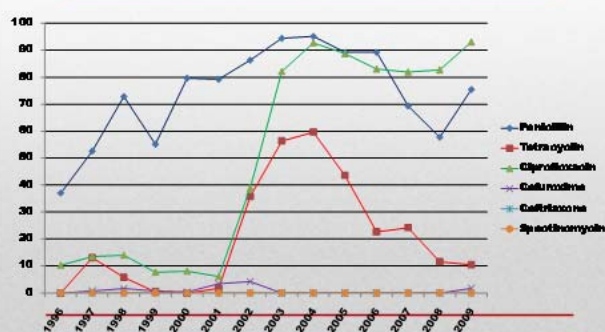
In mid 1996 cefuroxime, the oral third generation cephalosporin was introduced to the treatment schedule in STD clinics in Sri Lanka and still remains the drug of choice.

Foreseeing the problem, the World Health Organization established a global Gonococcal Antimicrobial Surveillance Programme (GASP) to ensure implementation of an evidence-based response. Since I was already working on this subject, when I heard about the WHO WPR GASP which was established in 1992, I wrote to late Prof. John Tapsall, a world authority on antimicrobial susceptibility of gonococci. I informed him our willingness to join the programme although we were not in their region. There was no SEAR program at that time. He was a little surprised about my request but agreed to accommodate us.

As a result of these negotiations, Sri Lanka has become the only country in the region which has very systematic laboratory confirmed susceptibility data dating back to 1995. As you may have seen from the studies, the lookout for gonococci resistant to antibiotics had begun even before that.



Antimicrobial Resistance



This is the most significant contribution that Sri Lanka has made to address the problem of gonococcal antimicrobial resistance in the world. We have actively monitored the susceptibility to a series of antibiotics and produced reliable data from 1996 to date. As you may see here these are the levels of resistance to six antibiotics from different classes. Resistance to penicillin, tetracycline and ciprofloxacin is so high that they cannot be even considered in the treatment protocols. We are now left with cefuroxime, ceftriaxone and spectinomycin. Looking at it in a different way, you will see here that the cefuroxime, ceftriaxone and the spectinomycin levels fall almost on the zero resistance line but the others are increasing steadily.

If we consider them separately, you see here the resistance to penicillin is extremely high and the linear trend is rising gradually. Penicillinase producing *Neisseria gonorrhoeae* (PPNG) which are responsible for the plasmid mediated resistance too have been extremely high over the years.

When analyzing the trends of resistance to tetracycline and the fluoroquinolones they too show a dramatic increase over the years.

Because cephalosporins are the only class of antibiotics that can be used, it is critical that susceptibility to these drugs be actively monitored. With this objective in mind, a study was undertaken in 2004 to determine the Minimum Inhibitory Concentration (MIC) of cefuroxime and ceftriaxone. (Detection of Minimum Inhibitory Concentration of cefuroxime and ceftriaxone for *Neisseria gonorrhoeae*, Dissertation by Malika Karunaratne)

Although none of the 108 isolates that were tested were resistant to cephalosporins, about 60% of them were closer to the higher end of 0.25 to 1.0 mcg/mL of MIC.

With the widespread use of oral cephalosporins reduced susceptibility to cefuroxime has now emerged.

Sri Lanka is now witnessing even high level resistance of gonococci to oral cephalosporins.

We have reported two cases of failed cefuroxime treatment in gonorrhoea with laboratory confirmed high level resistance.

These are the AST results of the 1st isolate with a high level of resistance to cefuroxime.

	Zone diam.	Result
Penicillin (0.51U)	0 mm	R
Tetracycline (10 µg)	18 mm (>10)	S
Ciprofloxacin (1 µg)	0 mm	R
Nalidixic acid (30 µg)	0 mm	R
Spectinomycin (100 µg)	25 mm (>18)	S
Cefuroxime (30 µg)	18 mm (<31)	R
Ceftriaxone (0.5 µg)	22 mm (>18)	S

The results of the 2nd isolate were similar. The MIC to cefuroxime was > 8 mcg/ml (agar dilution method)

We are witnessing high level resistance to cephalosporins in gonococci isolated in Sri Lanka. This I think is a grave situation. MDR and XDR GC may not be very far from us. Therefore, I hope this work that I initiated in 1992 will be carried out further by my successor and the other staff in the lab with utmost devotion and dedication. I wish them success.

Acknowledgements

- Staff of the Central Reference Laboratory of the National STD/AIDS Control Programme.
- Special thanks to – Chandrika Jayakody and Hemali Attenayake who did most of the bench work of the data I presented today.

Ladies and gentlemen, Dr. Wickremasinghe was a beloved and a dedicated teacher to most of us, a sincere colleague to others and a dear friend to all.

It was during my tenure as the President of the Sri Lanka College of Microbiologists in 2004 that we had the 1st Siri Wickremasinghe memorial oration on his 1st death anniversary. It was delivered by his good friend from Fairfield Hospital in Melbourne Dr. Norbert Ryan. Since then we have paid tribute to him every year and we will continue to do so for many years to come. I am certain that this is how he would have wanted to be remembered. There are two other very special people whom I wish to thank today. Late Dr. Nihal Perera, who taught me the ABC of Microbiology and late Dr. Gamini Jayakuru, who helped me to get myself acquainted with the STD clinic setting.

Thank you ladies and gentlemen.

THE FUTURE OF HIV VACCINES

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Approximately 33 million people live with the human immunodeficiency virus (HIV) and 2.6 million new infections are acquired each year (1). The development of an effective HIV vaccine that induces robust immunity represents a major global public health challenge. Large human efficacy trials of simple antibody-based and cytotoxic T cell based vaccines have failed to provide any protection (2,3,4). The recent RV144 HIV vaccine efficacy trial in Thailand using a prime-boost combination of vaccines, however, showed modest efficacy (31%, $p = 0.04$ on the primary analysis). Although the efficacy was marginal, the study has provided considerable hope that a vaccine to prevent infection by HIV may be feasible [5].

HIV infection is usually acquired sexually across genital or rectal mucosal surfaces. However, most candidate HIV vaccines to date have used vectors or delivery routes (eg. intramuscular injections) that are unlike to induce robust immunity at the common mucosal portals of entry. HIV vaccines that are delivered to (and preferably replicate at) mucosal surfaces are the most likely to induce effective mucosal immunity but these vectors are not at advanced stages of testing (6).

Considerable evidence from studies of HIV infected people and monkey models of SIV infection have demonstrated the importance of CD8 T cell immunity in controlling HIV infection in humans and simian immunodeficiency (SIV) infection in nonhuman primates (7). We predict that HIV vaccines inducing CD8 T cells that rapidly home to mucosal sites of viral entry will likely result in a more effective vaccine.

Our group is currently studying monkey models of HIV to measure the homing of vaccine-induced antiviral T cells to mucosal sites (8). The ability of vaccine strategies to induce mucosal HIV specific CD8 T cells can be studied by measuring the expression of a homing marker, $\alpha 4\beta 7$ integrin on HIV specific CD8 T cells. This homing marker plays an essential role in the migration of CD4 and CD8 T cells to mucosal tissues, including the gut. The gut lymphoid tissues are a primary site of CD4 T cell loss during acute HIV infection (9). Unfortunately, $\alpha 4\beta 7$ integrin also serves as a co receptor or target for HIV [10]. The destruction of $\alpha 4\beta 7$ expressing CD4 T cells in blood has been shown to correlate with the depletion of gut CD4 T cells in monkey models (11).

Inducing the right levels of $\alpha 4\beta 7$ expression on CD4 T cells and CD8 T cells creates further challenges for HIV vaccine design. We predict that the optimal HIV vaccine will induce high level mucosal HIV specific CD8 T cells on one hand without generating more targets or ("fuel") for HIV (i.e. an increase in $\alpha 4\beta 7$ on activated HIV-specific CD4 T cells) (12), that will lead to greater viral replication.

One way to reliably induce mucosal T cell immunity is to immunize with live vectors via mucosal routes. We have been studying recombinant attenuated influenza viruses modified to express HIV antigens (1). These viruses replicate efficiently in the respiratory tract that leads to induction of mucosal immunity at genital sites. Upon virus exposure of the genital mucosa of monkey models, mucosal-homing CD8 T cells dip during acute infection, suggesting they are homing to mucosal tissues. Recent reports on recombinant cytomegalovirus (CMV) vectors also show great promise in monkey models (2). These CMV vectors continually replicate and maintain high levels of activated T cells at multiple sites. A challenge for all live vector HIV vaccines is ensuring that they are safe and work effectively in people with pre-existing immunity to the vector. Human trials of recombinant adenovirus vector HIV vaccines however (3) showed they were less effective and even could be promote infection in people with preexisting immunity to adenovirus.

Another additional major challenge for all HIV vaccines is the ability of the incoming HIV virus to mutate to escape immune responses (4). Immune escape occurs for both antibody and T cell based vaccine strategies and likely occurs at both peripheral blood and mucosal tissues (5). Reducing replication to very low levels during early infection reduces the likelihood of escape and will be critical in future vaccine studies.

Neutralizing antibodies against HIV would be an ideal component of a vaccine, although inducing neutralizing antibodies that recognize a broad array of field strains of HIV has been impossible to date. Our group has been studying non-neutralizing antibodies that activate effector cells such as Natural Killer cells – so called "Antibody-dependent cellular cytotoxicity antibodies". These antibodies are more common in people who progress slowly to AIDS and there is some evidence that they are effective in the vaccine setting.

In summary, a major challenge for improved HIV vaccines is to understand how HIV vaccine regimens can induce effective HIV-specific immunity. The ability to quash the initial local mucosal replication of HIV following exposure should lead protective immunity.

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CONTAINING ANTIMICROBIAL RESISTANCE IN SRI LANKA: USE OF NATIONAL ANTIBIOTIC POLICY AND INFECTION CONTROL PRACTICES

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An antibiotic is a substance that inhibits growth of bacteria or selectively kills them. Since bacteria live and multiply inside the human cells, the drug must act on the bacterial cell and cause no harm to human cells. This selective action is achieved by getting the drug to act on sites present on bacterial cells and absent on the human cells, such as the cell wall. When the antibiotic acts on biochemical pathways like protein synthesis, a selective advantage is obtained because the ribosomal components taking part in protein synthesis differ in bacterial cells, as compared with human cells.

When the first antibiotic penicillin was discovered and introduced into the world, the main bacteria causing health problems were those that caused respiratory tract infections and skin and soft tissue sepsis. This prototype penicillin saved millions of lives.

But alongside this beneficial effect, one of the problems encountered was that the ubiquitous *Staphylococcus aureus* started producing the enzyme penicillinase, which destroyed the prototype benzyl penicillin. This was the start of the development of antibiotics or antimicrobial resistance (AMR). This also initiated the development of various types of antibiotics against the changing types of disease-causing bacteria.

This phenomenon of antibiotic resistance showed the medical world that they were dealing with a population of living organisms which had immense powers of adaptability for survival. The discovery of the anti-staphylococcal penicillin group (like methicillin) followed. Since the killing power of cloxacillin and fluorinated cloxacillin-flucloxacillin was superior, they were used in preference to methicillin, which was the standard or prototype anti-staphylococcal penicillin.

Around this time, another group of bacteria (Gram negative bacilli or rods) were posing problems of urinary tract infections and also diarrhoeal diseases. A broad spectrum penicillin group, the ampicillins were then developed. Alongside this development of the broad-spectrum ampicillin family, the anti-pseudomonal penicillins and other groups of antibiotics (macrolides, aminoglycosides, cephalosporins and tetracyclines) were developed for clinical use. The cephalosporins give broad spectrum cover against several bacteria.

Management of antimicrobial resistance

Antimicrobial resistance is one of the biggest challenges facing health care globally.

Although antibiotics have saved millions of lives, poverty, ignorance, inadequate access to drugs, poor health care delivery, especially in developing countries, have limited the control of infections. Sometimes we cannot provide reliable susceptibility data on which rational use antibiotics can be based.

Management of antimicrobial resistance requires many skills. Infection control measures would place barriers on the exchange of resistant bacteria from patient to patient, patient to health-care worker (HCW) and HCW to the patient. Skills are needed in the effective use of antibiotics, taking into consideration their pharmacokinetics and pharmacodynamics. Laboratory skills are required to identify bacteria, measure their antibiotic resistance and monitor the spread in hospital and in the community. These require integrated teamwork. Attention has to be paid to control the addiction of antibiotics to animal feeds as growth promoters, as this also helps in the selection of resistant bacteria.

It is not only drug use but also the dose and duration of use which play a role in antibiotic resistance. Appropriate antimicrobial use is the use that maximizes therapeutic impact while minimizing toxicity and development of resistance. The appropriate use is facilitated by promoting the use of informed guidelines by clinicians, improving diagnostic techniques, and also informing consumers about the proper use and the limitations of antibiotics.

Development of antibiotic policy in Sri Lanka

In Sri Lanka, at the request of the Ministry of Health, guidelines were prepared for rational use of antibiotics. They were presented in tabular form for easy reading and distributed by the ministry to medical officers. Recently, at the request of the Sri Lanka Medical Association, we developed an updated set of guidelines for which contributions were made by specialists in each field. Although guidelines are available, good microbiological laboratory backup is not available in many peripheral hospitals. A strict antibiotic policy is not in place at present.

An antibiotic policy would not only prevent the development of antibiotic resistance but also guide the clinician to use the antibiotic only when necessary e.g. the cause of upper respiratory infections is mainly viral and antibiotics are not necessary and symptomatic measures would suffice. Further, the antibiotic policy would help prevent the side effects related to antibiotics

and reduce treatment costs. It has been shown that the second-highest expenditure item after payment of salaries of staff is antibiotics. The types of policy could be restrictive - where doctors are not permitted to use reserve antibiotics for minor infections. A restrictive policy would also not permit the use of glycopeptide antibiotics meant for resistant bacteria like methicillin-resistant *Staphylo-coccus aureus* (MRSA).

Some countries rotate antibiotic use, i.e. use a certain antibiotic for a particular period and replace this with another during the successive period. This type of cyclic policy is not favoured. Some doctors do not like policies in which their prescribing rights are curbed by a set of guidelines drawn by a chosen set of doctors. In developing countries the availability of over the counter (OTC) antibiotics has been a tremendous impediment to rationalizing antibiotic use. It is felt that an antibiotic policy should be for a particular hospital or a group of hospitals in a province where there is a consultant microbiologist available, and adherence to this policy has to come through consensus. An antibiotic policy committee needs to be appointed to take decisions on antibiotic use and also to implement the decisions of the committee. Review of the antibiotic-resistance patterns need to be done periodically to change the antibiotics of the guideline accordingly. The provincial committee could monitor the antibiotic use in satellite hospitals in the province. In developed countries each hospital has its own antibiotic policy.

Role of laboratories in generating evidence

The information needed to manage resistance comes from the microbiologist and the microbiology laboratory. The laboratory will isolate bacteria from clinical samples and identify them to species level. Antibiotic sensitivity testing need to be performed to check antimicrobial resistance. The microbiology laboratory could generate the information and use a resistance-monitoring programme like WHONET to store the data. The laboratory can be linked to other WHO Collaborating Centers. Improved diagnostic testing will not only help detect resistance but also enhance correct antibiotic use and patient care. The microbiology laboratory would need to address appropriate specimen collection, performance of accurate testing, interpretation and reporting of antibiotic sensitivity (susceptibility) tests done on clinical samples. There has to be close communication between the microbiologist and clinicians to interpret the significance of isolates, choosing the appropriate antibiotic, investigation of infectious disease outbreaks and infection control measures.

Impact on patient care and hospital-associated infections (HAI)

A HAI is an infection which the patient acquires after 72 hours of hospital stay and which he did not have at the time of admission to hospital. HAI is a challenge to patient safety. If a patient acquires an infection while in hospital it is going to increase his hospital stay or make him more ill, or he may even die of the HAI. Gram negative bacteria have features which are of main concern to health care providers. These organisms are highly efficient at acquiring genes that code for drug resistance, particularly in the presence of antibiotic selection pressure. They have available a range of resistance mechanisms against the same antibiotic or a single mechanism to affect multiple antibiotics.

Since HAI cannot be totally eliminated, all hospitals in Sri Lanka have embarked on methods to control HAI. This is done through an infection control committee, which is headed by the hospital's Microbiologist and would have infection control nurses (ICN) and other consultants. Pharmacists are also included to help in implementing antibiotic policies.

Among the main measures is the reduction of the "bio-burden" or the quantum of microorganisms in all areas of the hospitals. General measures would include hand-washing, safety precautions made up of universal precautions plus body substance isolation (BSI), patients isolation policies and laundry care.

By far the single most important aspect of controlling of HAI is hand washing. When we speak of curtailing the spread of resistant bacteria like MRSA or multidrug resistant (MDR) Gram negative rods, hand washing will hold top position. Although hand washing with soap was the time tested method, alcoholic rubs have made this task easier. Posters have been developed at the National Hospital of Sri Lanka (NHSL) as per WHO guidelines to insist on the five moments for hand hygiene. There is little doubt that hand washing is at the top of list of priorities in a health care setting; it is a simple effective way to reduce HAI and combat the development and spread of antibiotic resistant bacteria.

(This is an extract of an article written by Dr. S. D. Atukorala for the WHO publication "Health in South East Asia" in March 2011).

ANTIBIOTIC RESISTANCE SURVEILLANCE PROJECT IN SRI LANKA – ARSP

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Antimicrobial resistance is a serious problem occurring worldwide. Emergence and rapid spread of resistance is a common occurrence in developing countries with uncontrolled antibiotic usage and poor infection control practices.

In Sri Lanka there was no existing system to gather this important information on a multi center basis. This long felt need was addressed by the Sri Lanka College of Microbiologists by initiating a data base on a antibiotic resistance of various organisms causing the diseases by means of a multicentre surveillance. This activity in turn back the therapeutics and help to develop antibiotic policies and the prescription guidelines.

The Antibiotic Resistance Surveillance Project (ARSP) was carried out by the SLCM with the support of the Ministry of Health and funded by the Glaxo Wellcome Ceylon Ltd. This project was planned in three phases. The main objective of the project was to lay the foundation for a national data base on antimicrobial resistance in Sri Lanka. This was initiated (first phase) by formulating the data base for the common Gram negative organisms and their resistance patterns in patients with bacteremia in state sector hospitals in Sri Lanka.

A prospective study was conducted in seven centres initially, (National Hospital of Sri Lanka, Colombo South Teaching Hospital, National Cancer Institute, Medical Research Institute, Teaching Hospital Kandy, Provincial General Hospital Ratnapura and Faculty of Medicine, Colombo) from March 2009 to November 2010. It covered the antibiotic resistance patterns of the clinically significant Gram negative bacterial isolates identified from the patients with bacteraemia and septicemia.

The significance of the Gram negative isolates in a blood culture to be included in this study was decided by the relevant microbiologists. Isolates were considered as significant when taken from patients with high fever with a matching provisional diagnosis with a Gram negative isolate from 24-72h incubation, a pure growth of isolate of the same patient with the same organism from more than one blood culture, *Salmonella* spp. isolated from blood irrespective of duration of incubation period, and an isolate from a peripheral and central venous catheters with the same organism.

The surveillance sample included information on 817 isolates. Data was complete for analysis in 733 isolates only. Of this 733 isolates 488 were from adults (> 12 years) 109 were from children (1-12 years) and 136 were from infants (<1 year). Samples from intensive care units represented 18.35% of the sample. This comprised of 123 adult patients and 27 paediatric patients.

Out of the culture positive bacteremias, in a majority (33.7%), the origin of sepsis was not possible to determine. Enteric fever contributed to 10.2% of bacteraemias

while pyelonephritis (4.5%) and respiratory tract infections (4.5%) equally contributed. It was noted that the sepsis due to invasive procedures contributed to 2.3% of the infections while sepsis from wound infections was only 1.5%.

In Sepsis of unknown origin the commonest Gram negative organism isolated in adults was *Escherichia coli*. Most infections (1/8th of infections) were due to ESBL producing *Escherichia coli* (12.6%). ESBL producing *Klebsiella pneumoniae* accounted for 9.9%. Accordingly 22.5% of total infections were due to ESBL producing organisms.

The report of the first year was handed over to the Ministry of Health (MOH) and the funding agency, Glaxo Wellcome Ceylon Ltd in a small function held at the auditorium of Ministry of Health on 15th December 2011.

During the first phase certain steps were taken to confirm the sustainability of the activity. 'The surveillance of antibiotic resistance' has to run it without the financial support from outside the health system. The MOH supported the sustainability of the surveillance by agreeing to provide the identification systems through the MSD. This was a major breakthrough in the field which enabled all the microbiologists to use an internationally accepted bacterial identification system in their hospitals which in turn improved the quality of antibiotic resistance data produced.

The second phase of the project was to expand the identification systems to the Gram positive range while continuing the activities of the first phase the 2nd phase of ARSP commenced on 15th of October 2012. It covers Gram positive and Gram negative Blood culture isolates. The prime objective of this phase is to strengthen the data base of aetiological agents of blood cultures and the antibiotic susceptibility patterns. This phase included all the centers where the microbiologists are located.

Altogether there are 22 centers in the surveillance programme now covering seven provinces. As it is necessary to use sheep blood for growth media preparation for Gram positive organisms 4 sheep were bought from the fund and handed over to MRI. The second phase is now continuing and hope fully Sri Lanka will have the basic information on antibiotic resistance patterns when this phase is completed.

The 3rd phase of the project was designed initially to continue the surveillance activities with countrywide expansion. As many microbiologists are now in the provinces, considering the ability of producing a data base on a wider range covering a wide geographical distribution and many disease entities. Later it was decided to overlap the 3rd phase of the ARSP with the second phase and the 3rd phase will be conducted as National Laboratory Based Surveillance of Antibiotic resistance (NLBSA) with the support of the Ministry of Health.

CAVITARY LUNG DISEASE WITH EMPHASIS ON INFECTIVE CAUSES

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Cavitary lung disease is a common global health problem which contributes significantly to morbidity and mortality in any community. The impact of the cavitary lung disease is more serious on health care systems of the developing world where its etiology is usually infective making it a community health hazard.

In this article, we intend to discuss the etiology, diagnosis and management of cavitary lung lesions with particular emphasis on those with infective etiology, referring to Sri Lankan experience when relevant.

A cavity is a gas filled space, seen as a lucency or low attenuation, within pulmonary consolidation, a mass or a nodule (1).

Cavities occur as the end result of variety of pathological processes which occur in lung parenchyma, the commonest of which is expulsion of necrotic part of a lung parenchymal lesion via bronchial tree (2).

Cavities also can occur due to cystic dilatation of lung structures.

Aetiology

Causes of lung cavities –

1. Infective - bacterial, TB, fungal and parasitic
2. Non infective - Primary and secondary malignancy, lymphoma, autoimmune Wegeners granulomatosis, rheumatoid cysts, trauma, congenital cysts.

1. Infective causes

Cavitary infective agents reach the lungs mainly via two routes

Environmental organisms – via bronchial tree

From a septic focus in the body – haematogenous

Community acquired bacterial causes

In Sri Lanka the commonest infective cause of lung cavities is *Mycobacterium tuberculosis* (MTB). Common causative agents of pneumonia like *Streptococcus pneumoniae* and *Haemophilus influenzae* only rarely cause lung cavities. Certain characteristics of the pathogen help in formation of cavities. Serotype 3 is the commonest serotype of *Streptococcus pneumoniae* involved in lung abscess and subsequent cavity formation (3).

Mycobacterial infections – MTB is classically associated with cavitary lung diseases. High rates of MTB infections are seen with HIV patients and other immunodeficiencies. Clinical progression is sub acute and 30-50% would give rise to cavitation. Cavities vary widely in size. Since the pathogen is a strict aerobe lesions are commonly seen in upper lobes or apical part of lower lobe. Presence of cavity increases the infectiveness, higher risk of relapse and takes long time to become smear negative with treatment. CT scan is more sensitive in detection.



Figure 1. This 17 y old boy was treated for pneumonia 1 month back (L). He came back with fever and shortness of breath. Positive Mantoux and sputum positive for AFB. Second x-ray shows a fluid level(R).

Non tuberculous Mycobacteria – These are potentially pathogenic environmental mycobacteria. Out of these *Mycobacterium avium intracellulare complex* is the commonest in United States (4). Characteristic appearance of Lady Windermere's disease (radiologically) the presence of cavitation in association with right middle lobe and lingular bronchiectasis and nodules often suggests nontuberculous mycobacterial disease. Although we have encountered this typical radiological appearance in number of our cases, none of them was proved to be culture positive. This may be due to inactivity of the disease at the time of bronchiectasis. Other causes of non tuberculous mycobacteria are *Mycobacterium kansasii*, *Mycobacterium malmoeense* and *Mycobacterium xenopi*. Infection is not transmitted from person to person. Clinical presentation may be similar to MTB. Culture is crucial in identification.

Community acquired Methicillin Resistant *Staphylococcus aureus* (CMRSA) – increasingly reported in Sri Lanka. These carry Panton Valantine Leucocidin (PVL) gene. Occur in immunocompetent, children or young adults without any predisposing factor. Usually preceded or accompanied by skin infections. Infection result in bronchopneumonia with cavitation (Figure 2). On plain radiographs, cavitation was noted in 12% of the patients in one series. Mortality was 56% in this series (5).

Staphylococcus aureus bronchopneumonia is common in elderly with predisposing factors like diabetes mellitus, alcoholism or secondary to influenza.

Community acquired *Klebsiella pneumoniae* – Commonest site is upper lobe. Usually the patient is described as an elderly male with smoking and alcoholism. *K. pneumoniae* is not limited to the community. In recent studies immunosuppression was more common than earlier risk factors. This is a common cause of severe necrotizing pneumonia leading to multiple small cavities (6). Entire section of lung can be destroyed giving rise to massive pulmonary gangrene. Though massive pulmo-

nary gangrene is rare 50% of cases are associated with *Klebsiella pneumoniae*.

Community acquired *Pseudomonas aeruginosa* – Causes a rapidly progressive necrotizing pneumonia (6,7). There is predilection for lower lobes. In a prospective study on community acquired pneumonias *Pseudomonas aeruginosa* accounted for only 0.4% but carried 18% mortality. Investigators found pulmonary co-morbidities and feeding through enteral tube as risk factors.

Hospital acquired bacterial causes

In our experience *Pseudomonas aeruginosa* is the commonest organism encountered in hospital acquired bacterial pathogens. Secondary infection occurs in patients with pulmonary co-morbidity, immune suppression, following hospitalization or within 30 days of antimicrobial therapy.

Hospital acquired *Klebsiella pneumoniae* is frequently complicated by lung abscess, which generally appears as one or more cavities.

Hospital acquired MRSA is a cause of ventilator associated pneumonia or bronchopneumonia in a debilitated patient with invasive devices. This infection carries a high mortality.

Lung abscesses

Usually occur in patients with risk factors like senility, diabetes, alcoholism, poor dentition, prior aspiration. Caused by anaerobic and microaerophilic organisms of oral flora and often poly microbial. Usual pathogens are *Prevotella*, *Fusobacterium spp* and *Streptococcus milleri*.

Melioidosis – *Burkholderia pseudomallei* is increasingly encountered in Sri Lanka (8). Mostly affect lungs. Pathogen is endemic in South East Asia and Northern Australia. Infection occurs through contact with or ingestion or inhalation of contaminated soil or water. Other risk factors are occupation, diabetes mellitus, chronic renal failure and thalassemia. Infection can be latent for years.



Figure 2. CT scans of thigh and lungs showing cavities in a 12 y old boy with blood culture positive for CMRSA.

Actinomycosis – Commonest isolate reported in literature is *Actinomyces israelii*. Alcoholism and poor dental hygiene are risk factors. Many *Actinomyces* infections are polymicrobial (3). Slow progression of non specific symptoms makes the diagnosis difficult.

Nocardia asteroides infection also can lead to cavity formation. Organism enters by direct inhalation or by direct inoculation due to trauma. Pulmonary disease is the most common presentation of *Nocardia*. Underlying lung disease is a risk factor with immune suppression.

Rhodococcus equi and *Tularemia* are rare causes of cavitary lung disease (9). Identification of these pathogens remains difficult at the present state of Sri Lankan microbiology. *Rhodococcus equi* is commonly isolated from soil contaminated by horse manure. Infection can occur by inhalation, ingestion or by traumatic inoculation. *R. equi* is commonly described among immunocompromised patients.

Lung abscess may be due to distant sites of sepsis causing septic emboli. This is common with IV drug users, Lemierre syndrome (internal jugular vein thrombosis), infected IV catheters, pace maker wires, right sided prosthetic heart valves. Common pathogens are *Staphylococcus aureus*, *coagulase negative Staphylococci*, *Fusobacterium spp.*

In immunocompromised non Typhoidal *Salmonella* can occur.

Lemierre syndrome which is also known as post anginal sepsis is characterized by septic emboli in internal jugular vein. Lemierre syndrome is a rare condition seen in adolescents and young adults. Though rare in antibiotic era we have encountered a few cases secondary to throat infections.

In addition to bacterial causes fungal pneumonia can lead to cavity formation. Out of this list of pathogens aspergillosis followed by zygomycosis are the common causes in Sri Lanka. *A. fumigatus* is the commonest *aspergillus* spp. reported in the literature. Invasive pulmonary aspergillosis is a common and important infection after hematopoietic stem cell transplant or in the setting of hematological malignancy and neutropenia *Cryptococcus neoformans* also can cause lung cavities. Other causes of fungal aetiology are *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Coccidioides immitis* and *Paracoccidioides brasiliensis*. These fungi are restricted to geographical areas so travel history is important in suspected patients.

Parasitic infections that lead to cavity formation are *Echinococcus granulosus* and *Paragonimus westermani*.

2. Non infectious causes

Malignancies are one of the most important causes. Primary lung cancers mainly squamous cell carcinoma

is the commonest cause reported in literature and also in Sri Lanka. Metastases especially of squamous cell origin and lymphoma cause cavitation. Cavitation in malignancy is a bad prognostic sign. Sometimes a co existence of pulmonary infection and malignancy can occur.

Rheumatologic diseases

Wegener's granulomatosis is an auto immune disease with systemic vasculitis which involves upper and lower respiratory tract. Pulmonary nodules and infiltrates can occur with cavitation. Sarcoidosis can also lead to lung cavities. Very rarely rheumatoid arthritis and primary amyloidosis can give rise to cavities.

Miscellaneous diseases associated with cavities

Pulmonary embolism, pulmonary infarction and necrosis can occur by dislodged clots, by trauma, fat, air bubbles, septic emboli, or by injected foreign substances. Super infection with *Clostridium* species has been particularly associated with pulmonary embolism and usually causes a necrotizing, cavitary pneumonia.

Pulmonary Langerhans cell histiocytosis

The disease almost exclusively (over 90%) afflicts smokers, with a peak age of onset of between 20 and 40 years. Clinical presentation varies, but symptoms generally include months of dry cough, fever, night sweats, and weight loss. Thin-walled cystic cavities are the usual (10). Radiographic manifestation, observed in over 50% of patients by either plain chest radiography or computed tomography scans, but thicker-walled cavities are also commonly observed.

Super infection of necrotic lung cavities with Gram negative rods, *Clostridium* spp. and aspergilloma can occur (2).

Diagnosis

Diagnostic imaging

Radiological imaging plays a major role in diagnosis, management and follow up of cavitary lung diseases.

Plain chest radiograph (frontal and lateral) and computed tomography (CECT - contrast enhanced computed tomography and HRCT - high resolution computed tomography) are the main imaging modalities which are useful in diagnosis and management of cavitary lung lesions. PET/CT (positron emission tomography/ computed tomography) is occasionally used in cavitated lung nodule to verify the metabolic status in cases of suspected malignancy.

Plain chest radiograph is the initial investigation of choice due to its cost effectiveness and availability. Cavity is identified as a focal lucency in the lung parenchyma devoid of vascular markings surrounded by a rim of

opacification of variable thickness. Although most cavities are easily identified by plain radiographs, false negative results may occur due to lack of contrast and superimposition of superficial soft tissues, which are inherent to plain film radiography.

Computed Tomography with its ability to depict lung parenchymal and mediastinal anatomy exquisitely is the mainstay in solving the problems which may arise in plain film radiography of the chest. CT is also extremely useful in providing image guidance for tissue sampling if the need arises.

Imaging characteristics of lung cavities which are helpful in etiological diagnosis

i) Appearance and number of cavities

In an acute clinical setting of fever, cough and leucocytosis, a solitary cavitory lesion with surrounding consolidation is almost certainly a lung abscess while multiple such lesions are likely to represent multifocal consolidations with tissue breakdown as a result of septicæmic process or multiple infarcts due to septic emboli.

In a patient with protracted clinical features, a well defined solitary cavitory lesion is likely to represent a granulomatous lesion (TB) or a primary bronchial malignancy. Good history (age of the patient, smoking, contact history of TB), ESR, sputum AFB and Mantoux will be helpful in discriminating these two conditions.

Multiple cavities in a patient with protracted clinical features are more commonly due to metastatic deposits of squamous cell primary malignancy elsewhere in the body than infective or non infective causes like Wegeners granulomatosis or necrobiotic rheumatoid nodule.

ii) Anatomical location of the cavity/cavities

Post primary TB cavities are characteristically located in the apical and posterior segments of the upper lobes and apical segment of the lower lobes. Isolated cavity in the anterior segment of the upper lobes makes the diagnosis of post primary TB unlikely.

Multiple bronchiectatic cavities bilaterally in middle and lingular lobes are characteristically found in chronic lower respiratory infections due to non tuberculous mycobacteria typically in middle aged females (Lady Windermere Disease).

In acute bacterial infections *Klebsiella* and *Proteus* generally produce cavities in the upper lobes while cavities due to anaerobic organisms are often located in lower lobes and posterior segments.

Cavities due to tissue breakdown of pulmonary infarcts following septic emboli are more commonly found in the peripheral lung while cavitating metastatic deposits have no zonal predilection in lung fields, although more commonly found in lower lung fields due to preponderance of lung tissue in the bases.

iii) Wall thickness of the cavity

Many studies have proved that cavities with thicker wall show a greater propensity for malignancy. Extremely thick walled cavities (>15mm) are highly suspicious of malignancy while those with a wall thickness of 5-15 mm have almost equal chances of turning out to be benign or malignant lesion.

In an appropriate setting thicker wall cavities need to be further evaluated with CT, PET/CT and image guided biopsies.

iv) Contour of the inner lining (smooth or irregular) and nature of the contents

The inner wall of the cavity is generally smooth in TB lesions in contrast to the irregularity and nodularity found in malignancies. In cavities due to acute bacterial infections the inner lining is often poorly defined and shaggy.

Determination of contents of a cavitory lung lesion is of great importance in narrowing the differential diagnosis. Demonstration of an intracavitary air fluid level or intracavitary mass generally provides a diagnostic clue in fitting clinical setting. This is generally achieved by a horizontal beam chest radiograph or computed tomography of chest.

Fluid in a cavitory lung lesion could be serous, serosanguinous, pus or liquefied necrotic material. Air in the cavity with an air fluid level may be due to a gas forming organism in the cavity or due to cavity being connected to a bronchus. In an appropriate clinical setting a cavity with an air fluid level generally represents a lung abscess.

A mobile intracavitary body with an air crescent in the non dependent area of the cavity (air crescent sign) is diagnostic of a mycetoma in a dormant cavity. Non mobile intra-cavitary bodies may represent necrotic tumor fragments in carcinomas, sequestered necrotic lung tissue in necrotizing pneumonia as in *Klebsiella*. Collapsed membrane of a ruptured echinococcal cyst floating on top of the fluid provides the "water lily" sign (11).

v) Communication of the cavity with inner bronchial tree

Communication of the cavity with the bronchial tree is generally not apparent in plain radiography, though it is well depicted in spiral CT. A thin walled cavity connected to the bronchial tree is likely to be due to cystic bronchiectasis and a fluid level in such cavity indicates secondary infection.

Thick walled cavity with a bronchial communication with or without a fluid level is possibly an infected necrotic lesion which has discharged its contents in to the bronchial tree. However partially occluding intra bronchial

neoplasm may present with similar radiological appearance requiring broncoscopic evaluation in few selected cases.

v) Auxiliary radiological features in mediastinum and pleura

Plain radiographic and CECT finding of hilar or mediastinal lymphadenopathy in the presence of a cavitary lung lesion is highly suspicious of a malignancy or a lymphoma. However in an immunocompromised patient TB is a possibility especially if the lymph nodes show low density centre and peripheral enhancement in CECT.

Presence of acute and chronic changes in the pleura in association with a cavitary lung lesion makes the diagnosis of infection more likely than a malignancy, although the latter cannot be totally excluded.

Management

In the management of patients with cavitary lung lesions, well coordinated multidisciplinary team approach comprising specialists from pulmonology, microbiology, pathology and radiology is highly recommended.

Diagnosis, treatment and follow up of patients with cavitary lung lesions should be rational and protocol based to achieve cost effectiveness. In most cases of cavitary lung lesions, management strategies are straight forward and generally depend of presenting clinical features, basic haematological investigations, sputum analysis plain radiography.

Broncho-alveolar lavage (BAL) is a better specimen for culture than sputum in diagnosis of infective causes since sputum is not homogenized and processed in dilution in Sri Lanka.

More sophisticated and invasive investigations such as haematology for ANF, CT, PET/CT, image guided biopsies are reserved for management of complicated and resistant cases. In the developing world including Sri Lanka, where infective etiology is predominating antimicrobial therapy is the mainstay in managing cavitary lung lesions. Rational and adequate use of antimicrobials is of utmost importance in reducing morbidity and mortality.

Importance of simultaneous management of comorbidities where relevant should always be in the minds of attending management team. Management of non infective cavitary lung lesions depends on the etiology.

Radiographs – courtesy of Radiology Museum, Colombo South Teaching Hospital.

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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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Abstract

Introduction: Extended spectrum beta-lactamase (ESBL) production is important to determine antibiotic treatment of infections caused by Enterobacteriaceae. ESBL bacteremia is frequently associated with poor outcome when appropriate antibiotics are not used at the right time. Hence, the knowledge of ESBL prevalence is essential for clinical management.

Objectives: To determine the prevalence, risk factors and clinical outcome of patients with bacteremia caused by ESBL producing Enterobacteriaceae in a District General Hospital in Sri Lanka.

Method: All patients with a non repetitive isolation of Enterobacteriaceae from blood culture were included. Data was collected from patient records and lab records.

Results: Out of 109 bacteremic patients 56 (51%) isolates were positive for Enterobacteriaceae. ESBL prevalence among Enterobacteriaceae was 34%. The majority (68%) of ESBL bacteremia were community acquired and the commonest (74%) primary source was the urinary tract. 53% of ESBL patients had diabetes in contrast to 19% of non ESBL patients ($p=0.09$).

Inappropriate empirical antibiotic therapy was given to 63% of patients. 84% isolates were multidrug resistant to 3 or more drug classes. (mainly to beta-lactams, fluoroquinolones and co-trimoxazole). However, all the isolates were sensitive to amikacin and meropenem.

Conclusion: ESBL prevalence in bacteremia was high and UTI was the commonest source. Diabetes was a significant risk factor. Although amikacin and meropenem sensitivity was 100%, high multi drug resistance was noted. Routine ESBL detection and timely reporting should be continued in every microbiology laboratory. Meropenem should be used without delay for critically ill patients.

Introduction

Production of extended spectrum beta-lactamases (ESBL) is one of the most important resistance mechanisms that impede the antibiotic treatment of infections caused by Enterobacteriaceae. ESBLs are plasmid mediated beta-lactamases capable of efficiently hydrolyzing all beta-lactam antibiotics except carbapenems and cephamycins. ESBLs are specifically

inhibited by β -lactamase inhibitors such as clavulanic acid. Therefore, clavulanic is used for the detection of ESBLs. The plasmids encoding ESBLs often encode for resistance to other class of antibiotics also, thereby critically limiting the choice of antibiotics available for the treatment of these infections.

The prevalence of ESBL producing strains among Enterobacteriaceae has increased in recent years with a wide variation in different geographical areas and in different institutes. Risk factors for the occurrence of ESBL producing organisms include prior administration of antibiotics particularly 3rd generation cephalosporins, increased length of stay in hospital, focus of infection primarily urinary or respiratory tract infections, in-dwelling urinary catheters, diabetes mellitus, mechanical ventilation etc.

ESBL bacteremia is frequently associated with a poor outcome when appropriate antibiotics are not used at the right time. Hence, the knowledge of ESBL prevalence is essential for clinical management as well as for the prevention of resistance transmission by instituting correct infection control practices.

There are no reports in Sri Lanka on the prevalence of ESBL producing Enterobacteriaceae bacteremia among hospitalized patients, their risk factors and clinical outcomes. Therefore, we conducted this study to determine the prevalence, risk factors and clinical outcome among patients with ESBL producing Enterobacteriaceae bacteremias, in a District General Hospital in the North Western province of Sri Lanka.

Objectives

To determine the prevalence, risk factors and clinical outcome of patients with bacteremia caused by ESBL producing Enterobacteriaceae in a District General Hospital (DGH) in Sri Lanka.

Methodology

We conducted a descriptive cross sectional study at the DGH, Chilaw for 15 months from 01st of January 2012. Permission to conduct the study was obtained from the hospital administration and clinicians. Ethics clearance was granted from the Faculty of Medicine, University of Colombo. Data collection was done by retrieving information from laboratory records and from bed head tickets.

We included all patients from neonates to adults with a non repetitive isolate of Enterobacteriaceae from blood culture samples which we received as a part of their routine management. Following manual processing of blood cultures, standard isolation and identification procedures were done according to standard operating procedures.

Antibiotic susceptibility testing was done using Stoke's disk diffusion method. ESBL detection was done by the double disk approximation test (DDAT) using clavulanic acid. The CLSI phenotypic confirmatory test was used for the confirmation of ESBL production using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid while such discs were available.

A positive comprised a ≥ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid, versus its zone when tested alone. Once the combination discs ran out, the CLSI interpretive zone diameters for cefpodoxime 10ug <17 mm and ceftriaxone 30 ug < 25 mm in addition to DDAT was used. API identification testing was done to identify Enterobacteriaceae to species level.

Data collected included risk factors, demographic details, co-morbidities, primary source of infection and the infection was classified as community acquired or hospital acquired. Statistical analysis was done using the Statistical Package for the Social Sciences (SPSS) software package.

Results

DGH Chilaw has a bed strength of 547. Total number of admissions during the study period was 69,087. Total blood culture specimens received during this period was 2974.

Of those, 109 significant blood cultures were identified of which 56 (51%) were positive for Enterobacteriaceae. ESBL prevalence among bacteremic Enterobacteriaceae was 34% (19/56). *Escherichia coli* comprised 16/19 (84%) of the ESBL producers while the rest were *Klebsiella pneumoniae* (3/19). ESBL *E. coli* and *K. pneumoniae* accounted for 16/41(39%) and 3/8 (37%) of *E. coli* and *K. pneumoniae* bacteremia cases respectively. Age range of ESBL patients was between 11 months to 82 years and non ESBL ranged from 1 day to 85 years.

Table 1. comparison of ESBL and non ESBL

Variable	ESBL producers (n=19)	Non ESBL (n=37)	Total (n=56)	Chi-square test (p value)
<u>Gender</u>				
Male	11 (57.9%)	19 (51.4%)	30 (53.6%)	0.22 (0.64)
Female	08 (42.1%)	18 (48.6%)	26 (46.4%)	
<u>Primary source</u>				
Urinary tract	14 (93.3%)	16 (72.7%)	30 (81.1%)	N.A.
Other (excluding undetermined)	01 (6.7%)	06 (27.3%)	07 (18.9%)	
<u>Acquired from *</u>				
Hospital	06 (31.6%)	26 (72.3%)	32 (58.1%)	8.84 (0.003)
Community	13 (68.4%)	10 (27.7%)	23 (41.9%)	
<u>Co-morbidities</u>				
Diabetes	10 (52.6%)	07 (18.9%)	17 (30.4%)	6.75 (0.009)
Chronic renal failure	05 (26.3%)	05 (13.5%)	10 (17.9%)	N.A.
Chronic liver disease	02 (10.5%)	04 (10.8%)	06 (10.7%)	N.A.
Ischeamic heart disease	04 (21.1%)	05 (13.5%)	09 (16.1%)	N.A.
Hypertension	08 (42.1%)	06 (16.2%)	14 (25.0%)	N.A.
Malignancy	01 (05.3%)	02 (05.4%)	03 (05.4%)	N.A.
<u>Outcome</u>				
Discharged	15 (78.9%)	28 (75.7%)	43 (76.8%)	N.A.
Died	04 (21.1%)	09 (24.3%)	13 (23.2%)	

* There was 1 non ESBL case where this could not be established

N.A. = chi-square test cannot be applied as expected value in some cells < 5

The majority (68 % or 13/19) of ESBL bacteremia were community acquired. The commonest (74% or 14/19) primary source for ESBL bacteremia was the urinary tract in comparison to non ESBL patients where the urinary tract was the source in only 43% (16/37). Forty seven percent (9/19) of ESBL bacteremia was associated with urinary catheters while it was only 20% (11/37) in non ESBL. Fifty three percent (10/19) of patients were found to have diabetes in contrast to 19% of non ESBL patients and the difference was significant (p=009).

Inappropriate empirical antibiotic therapy was given to 63.0% (12/19) of patients with ESBL isolates. But once urine culture results were available antibiotics were changed to appropriate choice in 83% (10/12). Seventy nine percent (15/19) of patients were discharged home following treatment, and 4 patients died.

A total of 16/19 (84%) isolates were multidrug resistant (MDR) (resistant to 3 or more drug classes). The most prevalent MDR pattern was resistance to beta-lactams, fluoroquinolones and co-trimoxazole. However, all the isolates were sensitive to amikacin and meropenem.

Discussion

The most common cause of resistance to β -lactam antibiotics is the production of β -lactamases. The selective pressure caused by the use and overuse of antibiotics in the treatment of patients has resulted in the emergence of various β -lactamases. Extended-spectrum β -lactamase mediated (ESBLs) resistance among Enterobacteriaceae continues to be a major problem in health care facilities throughout the world. Prevalence of ESBLs varies not only from institute to institute but from ward to ward within the institution. Among Enterobacteriaceae, the major ESBL-producing organisms are *E. coli* and *K. pneumoniae*.

In India, the prevalence of ESBL production in *E. coli* and *K. pneumoniae* from bacteremic patients and from various clinical samples varied from 22% to 73% (1,3). The prevalence of ESBL producing *E. coli* in urinary isolates was 60% and multidrug resistance was 69% in a study done in Chennai, India (2). A similar ESBL prevalence (59%) was found in *Klebsiella pneumoniae* from urinary tract infections in the North-West of Pakistan (4). A large study done in more than 100 European intensive care units found that the prevalence of ESBLs in *Klebsiella* spp. ranged from as low as 3% in Sweden to as high as 34% in Portugal (5). National surveys have indicated the presence of ESBLs in 5 to 8% of *Escherichia coli* isolates from Korea, Japan, Malaysia, and Singapore and 12 to 24% in Thailand, Taiwan, the Philippines and Indonesia (6).

ESBL resistance is a problem not only in hospital acquired infections but for community acquired infections as well. ESBL bacteremia was more common in patients with hospital acquired than community acquired infections (85% versus 53%) (1). ESBL prevalence in

community acquired Enterobacteriaceae bacteremia in Southern Israel was 63.6% (7).

Abhilash's study has noted a high degree of resistance to multiple classes of antibiotics in ESBL producing Enterobacteriaceae and concluded that carbapenems were the most active antibiotics in-vitro (1). A study done by Ullah and colleagues (4) also reported a 71% prevalence of multidrug resistance in their strains with carbapenem sensitivity similar to the Chennai study (2). They also found that the commonest source of bacteremia was the urinary tract (45%). The 14-day mortality rate was 23% (1).

In the present study ESBL prevalence in Enterobacteriaceae bacteremia was high when compared to Irish study (5.4%) (9) but not as high as in India, where the prevalence was 73% (1). The most common ESBL producer was *E. coli*.

Our study has a similar ESBL prevalence in community acquired Enterobacteriaceae bacteremia as Israel study (7). Diabetes was found to be a common risk factor similar to Panhotra's study (8). UTI was the commonest source for ESBL bacteremia similar to other studies (1,5).

The majority of our patients were treated with cefuroxime, ciprofloxacin or cefotaxime until urine culture results were available and then the antibiotics were changed to gentamicin, amikacin or meropenem. In a significant number of patients blood cultures were positive only after 5 days of incubation and for those patients meropenem was not used because by that time patients were improved or discharged home.

Clinical outcome depends on the timely initiation of appropriate antibiotics. In this study 4 patients died (21%). It is significant to note that in two of these patients appropriate treatment was delayed for 4 days in spite of having ESBL positive urine result and this may have contributed to mortality. Timely identification and reporting of ESBL positive urine culture result may be life saving in our setting because more than 50% of patients were over 60 years with diabetes and their timely changed of antibiotics may have changed their outcome.

A high (84%) multi drug resistance was seen, which is more than the other studies (2,5,6). Amikacin and meropenem showed 100% in vitro sensitivity in our study. Prior use of antibiotics, particularly cephalosporins in community acquired ESBL bacteremia was not found because that information was not available with patients. But in hospital acquired bacteremia use of cephalosporins was noted.

Conclusion

ESBL prevalence in Enterobacteriaceae bacteremia was high in our hospital. The most common ESBL producer was *E. coli*. Majority of ESBL bacteremias were community acquired. Urinary tract was the commonest

primary source for ESBL bacteremia. Diabetes was a significant risk factor for acquisition of ESBL. Empirical antibiotic treatment was inappropriate in the majority of cases until urine culture results were available. In our study mortality was 21% in patients with ESBL bacteremia. Most ESBL strains showed multi-resistance to other classes of antibiotics.

Routine ESBL detection and timely reporting should be continued in every microbiology laboratory. Amikacin can be recommended for empirical treatment for patients' with risk factors suspected to have UTI. But for critically ill patients, meropenem should be used without delay. Further studies are required in future to establish more information because positive numbers was a limiting factor in this study.

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CASE REPORTS

DELAYED DIAGNOSIS OF CUTAENEOUS PROTOTHECOSIS IN AN IMMUNOCOMPROMISED PATIENT

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Case Report

A 49 year old man with a past history of cerebral lymphoma, systemic lupus erythimatosus and autoimmune haemolytic anaemia presented with nausea, vomiting, abdominal and back pain of 2 weeks duration. On presentation he was on rituximab and steroid therapy for his autoimmune haemolytic anaemia. Physical examination revealed some enlarged right axillary lymph nodes. His abdominal, respiratory, neurological and cardiovascular examinations were unremarkable. It was also noted that he had a non-healing painful, nodular/ulcerative lesion on his left index finger and elbow (Figure 1) for which he was treated with multiple courses of beta-lactam antibiotics with no success. These lesions had gradually developed over the preceding months after he had accidentally cut his finger while chopping onions. Finger lesions were biopsied at that time at another institution and were reported to have cultured a non-albicans *Candida* species which was disregarded as a possible contaminant.

His initial investigations revealed a WCC of $9.9 \times 10^9/L$ (with neutrophils of $8.9 \times 10^9/L$) and haemoglobin of 63g/L and an ESR of 54 mm/hr. He had an initial creatinine of 300 $\mu\text{mol/L}$ and urea of 19 mmol/L, indicating acute renal failure of unknown origin. CT scan of chest/abdomen/pelvis showed enlargement of abdominal lymph nodes with bilateral ureteric obstruction secondary to lymphadenopathy as the cause of his acute renal failure. Bilateral ureteric stents were inserted to relieve the ureteric obstruction. The subsequent PET scan revealed relapse

of lymphoma with widespread involvement of abdomen and axillae. This was confirmed by biopsy and histology of the axillary nodes which was consistent with diffuse large B-cell lymphoma. Bone marrow biopsy further confirmed this finding.

The skin lesions were biopsied and histology and routine bacteriological and fungal cultures were performed. Cultures yielded an abundant growth of soft yeast-like whitish colonies on Sabouraud's agar (Figure 2), which on wet preparation showed spherical sporangia containing endospores typical of *Prototheca species* (Figure 3). The organism was identified by API ID32C to genus level and further to species level with additional biochemical tests (galactose positive, trehalose positive) as *Prototheca wickerhamii*. Histopathology of the ulcer base revealed histiocytic and neutrophilic inflammatory infiltrate and abundant rounded organisms with internal sporulations consistent with protothecosis.

Patient was commenced on intravenous liposomal amphotericin B 3mg/kg/d and the lesions responded well to antifungal therapy resulting in a significant decrease in size. Three weeks after commencing liposomal amphotericin B, he was started on chemotherapy for his lymphoma. Patient became neutropenic following chemotherapy and developed neutropeinc sepsis with no obvious focus of infection and was further treated with meropenem and vancomycin in addition to liposomal amphotericin B but failed to recover and died of sepsis and multiorgan failure.



Figure 1. Left index finger and left elbow lesions.



Figure 2. White/gray colonies of *P. wickerhamii* on Sabouraud's agar.

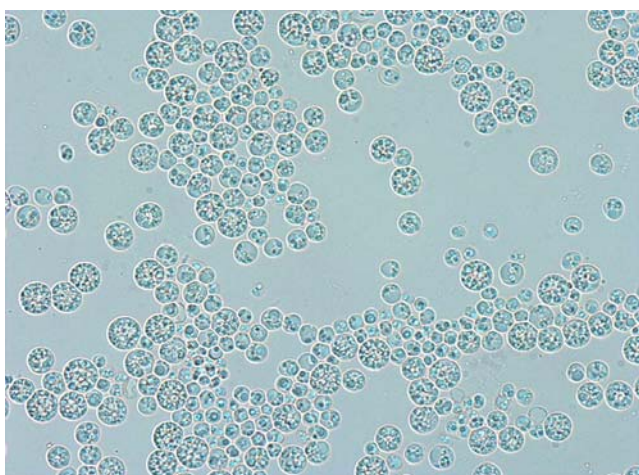


Figure 3. Wet preparation showing spherical sporangia of *Prototheca*.

Discussion and conclusions

Human protothecosis is a rare infection caused by members of genus *Prototheca* which are considered to be achlorophilic algae. They are spherical unicellular organisms which are distinguished from other algae by their lack of chloroplasts.

Prototheca spp are globally ubiquitous and can be isolated from various reservoirs such as the environment, animals and food. Human infection by *Prototheca* species was first described in 1964 by Davis and colleagues on a foot of a bare foot rice farmer from Sierra Leone. Human infections are due to contact with potential sources such as contaminated soil or water or by traumatic inoculation of the algae into subcutaneous tissue. Out of the many

species assigned to the genus *Prototheca*, *P. wickerhamii* and *P. zopfii* are the only species that have been reported to cause human infections and *P. wickerhamii* is the more common of the two.

The occurrence of protothecosis can be local or disseminated and acute or chronic, with the latter being the more common. It is classified into three clinical forms; cutaneous lesions, olecranon bursitis, and disseminated infections. Most of the protothecosis cases are simple cutaneous infections out of which majority occur in individuals who are immunocompromised by immunosuppressive therapy. In contrast, individuals presenting with olecranon bursitis are usually not immunocompromised but report trauma to the affected elbow. Disseminated protothecosis occurs in severely immunocompromised specially those with defective cell-mediated immunity.

Cutaneous protothecosis as in this patient develops slowly following trauma or in a patient with a skin or mucosal defect and can present as ulcerative lesions, plaques, pustules, papules, nodules and hypopigmented atrophic lesions on exposed areas of the body. Patients are subjected to various modes of treatments for long periods with no satisfactory results.

The definitive diagnosis depends on morphological identification of the organism in wet preparations of cultures and direct histopathologic examination of tissue sections. *Prototheca* species have simple nutritional requirements and grow readily on a variety of synthetic culture media; but common selective fungal culture media containing cycloheximide may inhibit growth of this organism. It produces soft wet white-to-light tan yeast-like colonies in Sabouraud dextrose agar which can be mistaken for *Candida* species. Microscopic examination of the organism in culture reveals spherical sporangia containing multiple endospores giving the organism the appearance of a spoked wheel. Optimum growth occurs between 25 and 37°C and may require up to 7 days of incubation for slow growing species. *Prototheca* organisms can be identified by API strip series applicable for yeasts, the Vitek yeast identification database, Vitek 2 test and RapidID Yeast Plus tests. The absence of growth on trehalose is a main diagnostic feature for differentiation between *P. wickerhamii* and *P. zopfii*.

There are no guidelines for performance and interpretation of in vitro susceptibility testing for these algae. In general, *Prototheca* spp show various susceptibility profiles but there is no direct correlation between in vitro susceptibility and clinical response; therefore in vitro susceptibility testing is not routinely recommended for patient management.

Treatment of protothecosis remains controversial. Antifungals such as ketoconazole, itraconazole,

fluconazole, conventional and liposomal amphotericin B are the most commonly used drugs to date. Usually treatment involves medical and surgical approaches and failure is not uncommon. So far, amphotericin B is the recommended first-line therapy for disseminated protothecosis and for severely immunosuppressed patients with *Prototheca* infection. The optimal dose and duration of therapy is uncertain.

In summary, human protothecosis is a rare opportunistic infection mainly affecting the immunocompromised patients which can be difficult to diagnose or misdiagnosed if not suspected clinically and therefore needs awareness

of the clinicians. Management is poorly defined but surgical excision and antifungal therapy are currently considered the mainstay of therapy.

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THE PERILS OF KEEPING PESTS AS PETS

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Case Report

A 13-year old girl presented to an outer metropolitan hospital in Perth with 3 days of fever, headache, vomiting, lethargy, polyarthralgia and a generalized maculopapular rash. Apart from a fever (T = 39.7°C) and a rash, examination was unremarkable.

Laboratory findings revealed an elevated white cell count (25.90x10⁹/L) with neutrophilia (91.9%) and a high C-reactive protein (89mg/L) together with normal liver function tests and a normal chest x-ray. Blood cultures were taken and she was conservatively managed and was discharged home as she improved within hours.

She represented to the emergency department on the same day with recurrence of symptoms. Her WCC remained elevated with a further rise in the C - reactive protein.

She was treated with ceftriaxone 1g daily to which she had a rapid response. Her blood cultures were negative at 48hrs and she was discharged home on a course of oral cephalexin without a specific diagnosis.

Blood cultures flagged positive 96 hours after incubation for elongated fusiform gram-negative bacilli which took another 48 hours to grow in sub culture. As the organism was biochemically unidentifiable, 16s rRNA gene sequencing was performed on culture and the organism was identified as *Streptobacillus moniliformis*.

Major cellular fatty acid profile of this isolate by gas liquid chromatography was 18:0, 10:0, 16:0, 18:1 which was partially compatible with *S.moniliformis*.

Antibiotic susceptibility tests performed by Etests demonstrated the following MICs: penicillin, 0.032 mg/L; ceftriaxone, 0.002 mg/L; doxycycline, 0.032 mg/L; clindamycin, 0.002 mg/L.

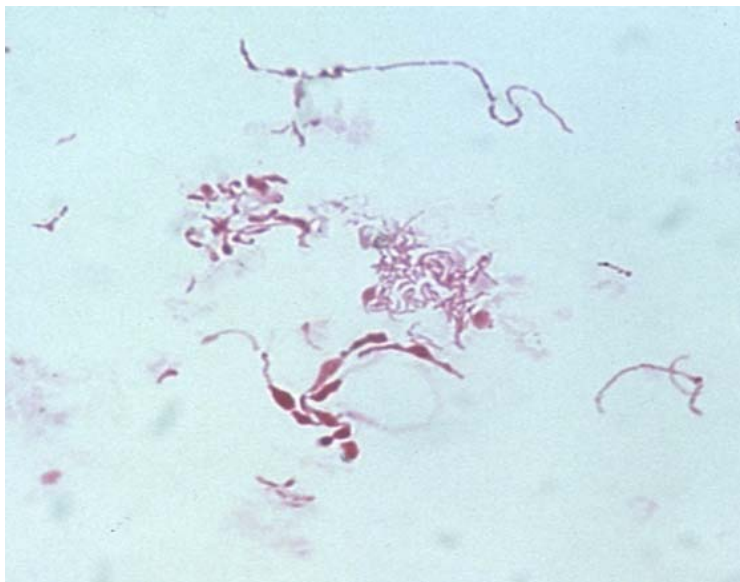
The patient was reviewed and was found to be well and her inflammatory parameters had returned to normal. She had no signs of complications of rat-bite fever. She was further treated with a 7-day course of oral amoxicillin-clavulanic acid.

On questioning, she revealed that she keeps pet rats, cleaned their cage everyday, cuddled and played with them.

Laboratory diagnosis

Streptobacillus moniliformis is an extremely fastidious facultative anaerobic organism that needs microaerophilic conditions to grow, making microbiological diagnosis difficult. It is inhibited by 0.05% sodium polyanethol sulfonate (SPS) that is added to most commercial aerobic blood culture bottles as an anti-coagulant.

Optimal growth requires addition of 20% blood, serum or ascitic fluid into growth medium. It grows slowly and takes about 2-7 days. Colonies show a typical "cotton ball" appearance in broth cultures and in solid media it forms circular, convex greyish colonies some of which may show "fried egg" appearance after few days of incubation. These L-phase variants can be induced by exposure to penicillin.



a



b

Figure 1.

- a. Gram Stain - Pleomorphic gram-negative bacilli in chains with areas of swelling (*moniliformis*- string of pearls) that form long filamentous cells (> 100um) that may fold in to coils or loops.
- b. Characteristic "cotton-ball" appearance in broth culture.

Discussion

Rat-bite fever is a rare zoonotic infection, characterised by fever, rash and arthritis (1) caused by *Streptobacillus moniliformis* or *Spirillum minus*. *Streptobacillus moniliformis* is responsible for most infections in North America, whereas *Spirillum minus* is the causative organism for most infections in Asia. Most reported cases of rat bite fever are from North America and Europe. There are only 7 cases reported from Australia since 1940, all of which were caused by *S. moniliformis* (2,3).

Historically, rat-bite fever occurred in those living in poverty, but now as rats are becoming popular pets and study animals, cases are increasingly seen in a wider group of people such as pet owners, laboratory technicians and pet shop workers, which makes rat-bite fever an emerging disease which needs increased awareness (4,5).

S. moniliformis is a part of the normal flora of the rodent's mouth and upper respiratory tract. Domesticated and wild rats are asymptotically colonized. Transmission to humans is via a bite or scratch; but 30% of the cases documented has no known bite or exposure.

Untreated rat-bite fever has a mortality rate of 10% but outcomes are good with appropriate antibiotic treatment. Reported complications of rat-bite fever include endocarditis, myocarditis, pericarditis, meningitis, hepatitis, nephritis, septic arthritis, pneumonia and focal abscesses, of which endocarditis carries the highest rate of mortality (53%) -(6).

Penicillin is the treatment of choice for rat-bite fever. Duration of treatment is for 7-14 days for uncomplicated

patients. Cephalosporins also have been used successfully in treatment (53%) (6).

Conclusions

Untreated rat-bite fever can result in severe sequelae. Therefore, it is important that clinicians and laboratory staff are aware of the possibility of this infection.

A lack of history of an animal bite should not dissuade clinicians from suspecting the disease.

Acknowledgements

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Dr. Joyce Gunawardene: A tribute

Dr. Joyce Gunawardene, former Consultant Microbiologist, NIHS passed away 3 months ago on the 5th of March 2013. Her death occurred gently and silently as the way she lived her life, bringing sadness and grief to all those who knew her well and to the countless people to whom she had given a helping hand. "Joyce" as she was affectionately known to her friends and colleagues, lived true to her name as she brought joy and comfort to all those who crossed her path. She was a lovely human being.

Joyce Isabel Gunawardene [nee Gunaratne] was born in Kalutara. She received her early education at Sri Sumangala Girls School, Panadura and then at Methodist College, Colombo. She entered the Faculty of Medicine Colombo, and passed out as a doctor in 1961. She met her life partner Dr. B. D. P Gunawardene, better known as 'Captain' while working as a Medical Officer at the Base Hospital, Balapitiya.

Joyce was selected for Bacteriology as a field for her post graduate studies. She was posted to the Institute of Hygiene [presently known as the National Institute of Health Sciences] Kalutara, after a short stint at the Medical Research Institute. In 1972, Joyce went overseas to the University of Manchester, U.K for her post graduate training where she obtained her Diploma in Medical Microbiology. She was appointed as a Consultant Microbiologist to the Institute of Hygiene Kalutara, where she served till her retirement.

Very few people know of her achievements as she never sought fame or publicity. She worked efficiently and selflessly behind the scenes and rendered an immense service to the country especially in the fields of Food Microbiology and Public Health. She was one of the pioneer Food Microbiologists in Sri Lanka and was responsible for the establishment and organisation of the national food laboratory at NIHS under the ongoing USAID /WHO project. She received an intensive training overseas under this project and she was in charge of this laboratory until her retirement. She played a major role

as a trainer in all categories of public health personnel namely Medical officers of Health, Public Health Nurses, Public Health Inspectors and Medical Laboratory Technologists. She was also involved in training of medical officers enrolled in the Diploma in Medical Microbiology course conducted by the Post Graduate Institute of Medicine. She was the author of several laboratory manuals which served as a useful guide to MLTTs and Food Technologists. She was a senior member and a Past President of the Sri Lanka College of Microbiologists.

Apart from her profession Joyce had many talents. She was an excellent seamstress. Smocking was her forte. I was fortunate that she sewed beautiful dresses with exquisite smoking for my daughter as well as for my granddaughter. She was also an expert cook.

I first met Joyce 33 years ago when I joined NIHS as a young post intern. Although she was more than 15 years older, she took me under her wing and this was the beginning of a truly wonderful friendship which lasted until her death. Joyce radiated boundless love and kindness to all those life she touched. An unkind word never crossed her lips and anger and hostility were alien to her. Her most endearing qualities were her gentleness and her innate goodness which ran within her like a skein of silver. Her compassion especially for the less fortunate was boundless. It was to her that they came in their hour of need. Her gentle, softly spoken words together with her serene temperament were a soothing balm to those who came to her and as a result she was loved and respected by all. Although Joyce is no longer with us, her loving spirit lingers on guiding and inspiring us. Farewell gentle friend, may your journey through Sansara be short and peaceful and may you reach the Supreme bliss of Nibbana.

Rukmal

(This appreciation was written by Dr. Rukmal Seneviratne)

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