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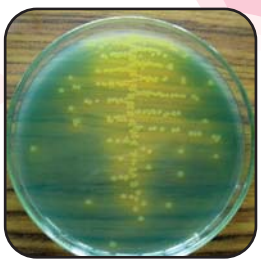

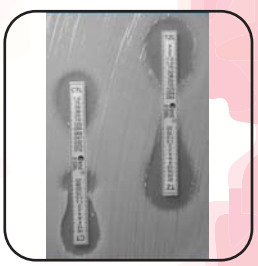
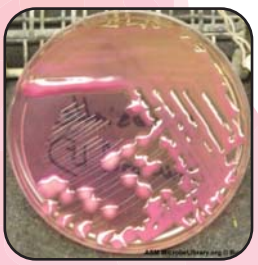
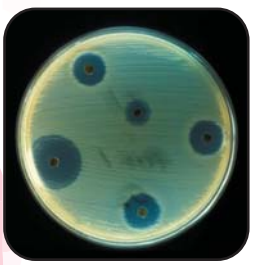

The Sri Lanka College of Microbiologists

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MESSAGE FROM THE PRESIDENT

I consider it a great pleasure and privilege to be able to write the welcome address for the 20th Annual Scientific Sessions of the Sri Lanka College of Microbiologists to be held from 14th to 16th September 2011 in Colombo.

The theme for the current year is "Antimicrobial Resistance - No action today, No cure tomorrow" in keeping with the theme of the World Health Organization for 2011. Emerging antimicrobial resistance is a global issue engulfing both the developing world and the developed world alike. But countries in the developing world have more serious problems with regard to antimicrobial resistance. Many of these are associated with overuse or inappropriate use of antimicrobial agents by clinicians and some of the resistance problems can be addressed by application of good clinical judgment by practicing physicians.

The membership of the Sri Lanka College of Microbiologists has recognized the importance of this responsibility and has been working for years to achieve the best they could, by conforming to good practices, sharing knowledge with colleagues in other fields of medicine, conducting regular CME activities for the membership and other medical colleagues, and carrying out teaching and training of undergraduates, postgraduates and para-medical personnel.

The Antimicrobial Resistance Surveillance Project of the College, during the last 3 years, has targeted Gram negative blood culture isolates of selected centers and contributed to the antimicrobial sensitivity data pool. This year the project has expanded to include Gram positive blood culture isolates with 70% coverage of the country.

Another great achievement in the current year is the joint initiative of the Ministry of Health and the Sri Lanka College of Microbiologists to spread this surveillance network to cover all state sector hospitals with Consultant Microbiologists and to include the results from other clinical specimens as well.

I am very happy to inform that our dedicated membership has been extremely active during the year revising the 'Laboratory Manual of the Sri Lanka College of Microbiologists' and revising and developing the 'Bio-Safety Manual for Medical Laboratories'. Both these revised and updated manuals will be available for use within the current year. We also conducted monthly CME programmes for doctors; and several workshops were held for Medical Laboratory Technologists in the peripheries. All these achievements were possible due to the untiring efforts of the past presidents, council members and the general membership.

Together with my council, I wish to thank all those who are presenting papers and posters, delivering plenary lectures and contributing to the symposia at the sessions. I wish to extend my sincere gratitude to the Chief Guest, Guest of Honour, guest speakers, distinguished guests and invitees for having accepted our invitation to grace this occasion.

I wish to express my sincere thanks to the very supportive joint secretaries, editor, members of the council and all the members of the College for the support and encouragement given to me during my tenure of office as the President of the Sri Lanka College of Microbiologists.

A special 'thank you' goes out to all our sponsors for their generous financial contributions.

I wish the College every success in future endeavors and years of fruitful service to Mother Lanka.

Dr Pranitha Somaratne
President
Sri Lanka College of Microbiologists

14th September 2011

20th Annual Scientific Sessions

The Sri Lanka College of Microbiologists

Inauguration

14th September 2011 at 6.15 pm

Berjaya Mount Royal Hotel
Mount Lavinia

Pre-congress workshop on Antimicrobial Resistance

14th September 2011 from 8.30 am to 12.30 pm

Scientific Programme

15th & 16th September 2011

Aldo Castellani Auditorium

Medical Research Institute,
Colombo 8.

INAUGURATION

6.30 pm - Invitees take their seats

6.45 pm - Arrival of the Chief Guest
Introduction of Members of the Council

6.50 pm - Ceremonial procession

6.55 pm - National Anthem

7.00 pm - Traditional lighting of the oil lamp

7.05 pm - Welcome Address
Dr. Samanmalee Gunasekara
Hony. Joint Secretary

7.10 pm - Address by the Chief Guest
Prof. Uditha Liyanage
Director, Postgraduate Institute of Management

7.30 pm - Address by the Guest of Honour
Prof. Lily Therese
Consultant Microbiologist, Sankaranethralaya Eye Hospital, Chennai

8.00 pm - Address by the President
Dr. Pranitha Somaratne
Consultant Microbiologist

8.30 pm - Vote of Thanks
Dr. Thamara Wijesuriya
Hony. Joint Secretary

8.35 pm - Cultural Show

8.45 pm - Ceremonial Procession leaves

8.50 pm - Reception
(Sponsored by PCL Solutions Pvt. Ltd)

20th Annual Scientific Sessions

The Sri Lanka College of Microbiologists

14th - 16th September 2011

Aldo Castellani Auditorium, Medical Research Institute, Colombo 8.

"Antimicrobial Resistance: No Action Today, No Cure Tomorrow"

Pre-congress workshop on "Antimicrobial Resistance" – 14th September 2011

8.00 am - 8.30 am	**Registration**
8.30 am - 9.15 am	**Patterns of antimicrobial resistance and its outcome - current situation in Sri Lanka** Dr. Geethika Patabendige *Consultant Microbiologist, National Cancer Institute of Sri Lanka, Maharagama* ***Chairperson:*** **Dr. Kanthi Nanayakkara**
9.15 am - 10.00 am	**Antibiotics in animal husbandry** Dr. Gnana Gunawardene *Veterinary Surgeon, Veterinary Research Institute, Gannoruwa* ***Chairperson:*** **Dr. Varuna Navaratne**
10.00 am - 10.15 am	**Tea**
10.15 am - 11.15 am	**Antibiotic resistance and automation in Microbiology** Dr. T. D. Chugh *Head of Department of Microbiology,* *BL Kapur Hospital, New Delhi* ***Chairperson:*** **Dr. Kumudu Karunaratne**
11.15 am - 12 noon	**Prebiotics and Probiotics** Dr. H. T. Wickramasinghe *Consultant Peadiatrician* ***Chairperson:*** **Dr. Preethi Perera**

15th September 2011

8.30 am - 9.00 am **Registration**

9.00 am - 10.00 am **Free Paper Session 1 – Bacteriology**

Chairpersons: Dr. Ajith Nagahawatta and Prof. Neluka Fernando

OP 1 Causative organisms and demographic data of infective endocarditis in a tertiary care hospital in Sri Lanka

Chandrasiri NS[1], Athukorala GIDDAD[1], Rathnayaka NR[1], Feroza MBF[1], Jayawardhana JMDD[1], Perera P[2], Perera UPN[1],
[1]Department of Microbiology, Colombo South Teaching Hospital, Kalubowila, Sri Lanka, [2]Medical Research Institute, Colombo, Sri Lanka.

OP 2 Antibiotic susceptibility pattern and minimum inhibitory concentration for vancomycin in MRSA isolates at National Cancer Institute, Sri Lanka.

Dissanayake BN, Patabendige CGUA
National Cancer Institute, Sri Lanka.

OP 3 An assessment of diagnostic accuracy of semi quantitative culture of refrigerated urine samples

Wadanamby JMRWW[1], Piyananda MGP[2], Azam MMNM[2], Lankika TLI[1], Priyantha KGS[1], Ireshika GDI[1], Udukumara HPN[1], Krishantha HTG[1]
[1]Department of Microbiology, Base Hospital Angoda, [2]Department of Microbiology, Base Hospital Mulleriyawa.

OP 4 Quality control of CLSI and Stokes' method of antibiotic susceptibility testing – a preliminary study

Mendis BCG, Thevanesam V, Ekanayake WMA
Department of Microbiology, Faculty of Medicine, University of Peradeniya.

10.00 am - 10.15 am **Tea**

10.15 am - 11.00 am **Plenary lecture 1**

1st line antimicrobials for tuberculosis

Prof. Lilly Therese
Consultant Microbiologist, Head of Department, Vision Research Institute, Sankaranethralaya Eye Hospital, Chennai, India.

Chairperson: **Dr. Jayanthi Elwitigala**

11.00 am - 12.00 noon **Free Paper Session 2 – Virology**

Chairpersons: **Prof. N P Sunil Chandra and Dr. Geethani Wikramasinghe**

OP 5 Immunogenicity study to assess rabies neutralizing antibodies in previously immunized patients

Perera KADN, Rajapakse YN, Nanayakkara N, Wimalaratne OV, Liyanage AD
Department of Rabies Diagnosis and Vaccine Quality Control, Medical Research Institute, Colombo.

OP 6 Comparison of recombinant protein and cell lysate antigens for detection of anti-chikungunya (CHIK) IgM antibody

Athapaththu AMMH[1], Khanna N[2], Inouve S[3], Tun MMN[3], Gunasena S[4], Abeyewickreme W[1], Hapugoda M[1]
[1]Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka, [2]International Centre for Genetic Engineering and Biotechnology, New Delhi, India, [3]WHO Collaborative Centre for Viral Reference and Research, Institute of Tropical Medicine, Nagasaki University, Japan,
[4]Department of Virology, Medical Research Institute, Colombo 8, Sri Lanka.

OP 7 Detection of influenza A and B antigens in paediatric patients
Pandithasundara H[1], *Noordeen F*[1], *Senavirathna SK*[1], *Abeykoon SB*[1], *Faizal MAM*[2], *Morel AJ*[3], *Mudiyanse RM*[4]
[1]Department of Microbiology, Faculty of Medicine, University of Peradeniya, [2]Sirimavo Bandaranayaka Specialized Children's Hospital, Peradeniya, [3]Teaching Hospital, Kegalle, [4]Department of Paediatrics, Faculty of Medicine, University of Peradeniya.

OP 8 Clinical and virological features of dengue in 2010
Hapugoda MD[1], *Manamperi H*[1], *Gunasena S*[2], *Athapaththu AMMH*[1], *Premawansa G*[3], *Wellawaththage C*[1], *Jayarathna TDSS*[1], *Abeyewickreme W*[1]
[1]Faculty of Medicine, University of Kelaniya, Ragama, [2]Medical Research Institute, Colombo 08, [3]North Colombo Teaching Hospital, Ragama.

12 pm - 12.15 pm Clinical waste management using an incinerator – PCL Solutions (Pvt) Ltd.

12.15 pm - 1.15 pm Lunch

1.15 pm - 2.15 pm

Symposium 1 – Role of the microbiology laboratory in elimination of parasitic infections

Moderators: **Prof. M. M. Ismail and Dr. Sagarika Samarasinghe**

Control of soil transmitted helminth infections
Prof. Nilanthi de Silva
Professor of Parasitology, Faculty of Medicine, Ragama, University of Kelaniya.

Role of the microbiology laboratory in the elimination of parasitic diseases – Malaria and Leishmaniasis
Prof. Renu Wickramasinghe
Professor in Parasitology, Faculty of Medicine, University of Sri Jayawardenepura, Nugegoda.

Role of the parasitology laboratory in the elimination programme of filariasis in Sri Lanka
Prof. Mirani Weerasuriya
Professor of Parasitology, Faculty of Medicine, Galle, University of Ruhuna.

2.15 pm - 3.15 pm

Free Paper Session 3 – Parasitology and Virology

Chairpersons: **Dr. Omala Wimalaratne and Prof. Nadeera Karunawera**

OP 9 Measles and rubella surveillance in Sri Lanka from January 2010 to June 2011
Wickramasinghe G, Kumarasinghe KADM, Wijerathna T, Gunarathna K, Withanage WNL
Medical Research Institute, Colombo 8.

OP 10 Seroprevalence of chikungunya infection in adults 20 years and above in selected wards of the Colombo municipality area
Kumarasinghe KADM, Gunasena S, Abeysinghe N, Fernando C
Medical Research Institute, Colombo 8.

OP 11 Comparison of laboratory techniques for diagnosis of hookworm infections.
Manamperi NH[1], *Anjalee KGC*[2], *Gunawardena NK*[1], *Sudusinghe H*[1], *Nilaweera THWT*[1], *de Silva NR*[1]
[1]Department of Parasitology, Faculty of Medicine, University of Kelaniya, [2]Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Peradeniya.

OP 12 Determining the geographical origin of *Plasmodium vivax* using five microsatellite markers, instead of twelve: a more cost effective tool
De Silva C, Karunaweera N, Gunawardena S
Department of Parasitology, Faculty of Medicine, University of Colombo, Sri Lanka.

3.15 pm - 4.30 pm **Symposium 2 – Bio-security and Bio-safety**

Moderators: **Dr. Geetha Nanayakkara and Dr. Rohini Wadanamby**

Biosafety programmes – policies and goals
Dr Geethani Galagoda
Consultant Virologist, Medical Research Institute, Colombo.

Transport of biological substances and International Health Regulations
Dr Lilani Karunanayake
Consultant Microbiologist, Medical Research Institute, Colombo.

Interactive session on bio-safety and bio-security equipment and emergency procedures
Dr Sunethra Gunasena
Consultant Virologist, Medical Research Institute, Colombo.

4.30 pm Tea and close of proceedings of day 1

16th September 2011

8.30 am - 9.30 am **Free Paper Session 4 – Bacteriology and Mycology**

Chairpersons: **Dr. Maya Atapattu and Dr. Pankaja Kalukottege**

OP 13 A study of paediatric patients with candidaemia at the Lady Ridgeway Hospital
Karunaratne GKD[1], Kathriarachchi K[1], Perera P[2], Jayasundera TSK[1]
[1]Lady Ridgeway Hospital, Colombo 8, [2]Medical Research Institute, Colombo 8.

OP 14 A study to determine the species of malassezia causing pityriasis versicolor and their comparable response to single dose therapy of fluconazole and seven day therapy of itraconazole in a teaching hospital in the Western Province
Dissanayake DMS[1], Perera P[2], Karunasekara P[1], Perera M[2]
[1]Dermatology Clinic , CSTH, Kalubowila, [2]Mycology Department, MRI, Colombo 8.

OP 15 Experience with an automated liquid culture system for Mycobacteria species
Senanayake SPS, Elwitigala JP
National TB Reference laboratory, Welisera.

OP 16 Exploring the antimicrobial activity of Triphala – a traditional medicine formulation
Manoraj A, Thevanesam V, Ekanayake WMA
Department of Microbiology, Faculty of Medicine, University of Peradeniya.

SCIENTIFIC PROGRAMME

9.30 am - 10.15 am

Plenary lecture 2 – Viral Encephalomyelitis

Dr. Channa Senanayake
Senior Lecturer, Faculty of Medicine, University of Colombo, Colombo 8

Chairperson: **Dr. Nalini Vithana**

10.15 am - 10.30 am Tea

10.30 am - 11.30 am

Symposium 3 – Quality Assurance and Accreditation of Medical Laboratories

Moderators: **Prof. Lalitha Mendis and Dr. Ranjith Perera**

Introduction to accreditation
Prof. Vasanthi Thevanesam
Professor of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya.

Improving quality assurance with ISO guidelines
Mr. Rahal Widanagamage
Senior Assessor, Sri Lanka Accreditation Board, Bauddaloka Mawatha, Colombo 7.

Emerging needs in laboratory assessments
Dr. K. A. C. Wickramaratne
Senior Lecturer, Faculty of Medicine, University of Ruhuna, Galle.

11.30 am - 12.30 pm

Free Paper Session 5 – Bacteriology

Chairpersons: **Prof. Emil Wijewatha and Dr. Sujatha Mananwatte**

OP 17 Sero-prevalence of leptospirosis among paddy-field workers in Kalutara district
Senanayake T[1], Somaratne P[2], Premaratne R[3], Perera R[4], Gunaratne N[4]
[1]Postgraduate Trainee in Medical Microbiology, [2]Consultant Microbiologist, Medical Research Institute, Borella, [3]Deputy Director, Anti-Malaria Campaign, [4]Medical Laboratory Technician, Medical Research Institute, Colombo 8.

OP 18 Prospective study of patients with infective endocarditis
Piyasiri DLB, Chandrasiri P
National Hospital of Sri Lanka, Colombo.

OP 19 A comparison of drug susceptibility of Mycobacterium species isolated at National TB Reference Laboratory, Sri Lanka between 2000 and 2010
Jayawardena KDJHM, Elwitigala JP
National TB Reference Laboratory, Welisera.

OP 20 The incidence and characteristics of extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella* species among urinary isolates in a tertiary care hospital
Dissanayake DMBT[1], Fernando SSN[1], Chandrasiri NS[2], Mahendra R[1]
[1]Department of Microbiology, University of Sri Jayewardenepura, Gangodawila, Nugegoda, [2]Colombo South Teaching Hospital, Kalubowila.

12.30 noon - 1.30 pm Lunch

1.30 pm - 2.00 pm Antimicrobial resistance surveillance project report
Dr. Shirani Chandrasiri
Consultant Microbiologist, Colombo South Teaching Hospital, Kalubowila.

Chairperson: **Prof. Nelun De Silva**

2.00 pm - 3.00 pm

Symposium 4 – Leptospirosis

Moderators: **Dr. Philomena Chandrasiri and Dr. Malika Karunaratne**

Leptospirosis in the Central Province of Sri Lanka and specific management issues
Prof. S. A. M. Kularatne
Professor of Medicine, Faculty of Medicine, University of Peradeniya, Peradeniya.

Epidemiology of Leptospirosis - is it changing?
Dr. Jagath Amarasekara
Consultant Community Physician, Epidemiology Unit, Colombo 10.

Laboratory diagnosis of leptospirosis – an update
Dr. Pranitha Somaratne
Consultant Microbiologist, Medical Research Institute, Colombo 8.

3.00 pm - 3.30 pm

Interactive session
Dr. Kushlani Jayathilleke
Consultant Microbiologist, Sri Jayawardenepura General Hospital, Nugegoda.

Chairperson: **Dr. Enoka Corea**

3.30 pm - 4.00 pm

Awards and closing ceremony

4.00 pm

Tea

LIST OF GUEST SPEAKERS

Prof. Lilly Therese
Consultant Microbiologist, Sankaranethralaya Eye Hospital, Chennai, India

Dr. T. D. Chugh
Head of Department of Microbiology, BL Kapur Hospital, New Delhi, India

Prof. S. A. M. Kularatne
Professor of Medicine, Faculty of Medicine, University of Peradeniya, Peradeniya

Dr. H. T. Wickramasinghe
Consultant Paediatrician

Dr. Gnana Gunawardene
Veterinary Surgeon, Veterinary Research Institute, Gannoruwa

Dr. K. A. C. Wickramaratne
Senior Lecturer, Faculty of Medicine, University of Ruhuna, Galle

Mr. Rahal Widanagamage
Senior Assessor, Sri Lanka Accreditation Board, Bauddaloka Mawatha, Colombo 7

Dr. Jagath Amarasekara
Consultant Community Physician, Epidemiology Unit, Colombo 10

Prof. Vasanthi Thevanesam
Professor of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya

Prof. Mirani Weerasooriya
Professor of Parasitology, Faculty of Medicine, University of Ruhuna

Prof. Nilanthi de Silva
Professor of Parasitology, Faculty of Medicine, University of Kelaniya

Dr. Channa Senanayake
Senior Lecturer, Faculty of Medicine, University of Colombo, Colombo

Dr. Pranitha Somaratne
Consultant Microbiologist, Medical Research Institute, Colombo 8

Prof. Renu Wickramasinghe
Professor in Parasitology, Faculty of Medicine, University of Sri Jayawardenepura, Nugegoda

Dr. Sunethra Gunasena
Consultant Virologist, Medical Research Institute, Colombo

Dr. Geethani Galagoda
Consultant Virologist, Medical Research Instituite, Colobmbo 8

Dr. Geethika Patabendige
Consultant Microbiologist, National Cancer Institute of Sri Lanka

Dr. Shirani Chandrasiri
Consultant Microbiologist, Colombo South Teaching Hospital, Kalubowila

Dr. Kushlani Jayathilleke
Consultant Microbiologist, Sri Jayawardenapura General Hospital, Nugegoda

Dr. Lilani Karunanayake
Consultant Microbiologist, Medical Research Institute, Colombo

OP 1

Causative organisms and demographic data of infective endocarditis in a tertiary care hospital in Sri Lanka

[1]*Chandrasiri NS*[1], *Athukorala GIDDAD*[1], *Rathnayaka NR*[1], *Feroza MBF*[1], *Jayawardhana JMDD*[1], *Perera P*[2], *Perera UPN*[1]

[1]Department of Microbiology, Colombo South Teaching Hospital, Kalubowila, [2]Medical Research Institute, Colombo.

Objectives

To determine the causative agents and risk factors of infective endocarditis (IE) and to develop empiric antibiotic guidelines for IE patients in Colombo South Teaching Hospital (CSTH).

Methods

CSTH is a tertiary care hospital having 1100 beds. A descriptive prospective study was carried out from 1st January 2006 to 30th November 2010. Patients suspected of having IE, with a positive blood culture for a possible pathogen causing IE were included. Manual blood cultures were done. Identification (ID) of isolates and antibiotic sensitivity was done using standard techniques. American Heart Association Guidelines published in 2007 were used in the diagnosis of IE.

Results

Nineteen blood culture positive, suspected IE patients were found during the study period. Out of the 19 patients, 15 (79%) had definitive IE, and rest had possible IE. Sixteen (84.2%) had vegetations visualized by echo-cardiography. Nine (47.4%) had 2 positive blood cultures. None had history of rheumatic heart disease (RHD) but, 14 (73.7%) had a cardiac valvular lesion at the time of diagnosis. All had native valve endocarditis. Sixteen (84.2%) were males.

Twelve (63.2%) isolates were streptococcal species. Of them 6 (50%) were viridians group streptococci (VGS), 2 (16.7%) were *Streptococcus bovis* (SB), 2 (16.7%) were other group D streptococci (GDS), one (8.3%) was *Streptococcus agalactiae* (SA) and one was an Enterococcus (ES) species. Of the 5 isolates for which MIC for penicillin was performed, 2 were VGS (MIC 0.005µg/ml and 0.25µg/ml), 2 were SB (MIC 0.002µg/ml and 0.16µg/ml) and one was GDS (MIC 0.032µg/ml). The SA and ES isolates were sensitive to penicillin by disc diffusion. Other isolates included 3 (15.8%) *Salmonella typhi* (ST), one (5.3%) *Salmonella paratyphi A* (SPA), one (5.3%) MSSA, one (5.3%) HA-MRSA and one (5.3%) *Candida gulliermondii*. During this period SPA was the commonest isolate from enteric fever (147:52).

Conclusion

The rate of ST isolation from IE is higher than in other studies. A combination of c. penicillin and gentamicin should be the initial therapy but non responders need ceftriaxone as this will cover *Salmonella* spp. or HACEK organisms.

OP 2

Antibiotic susceptibility pattern and minimum inhibitory concentration for vancomycin in MRSA isolates at the National Cancer Institute, Sri Lanka

Dissanayake BN, Patabendige CGUA

National Cancer Institute, Maharagama.

Objectives

To determine the antibiotic susceptibility (ABST) pattern of MRSA isolates in the National Cancer Institute, Sri Lanka (NCISL) and to determine vancomycin minimum inhibitory concentrations (MIC) for these isolates.

Methodology

This study was conducted at the NCISL from January to April 2009. Ninety three (93) *Staphylococcus aureus* isolates were recovered during this period. Resistance to methicillin was detected using cefoxitin 30µg discs according to the CLSI guidelines. Vancomycin MIC was performed using Etest strips (Solna, Sweden). Results were interpreted according to the CLSI guidelines (2008).

Results

Higher resistance rates were observed for erythromycin (94.62%) followed by clindamycin (78.49%) of which 67 (91.78%) were due to inducible resistance. Resistance to gentamicin was 60.22% and that for cotrimoxazole and ciprofloxacin were 63.44% and 58.07% respectively. Resistance to amikacin and netilmicin were 40.86% and 37.63% respectively. Resistance to fusidic acid was 22.58% and was remarkably low (2.16%) for rifampicin. No resistance was observed for linezolid.

Table 1. Distribution of vancomycin MICs among isolates

MIC (µg/ml)	0.25	0.32	0.38	0.5	0.75	1	1.5	2
Number of isolates	1	3	4	12	22	31	14	6

MIC_{50} – 1.0 µg/ml MIC_{90} – 1.5 µg/ml

Conclusion

Resistance to erythromycin and clindamycin was considerably high in this institute, while it was very low to rifampicin and linezolid. None of the isolates were found to be intermediate sensitive or resistant to vancomycin.

OP 3

An assessment of the diagnostic accuracy of semi quantitative cultures of refrigerated urine samples

Wadanamby JMRWW[1], *Piyananda MGP*[2], *Azam MMNM*[2], *Lankika TLI*[1], *Priyantha KGS*[1], *Ireshika GDI*[1], *Udayakumara HPN*[1], *Krishantha HTG*[1]

[1]Department of Microbiology, Base Hospital Angoda, [2]Department of Microbiology, Base Hospital Mulleriyawa.

Introduction

Semi quantitative culture of urine is used to diagnose urinary tract infections since 1957, following a publication by Kass. Urine samples are not processed after working hours in many centres. Such samples are kept in the refrigerator till the next day, either in the lab or in the ward. We decided to assess the accuracy of this practice.

Objectives

To determine any difference in the result of urine culture of MSU cultured on receipt and after overnight refrigeration.

To determine the diagnostic accuracy of semi quantitative culture of refrigerated urine samples.

Methodology and settings

The study was carried out in the microbiology departments of the Base Hospital, Mulleriyawa (BHM) and Base Hospital Angoda (BHA). Urine samples, routinely received within 1-2 hrs after collection, in both hospitals over a period of about 2 months were included. Already refrigerated samples were excluded. Samples were divided into two. One was semi quantitatively cultured on day one and the other the next day after overnight refrigeration. The presence of growth, the colony types and colony counts were recorded and compared.

Results

Total number of samples in both BHA and BHM – 338

Number of samples that showed similar results on both occasions – 292 (86%)

(no bacterial growth =197, mixed growth= 48, insignificant growth = 2, coliform $>10^5$ = 38, acinetobacter$>10^5$ = 3, pseudomonas $>10^5$ = 2, diphtheroids = 1)

Number of samples which showed dissimilar results – 52 (14%)

(7 positives (2%), and 5 negatives (1.4%) in the overnight refrigerated samples. 40 samples showed differences but did not alter the final results)

Conclusion

This study shows that refrigerated urine samples can be cultured with negligible error rates. Instances when there is undue delay in transferring samples or where urine is collected after working hours may adopt this method safely.

OP 4

Quality control of CLSI and Stokes' method of antibiotic susceptibility testing – a preliminary study

Mendis BCG, Thevanesam V, Ekanayake WMA

Department of Microbiology, Faculty of Medicine, University of Peradeniya.

Introduction

Antimicrobial susceptibility testing (ABST) in clinical diagnostic laboratories is a routine procedure of proven clinical benefit. Although a change from the Stokes' method to the CLSI (Clinical and Laboratory Standards Institute) method is recommended in Sri Lanka, several constraints exist in carrying out the recommended quality control (QC) procedures in Sri Lankan microbiology laboratories.

Objective

To determine the performance of the 2 methods using the recommended quality control procedure for each method.

Design, setting and methods

A daily QC was carried out for both methods using five antibiotics – chloramphenicol, amikacin, ceftazidime, gentamicin, cefuroxime – with *Escherichia coli* (ATCC 25922) for CLSI and *Escherichia coli* (NCTC 10418) for Stokes respectively for a 30 day period. QC of the CLSI method was assessed using Shewhart charts. Optimal inoculum and inhibitory zones within 8-15mm radius described in the Stokes' method as being satisfactory for reporting of results was used as the QC measure of the Stokes' method.

Results

CLSI method:
Using the Shewhart chart, 2, 2, 7 and 16 readings for chloramphenicol, ceftazidime, amikacin and gentamicin respectively were outside the given QC range. With 4 of the 5 antibiotics tested, the zone diameters were consistently below the mean (chloramphenicol – 5/30; amikacin – 27/30; gentamicin – 30/30; cefuroxime – 11/20) indicating a systematic error.

Stokes' method:
Chloramphenicol, amikacin and ceftazidime met the QC requirements of the Stokes' method. Gentamicin and cefuroxime had 2 and 3 of 30 readings respectively below the expected range.

Conclusion

Further work is needed before establishing the CLSI method in routine laboratories unable to perform daily QC on all antibiotics tested. The Stokes method may provide a more robust and flexible method for ABST in resource limited laboratories.

OP 5

Immunogenicity study to assess rabies neutralizing antibodies in previously immunized patients

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Introduction

A significant number of patients (11%) who seek anti rabies post exposure therapy (PET) following a re-exposure, had received either a full or the first three doses (partial course) of anti rabies vaccine (ARV) previously. There are no recommended guidelines on treatment of patients

Objectives

To assess rabies virus neutralizing antibody titre (RVNAT) in patients who received PET at different times previously and to assess the booster response following subsequent intradermal (ID) ARV therapy.

Methodology

Eighty two patients exposed to suspected rabid animals with a past history of ARV therapy were enrolled. Patients were categorized into four groups depending on duration following the last dose of previous ARV (Group - 1: <6 months; 2: 6 months - 2 years; 3: 2-5 years; 4: >5 years). RVNAT were determined by rapid fluorescent focus inhibition test on day 0 (D0) and day 14 (D14) post-vaccination following 2 or 3 ARV booster doses.

Results

Except for 4 patients in groups 2 and 3, all others had RVNAT >0.5IU/ml (WHO recommended minimum protective level) on D0. All patients, showed high antibody titres on D14 following booster doses.

Discussion and recommendations

Four patients whose RVNAT was <0.5IU/ml on D0 had received only a partial course of ARV previously. As all subjects developed accelerated anamnestic antibody response on D14, recommendation of a full course of PET following a previous partial course of ARV within five years seems to be an over treatment. With these results, we could recommend two booster doses of ID ARV upto five years after even a partial course of ARV.
MRI research grant No.16-2007 is acknowledged.

OP 6

Comparison of recombinant protein and cell lysate antigens for detection of anti-chikungunya (CHIK) IgM antibody

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Introduction

Chikungunya (CHIK) virus specific antigen which has high specificity and low cross reactivity with other related diseases is required for laboratory confirmation.

Objective

To compare two antigens for detection of anti-CHIK antibody.

Design, setting and methods

In this study, two antigens (viral cell lysate and recombinant protein) were evaluated for detection of anti-CHIK antibody by using IgM ELISA. A novel recombinant protein antigen was designed based on envelope domain, a critical antigenic region of the major structural protein. This protein was expressed in *Escherichia coli* and resultant protein was affinity purified and ~10mg with >95% of purity per liter of culture was obtained. Cell lysate antigen was prepared using a crude culture fluid. Two antigens were evaluated separately using a panel of well characterized serum samples obtained from the Dept. of Virology (WHO Reference Centre for Viral Reference and Research), Institute of Tropical Medicine, Nagasaki University.

Results

A total of 64 serum samples confirmed as positives and 22 confirmed as negatives were used to evaluate the antigens. Specificity and sensitivity of the recombinant protein antigen was 48% and 90% respectively. Specificity and sensitivity of the viral lysate antigen was 17% and 100% respectively.

Conclusion

Viral lysate antigens can cause biohazard risk, high production cost and cross reactivity with other organisms of the same genus/family. Recombinant protein antigen which shows high specificity and sensitivity used in this study is important to overcome problems associated with viral lysate antigen. Testing of a large number of samples is needed to reconfirm this finding.

Acknowledgment

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OP 7

Detection of influenza A and B antigens in paediatric patients

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Introduction

Influenza A virus (H1N1) caused a worldwide pandemic from 2009 to 2011. Symptoms of this respiratory illness ranged from classical flu to severe pneumonia. In Sri Lanka, too, there were suspected and laboratory confirmed cases of influenza in the period, with varying severity. Of the clinically reported influenza cases, it is important to identify how many were positive for influenza A virus to make the initial link of the presenting illness to the suspected pathogen.

Objective

To detect influenza A/B antigens in clinically suspected flu patients.

Method

From June to Dec 2010, nasopharyngeal aspirates (NPA) and clinical details were collected from 70 children suspected of having severe flu (age = 2 months-15 years) from Teaching Hospitals Peradeniya and Kegalle and Sirimavo Bandaranayake Specialized Children's Hospital. Laboratory diagnosis was performed using QuickVue Influenza A+B chromatographic test kit (USA) which allows detection of influenza A/ B antigens in NPAs (Sensitivity = 95%; Specificity = 95%).

Results

Of the 70 patients, 11 had cough, cold and fever; 5 had cough, cold, fever and wheezing and others had cough or cold without fever. Six patients had severe respiratory tract infection with 2 requiring ventilation. Of the 70 patients only 5 were positive for influenza A or B (7.14%). Although there was a suspicion, we were able to detect influenza A in only 3 patients. This shows the over suspicion of a pathogen because of panic and awareness during an epidemic. Most importantly, majority of the respiratory infections/diseases reported in the study might have been due to other infective causes which need further investigation.

Acknowledgement

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OP 8

Clinical and virological features of dengue in 2010

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Introduction

Dengue is an important viral infection in Sri Lanka. All 4 serotypes co-circulate in Sri Lanka.

Objective

To study the clinical and virological features of dengue in 2010.

Design, setting and methods

A hospital-based study was carried out at North Colombo Teaching Hospital, Ragama in 2010. Patients clinically suspected of having dengue, with fever less than 5 days were recruited. Acute and convalescent blood samples were collected within 7 days after obtaining informed written consent. Demographic, clinical information and laboratory results were obtained. Acute serum samples were tested using molecular (RT-PCR and Semi-Nested PCR) and serological (ELISAs and HAI) assays. Convalescent samples were tested by serological assays.

Results

Of 209 patients enrolled, 93 % (195/209) were laboratory confirmed as recent positive cases of dengue viral infection; of these, 5% (9/195) were classified as dengue fever; 85%(165/195) dengue haemorrhagic fever (DHF) and 0.5% (1/195) dengue shock syndrome. Mean platelet

value and packed cell volume (PCV) in laboratory confirmed dengue patients were 56,107/mm^3 (range 10,000-306,000) and 42%(range 34-61%) respectively. Patients infected with DHF showed both primary (n=45) and secondary (n=102) infections. Interestingly, secondary infection was not significantly correlated with DHF (χ^2=0.3:p=0.6). DEN-1 was responsible for the majority of cases, with a minority due to other three serotypes; all serotypes contributed to severe disease.

Conclusion

DEN-1 was responsible for the majority of cases in 2010 but it circulated at a low level during previous epidemics. Majority of patients had severe clinical symptoms. In this epidemic, the clinical presentation of dengue differed according to the geographic region and viral serotype.

Acknowledgments

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OP 9

Measles and rubella surveillance in Sri Lanka from January 2010 to June 2011

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Introduction

Measles and rubella are two important causes of fever and rash. Several outbreaks were reported recently. Confirmation of infection is mainly by serological investigations. Different sampling techniques have been introduced by the WHO global measles programme.

Objectives

1. To ascertain the etiology of cases presenting with fever and rash.
2. To study the epidemiological pattern.
3. To assess different specimens and methods of diagnosis.

Methodology

A retrospective descriptive study was carried out from January 2010 to June 2011. 188 Serum samples and 13 oral fluid (OF) samples were tested for virus specific IgM by ELISA. 60 nasal/throat swabs (NTS) were subjected to virus specific RT-PCR. 13 PCR products were sent to the Regional Reference Laboratory (RRL) in Thailand for genotyping. Virus isolation was attempted on 16 NTS. Age, sex and residential area were noted.

Results

35/188 (18.61%) serum samples were positive for measles IgM and 66/188 (35.1%) were positive for rubella IgM. Of the positive rubella cases 20 (30.3%) and 46 (69.7%) were in 2010, 2011 respectively. Of the 13 OF specimens 6 (46.15%) were positive for rubella IgM and 1 was positive for measles IgM.

42.8% of confirmed measles cases were in the 26-30yrs age group and 75.7% of rubella cases were in the 16-25yrs age groups. PCR testing of NTS showed 23/60 (38.33%) positivity to measles and 25/60 (41.66%) for rubella. Genotyping of viruses at RRL revealed measles virus belonged to D8 in both 2010 and 2011 and 2B for rubella virus in 2011.

Conclusions

1. Resurgence of measles and rubella infection were observed in 2010/2011 with 4 rubella outbreaks in the older age group.
2. Both OF and NTS can be considered as alternative specimens.
3. NTS are better than OF specimens for molecular diagnosis and genotyping.
4. Genotype D8 and 2B are the circulating strain of measles and rubella viruses in SL at present.

OP 10

Seroprevalence of chikungunya infection in adults 20 years and above in selected wards of the Colombo municipality area

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Introduction

Chikungunya virus caused several outbreaks from 2005-2007 in the Indian Ocean islands, India and other South East Asian countries. Sri Lanka was affected from October 2006 to February 2007 with 37000 suspected cases.

Objectives

To describe the epidemiological and clinical features of chikungunya infection in Sri Lanka, to determine the seroprevalence of chikungunya in adults of selected wards in the Colombo municipality (CMC) area and to determine the proportion of asymptomatic chikungunya among seropositive adults.

Methodology

The study was conducted in two phases. 300 adults, 20 years and above who lived in CMC were included in phase 1. They were selected from 20/47 wards, proportionate

to the size of population. Epidemiological and clinical features were obtained using a questionnaire. From the study subjects in phase 1, 100 from 7 wards were randomly selected for phase 2. 3 ml of blood was collected from each. Haemaglutination inhibition test was performed.

Results

Of 300 adults, 66% had symptoms of presumptive chikungunya. Fever was the most common (99.5%). Joint pains were seen in 89% of the individuals. A macular papular skin rash was reported in 20% of the participants. Of 198 symptomatic individuals, 5.5% had psychosomatic manifestations and 3% had neurological manifestations. Persistent joint pain was present in 21.7%.

Anti-chikungunya HI antibody prevalence among adults in selected wards was 57%. 45% of asymptomatic individuals were positive for anti chikungunya antibodies.

Conclusions

Chikungunya was transmitted extensively affecting more than half of the population in CMC. Though fever with rash was the most significant, psychosomatic and neurological manifestations were not rare. Chikungunya virus can cause a significant proportion of asymptomatic infection.

OP11

Comparison of laboratory techniques for diagnosis of hookworm infections

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Introduction

Hookworm infection may be diagnosed by detection of eggs or culturing of larvae from stools.

Objective

To compare the efficacy of modified Kato-Katz (K-K) technique, saline smears, Harada-Mori (H-M) and nutrient agar culture plate (NACP) methods in diagnosis of hookworm infections.

Methodology

A total of 324 stool samples, from the plantation sector families in Ratnapura district were examined by modified Kato-Katz technique and saline smears, and cultured by Harada-Mori and on NACP according to standard protocols, at the Faculty of Medicine, Ragama. Harada-Mori and NACP were maintained for 7-10 days and larvae or larval tracts observed by a stereomicroscope. Positivity in any two of the four techniques was considered the gold standard positive. Statistical analysis was done using SPSS version 16.

Results

A total of 172 (53.1%) samples were positive by at least one method. The positivity rates with K-K, H-M, NACP and saline smears were 42.3% (137/324), 37.7% (122/324), 23.5% (76/324) and 22.2% (72/324) respectively. The highest sensitivity (89.8%) and lowest specificity (88.3%) was seen in modified K-K, the lowest sensitivity (50.4%) in saline smears and the highest specificity in NACP (98.5%). Detection rates with saline smears, H-M and NACP rose with increasing intensity of infection as determined by K-K technique. Harada-Mori had the highest detection rate (70.7%) in light infections. It also detected 11.8% of K-K negative samples.

Conclusions

The modified K-K technique is a reasonably good diagnostic method for detection of hookworm infections. A combination of methods will increase the diagnostic accuracy in hookworm infections.

OP 12

Determining the geographical origin of *Plasmodium vivax* using five microsatellite markers, instead of twelve: a more cost effective tool

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Introduction

Twelve previously validated microsatellite markers have proved to be useful in revealing the geographic origin and population structure of *P. vivax* parasites, which is a costly method. Aim of this study was to determine the minimum number of markers required to achieve the same outcome.

Methods

Data from 425 isolates genotyped using a previously validated panel of 12 microsatellite markers (MS1, MS2, MS3, MS4, MS5, MS7, MS8, MS10, MS12, MS15, MS16 and MS20) were used. Different combinations of microsatellite haplotypes (varying from 3 to 5) were tested using 2/3rds of isolates as a model for predicting the ancestry by using the STRUCTURE software. Virtual heterozygosity (HE) and standardized index of association (ISA) was also calculated using LIAN 3.5 software.

Results

A combination of 5 microsatellite loci (MS1, MS2, MS5, MS15 and MS16) was identified which gave comparable results to previous. Of the 142 isolates that were tested on this model, percentages of test isolates that were correctly identified to have a predominant ancestry from either Asian or African origin were: 72.3% (n=34) for Sri Lanka (Asian), 62.5% (n=35) for Myanmar (Asian) and 76.9% (n=30) for Ethiopia (African), giving an overall predictive power of 69.7% (n=99). Mean genetic diversity (HE) was: 0.6363 (Ethiopia), 0.7627 (Myanmar) and 0.8195 (Sri Lanka). Significant linkage disequilibrium was maintained for the Asian region (ISA=0.0126; P=0.001).

Conclusion

Microsatellite analysis with 5 markers appears to give comparable results to the 12 markers previously tested in determining the geographical origin of P. vivax parasite isolates, at least to continent level (Asian or African).

Acknowledgements

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OP 13

A study of paediatric patients with candidaemia at the Lady Ridgeway Hospital

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Objectives

To analyse the epidemiological data of patients with candidaemia and to speciate the isolates of *Candida* spp.

Method

Lady Ridgeway Hospital (LRH) performs blood cultures using both in-house media and an automated system (Bactec). Data were collected for a period of 6 months from November 2010. Criteria for clinical significance were either a patient with two consecutive blood cultures positive for *Candida* or a clinical condition requiring antifungal therapy. Species identification was done at the Department of Mycology, Medical Research Institute (MRI).

Results

Of 22 patients with positive blood cultures, 17 were clinically significant (73.9%). Others were contaminants. Six patients were neonates while 9/17 were in the age group of 1 month to 1 year. Male: Female ratio was 10:7. Six (35.2%) patients were from ICU while others were inward patients. Patients had a variety of clinical conditions but candidiaemia was more common among patients with congenital heart disease (6/17), lower respiratory tract disease (4/17) or sepsis (3/17). *Candida albicans* was isolated in only 2 (11.7%) patients while other isolates were non-albicans *Candida*. Of 8 germ tube negative isolates tested, one was *Candida albicans*, one was *Candida parapsilosis* and the other 6 were *Candida tropicalis*. 13 specimens were isolated from Bactec bottles. All Bactec bottles indicated positive within 48 hours after receipt of specimen while manual cultures took 2-4 days.

Conclusion

Non-albicans *Candida* is more common than *Candida albicans* among candidaemic patients at LRH.

OP 14

A study to determine the species of malassezia causing pityriasis versicolor and their comparable response to single dose therapy of fluconazole and seven day therapy of itraconazole in a Teaching Hospital in the Western Province

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Introduction

Pityriasis versicolor is a common, recurrent fungal skin infection in the tropics. Itraconazole and fluconazole are effective medications used in pityriasis versicolor.

Objectives

To compare the efficacy and side effects of a single dose of fluconazole and a seven day course of itraconazole in the treatment of pityriasis versicolor, to assess the relapse rate within six months of follow up and to identify the species of malassezia causing pityriasis versicolor.

Method

One hundred and twenty patients were randomly divided into two groups and treated with either itraconazole 200mg daily for 7 days (group 1) or 400mg single dose fluconazole (group 2). Clinical and mycological cure was assessed and compared at six weeks. Culture was done to identify species. Side effects to drugs were assessed clinically and biochemically before and after treatment.

Results

One hundred and eighteen patients (90%) (group 1–53, group 2–55) completed the study. Fluconazole and

itraconazole treated groups took 2.5 and 2.8 weeks ($p<0.05$) for mycological clearance and disappearance of scaling took 3.8 and 4.3 weeks ($p<0.05$) respectively. Pruritis disappeared in 2.7 weeks in both groups. Hypopigmentation started to disappear after 8.4 weeks in group 2 and 8.6 weeks in Group 1. No patient had clinical or biochemical evidence of side effects to therapy. Malassezia obtusa was the commonest species isolated. Itraconazole treated group had a recurrence rate of 3.76% while fluconazole had 18 %.

Conclusions

A single dose fluconazole was more effective than a seven day course of itraconazole for the treatment of pityriasis versicolor though fluconazole treated group had a higher recurrence rate. Both medications were free of side effects. The commonest species isolated in this study was *Malassezia obtusa*.

OP 15

Experience with an automated liquid culture system for Mycobacteria species

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Introduction

The cornerstone of tuberculosis control is case detection. Case detection by TB culture is performed in the resource poor setting by the conventional method using Lowenstein Jensen (LJ) medium. This test takes 4-8 weeks creating difficulties for the clinicians to manage the patients.

The automated liquid culture methods are superior to culture on solid media in terms of their speed and precision. No performance data on this exist for Sri Lankan setting. Therefore the automated culture system (BacT/Alert3D) was compared with LJ method with a view to obtain performance data useful for TB control activities.

Objectives

To assess the positivity rate of the BacT/Alert3D.

To compare the positivity rates of the BacT/Alert3D and LJ.

To compare the time intervals to obtain positivity.

Methodology

284 samples [sputum (182), bronchial washings (07), pleural fluid (16), CSF (42), and other samples (37)] were processed by both LJ and BacT/Alert3D simultaneously. Sterile fluids were directly inoculated to BacT/Alert3D and LJ medium. Others were processed using the NALC/NaOH method.

Method	Positive % (n)	Contamination % (n)	Negative % (n)
LJ	14.78% (42)	0.35% (01)	84.97% (241)
BacT/ Alert3D	28.87% (82)	14.44% (41)	56.69% (161)

Results

Only 47.56% (39) of the BacT/Alert3D positives were positive in LJ while 98.75% (159) negatives were negative in LJ. The average duration to obtain a positive in automated system and LJ was 14 and 23 days respectively.

Conclusions

The sensitivity of detection in BacT/Alert3D is double that of LJ. Contamination is higher in BacT/Alert3D. On average detecting a positive is 9 days faster in the automated system.

OP 16

Exploring the antimicrobial activity of Triphala – a traditional medicine formulation

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Introduction

Triphala, a traditional medicine, consisting of equal parts *Terminalia chebula* (aralu), *Terminalia bellirica* (bulu) and *Emblica officinalis* (nelli) is widely used in both Siddha and Ayurveda practice for treatment of wound infections.

Objective

To explore the antimicrobial activity of Triphala.

Design, setting and methods

The antimicrobial activity of Triphala was examined using the cut well and agar dilution methods. The fruits were bought from the local market. After removing seeds and mixing an equal weight of each fruit, an aqueous extract was prepared by boiling the fruits in distilled water to 1/8th the initial volume, meeting the conditions of traditional drug preparation.

The aqueous extract was examined at dilutions of1/20, 1/40, 1/80 and 1/160 against clinical isolates of methicillin sensitive and resistant *Staphylococcus aureus* (MSSA and MRSA), ESBL producing coliforms, *Candida albicans* and standard strains of *Escherichia coli* (NCTC 10418) and *Pseudomonas aeruginosa* (NTCC 10662). Extracts which showed a zone of inhibition at the 1/160 dilution were further diluted up to 1/800 and retested.

Results

Triphala was active against MSSA and MRSA at 1/400 dilution and 1/800 dilutions respectively. Activity against *P aeruginosa*, *E coli* and ESBL producing coliforms were seen at 1/100-1/200, 1/100 and 1/10-1/20 dilutions respectively. No activity was seen against *Candida albicans*.

Conclusion

This study confirmed the antibacterial activity of Triphala extract demonstrating activity against MSSA and MRSA at high dilutions. Additionally, activity against ESBL producing coliforms, *P. aeruginosa* and *E coli* was also shown. Further work to determine the spectrum of activity of Triphala and investigations into the plant constituents of Triphala are indicated in the light of the very encouraging findings of this study.

OP 17

Sero-prevalence of Leptospirosis among paddy-field workers in the Kalutara district

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Introduction

Leptospirosis, which is the most prevalent zoonotic infection worldwide, has become a prominent contributor to the communicable disease burden in Sri Lanka during recent years. The disease is now endemic in most parts of the country, especially in areas of agricultural farming. With the changing epidemiology of leptospirosis in the country, updates on sero-prevalence are of paramount importance for formulation of effective strategies and activities for prevention and control.

Objectives

To determine the sero-prevalence of leptospirosis among paddy-field farmers in the Kalutara district.

Method

Samples of 3-5ml of blood were obtained from 607 apparently healthy paddy-field workers having at least fifteen hours of exposure in the field per week, through a cluster sampling technique involving 54 clusters. Samples, transported to the MRI within 24 hours of collection, were subjected to MAT with the Patoc 1 strain of Leptospira. Epidemiological data were collected using an interviewer-administered questionnaire. The results were analyzed using Minitab.

Results

217 (36%) were sero-positive for leptospirosis. Only 9% of them had a history of clinical disease. 91% of the remainder did not have any family members or co-workers who had a history of disease. 91% of sero-positives were farmers, 8% helpers and 1% agricultural field officers. Male to female ratio was 9:1.

Conclusion

Asymptomatic infection with leptospira is much more common than clinically diagnosed cases, among regular paddy field-farmers. The results highlight the increased risk of infection in exposed populations such as regular paddy field workers, thus warranting a detailed sero-epidemiological analysis of 'at-risk' populations to determine the correlates of infection, for effective prevention and control.

OP 18

Prospective study of patients with infective endocarditis

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Introduction

Infective endocarditis (IE) is one of the major causes of fever of unknown origin. Data regarding IE in Sri Lanka is scarce.

Objectives

To follow up patients with IE, to assess aetiology, risk factors, treatment and outcome.

Method

Seventy eight in-patients with IE between 01/03/2010 and 01/06/2011 were followed up. At least three blood cultures were processed for each patient and they were followed up for a minimum 1 month after stopping treatment.

Results

Of 78 patients, 64 patients had native valve IE and 14 (18%) presented with prosthetic valve IE. 38 (48%) patients had identified risk factors including 15 (39%) after cardiac surgery, 6 (16%) following normal vaginal delivery/evacuation of retained products of conception. Other risk factors were recurrent IE [4 (10%)]. 44 patients had existing valvular or cardiac defects.

37 (47%) patients had positive blood cultures. Majority (12, 32%) were positive for viridans group of *Streptococci*, followed by *Enterococcus* spp (7, 19%). Other significant isolates were *Staphylococcus* spp, *Pseudomonas* spp, other *Streptococcus* spp, coliforms, *Acinetobacter*, etc. Most common type was mitral valve IE (40, 51%), followed by aortic valve endocarditis (25, 32%). 4 patients

presented with multiple valve vegetations. 44 developed complications, such as septic emboli, root abscesses, chordal rupture, etc. 59 (76%) recovered. 3 died. 10 are still being treated. Response was not known for 6 patients. Valve replacement was necessary for 9 patients who responded poorly to antibiotics.

Conclusions

Native mitral valve endocarditis is the commonest variety. Viridans *Streptococcus* spp accounts for majority of blood culture positives. With targeted treatment, planned management and follow up, majority of patients can be cured (76%).

OP 19

A comparison of drug susceptibility of *Mycobacterium* species isolated at National TB Reference Laboratory, Sri Lanka between years 2000 to 2010

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Introduction

Data on drug susceptibility of *Mycobacterium* species are of clinical importance to manage TB patients.

Objectives

To determine the drug susceptibility patterns of *Mycobacterium tuberculosis* and atypical mycobacterial isolates and the changing pattern of drug susceptibility of 2009-2010 from the patterns observed in 2000-2008.

Method

Susceptibility data from 2622 mycobacterium isolates between 2009 and 2010 were analyzed. This data was compared with the patterns of drug susceptibility in 2000-2008.

Results

A total of 21181 cultures were performed in 2009-2010. A mycobacterial growth was observed in 2622 cultures (12.38%). Multi drug resistance was observed in 1.42% of *Mycobacterium tuberculosis* culture isolates.

Drug susceptibility patterns

2000 - 2004 Isolate	Streptomycin	INAH	Rifampicin	Ethambutol
M. tuberculosis	9.8%	6.33%	6.30%	6.49%
Atypical mycobacteria	96.54%	98.85%	88.5%	95.4%

2005 - 2008 Isolate	Streptomycin	INAH	Rifampicin	Ethambutol
M. tuberculosis	9.2%	3.98%	3.05%	6.16%
Atypical mycobacteria	94.01%	96.41%	89.82%	97.01%

2009 - 2010 Isolate	Streptomycin	INAH	Rifampicin	Ethambutol
M. tuberculosis	9.91%	2.8%	1.82%	5.41%
Atypical mycobacteria	90.12%	95.88%	90.72%	98.97%

Conclusions

Maximum resistance was seen for streptomycin (9.4%). A gradual decrease in resistance to both rifampicin and INAH in *M. tuberculosis* was observed over the years which indirectly reflect the success of TB control activities. No deviations from the previous susceptibility patterns were observed for the other drugs.

OP 20

The incidence and characteristics of extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella* species among urinary isolates in a tertiary care hospital

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Introduction

Enterobacteriaceae are the leading cause of urinary tract infections (UTIs). Extended-spectrum beta-lactamases (ESBLs) production has been implicated as a major cause of drug resistance in these Gram negative bacteria.

Objective

To detect and describe some relevant characteristics of ESBL producing urinary isolates of *Escherichia coli* and *Klebsiella* species in a tertiary care hospital.

Method

A descriptive cross sectional study. Study period – January 2009 to April 2009. *E.coli* and *Klebsiella* urinary isolates from hospitalized and non-hospitalized patients were included. Presence or absence of identified risk factors in these patients were recorded and community acquired infections were identified. Antibiotic susceptibility tests and the ESBL phenotype detection

tests were performed according to the Clinical Laboratory Standard Institute guidelines (2008).

Results

A total of 286 isolates were studied and 32.87% produced ESBLs. ESBL rate in ICUs, genito-urinary unit, general wards and the out-patient department was 90.90%, 75%, 33.17% and 14.28% respectively.

Out of the 181 community acquired isolates 12.70% produced ESBLs. Urinary catheters, diabetes mellitus, previous antibiotic use, urinary tract abnormalities and recurrent UTIs were associated with higher risk of acquiring ESBL producing organisms (P<0.001).

Conclusions

Marked differences exist in the proportion of ESBL producers causing UTIs between different units. Common uropathogens have high resistance rates to multiple antibiotics. Resistance was considerably higher in ESBL positives compared to ESBL negatives. The rate of ESBL production in community acquired *E.coli* and *Klebsiella* species causing UTIs is 12.70%.

PP 1

An analysis of accidental exposures of healthcare workers to blood and blood stained body fluids at the Lady Ridgeway Hospital

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Lady Ridgeway Hospital.

Objective

To study data on accidental exposures to blood and blood-stained body fluids reported to the Infection Control Unit at the Lady Ridgeway Hospital (LRH), Colombo.

Method

Accidental exposures are reported to the Infection Control Unit at LRH. Information collected when reported were analysed for a period of 6 months from December 2010.

Results

39 incidents were reported, of which 28 recipients were nurses, 8 were doctors and 3 minor staff. 87.2% (34/39) were percutaneous injuries and 12.8% (5/39) were mucous membrane exposures. Procedures which lead to the exposure were recorded in 30 instances. 33.3% (10/30) occurred during drawing blood, 23.3% (7/30) were during IV cannulation, 20.0% (6/30) during cleaning procedures and disposal of sharps, and 13.3% (4/30) during surgery. The other 3 were during exchange transfusion, infiltration of a bite wound and administration of anti rabies vaccine.

Of the 39 incidents studied, 24 (61.5%) occurred in medical and surgical wards (12 in each), 10 (25.6%) in the theatre, 3 (7.7%) in the ICU, one (2.6%) in CSSD and the other (2.6%) in the garden. In 36 instances the source was patients. Source was unknown in the other 3. 94.8% (37/39) incidents were reported within 24hrs. All recipients had taken a full course of Hepatitis B vaccine prior to accidental exposure. Source and recipient blood samples were tested for HIV and Hepatitis B according to the protocol practiced in the hospital. Source patients were negative for HIV and Hepatitis B.

Conclusion

It is important to provide guidance and measures to reduce accidental exposures while encouraging reporting of incidents.

PP 2

Antimicrobial sensitivity of organisms causing enteric fever in a Teaching Hospital of Sri Lanka

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Sri Jayewardenepura General Hospital, Nugegoda.

Objectives

To determine the serotypes of enteric fever causing organisms isolated from blood culture, from 1st January to 31st December 2010 at the Microbiology Laboratory of Sri Jayewardenepura General Hospital, Nugegoda, Sri Lanka and to determine the antimicrobial sensitivity pattern (ABST) of those isolates.

Methodology

Blood cultures were performed manually using a commercial bottle. ABST was done using CLSI method. A retrospective analysis of the data was done using the computer database and the records entered in the books of the microbiology department of the hospital.

Results

Thirty two blood cultures from twenty nine patients yielded *Salmonella paratyphi A. Salmonella typhi* was isolated from one blood culture in September 2010. No other enteric fever causing organisms were isolated from blood cultures within this period. *Salmonella paratyphi* A was isolated from one patient in May, from two in July, twelve in September, eight in October, three in November and three in December.

All the *Salmonella paratyphi* A isolates had the same antibiogram, being sensitive to ampicillin, cefotaxime, ceftriaxone, cotrimoxazole and chloramphenicol and being resistant to nalidixic acid and ciprofloxacin. The single isolate of *Salmonella typhi* was sensitive to ampicillin, cefotaxime, ceftriaxone, cotrimoxazole, chloramphenicol, nalidixic acid and ciprofloxacin.

Conclusions

Salmonella paratyphi A was the predominant enteric fever causing organism isolated from blood cultures in Sri Jayewardenapura General Hospital, Nugegoda, Sri Lanka in 2010. All the *Salmonella paratyphi* A isolates were resistant to quinolones including ciprofloxacin.

PP 3

An unusually large outbreak

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Introduction

Salmonella paratyphi A is a human pathogen giving rise to the systemic disease, enteric fever.

Infections occur either sporadically or as limited outbreaks. Transmission is by direct or indirect contact with faeces, or rarely from the urine, of a symptomatic patient or a carrier, from contaminated food or contaminated hands or even flies.

Outbreaks related to water supplies have been reported. Organism excreted in the faeces of infected humans may contaminate ground water or surface waters. Insufficiently treated drinking water consumed by large, close communities is the main cause of epidemic waterborne disease caused by *Salmonella* species.

Case report

A hospital from a military base started sending samples of blood for culture from previously healthy young men with continued fever during the latter part of June 2010. At the beginning one or two samples were received each day. Gradually the numbers increased up to eight samples per day. This trend continued for several days and weeks till mid October. A total of 122 blood culture samples were positive for *Salmonella paratyphi* A. All isolates had the same antibiogram, sensitive to ampicillin, cefotaxime, co-trimoxazole, chloramphenicol and resistant to nalidixic acid (reported as ciprofloxacin resistant) proving a common source aetiology.

A detailed investigation was carried out with the help of the MOH, epidemiologists, officials from the town council and the military health authority, which eventually revealed that the outbreak was probably due to cross contamination of ground water pipes with the sewer system.

Conclusions

Unsafe drinking water could lead to extreme loss of man power and economic losses.

PP 4

A case of Legionnaires' disease

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Introduction

In 1976, an outbreak of pneumonia occurred following an American Legion Convention of war veterans in Philadelphia. A total of 221 attendees contracted *Legionella pneumophila* pneumonia, and 34 died.

Legionella pnuemophila infection can manifest as mild Pontiac fever or as Legionnaires' disease; a severe pneumonia with stupor and widespread pulmonary infiltrates to multi organ failure. The incubation period is two to ten days.

Case report

A 69 year old Austrian traveler was admitted to a private hospital in Kalutara with a history of fever, cough and shortness of breath and haemoptysis of five days duration. He had come to Sri Lanka 11 days ago and had travelled extensively within the country before reporting sick.

He had a high white cell count, CRP of 112 mg/L, a right sided consolidation in the chest X ray and an INR of > 6. The patient was treated with cefotaxime and clarithromycin. His condition deteriorated gradually with a CRP > 384 and was transferred to a private hospital in Colombo for ventilator support.

He was a patient known to have obstructive sleep apnoea, type 2 diabetes mellitus, hypertension, paroxysmal atrial fibrillation and had a permanent pace maker. Legionella urinary antigen was positive, reported by two different laboratories. Mycoplasma IgM antibody, *Streptococcus pneumoniae* capsular antigen in urine, repeated blood cultures and Influenza A H1N1 antigen were negative. Sputum culture yielded Candida species.

He was treated with IV clarithromycin for 10 days followed by 4 days of oral therapy. Moxifloxacin and meropenem were also given for 10 days. Serial CXRs showed initial worsening but later gradual improvement. The CRP was 48 mg/L and consolidation had cleared almost completely on discharge, in two weeks.

PP 5

An audit on National External Quality Assessment Scheme (NEQAS) for Bacteriology in Sri Lanka

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Medical Research Institute, Colombo.

Introduction

The Medical Research Institute has been the organizer of the National External Quality Assessment Scheme (NEQAS) in Bacteriology for the last 14 years in Sri Lanka. The objectives of NEQAS are to evaluate, compare and improve the quality of diagnostic microbiology.

Objectives

To analyse the performance of NEQAS in Sri Lanka.

Method

NEQAS surveys are conducted quarterly each year. Participating laboratories are provided with three bacterial cultures for identification and to perform antibiotic sensitivity testing (ABST). Results are analyzed and scored.

The scoring system divides the laboratories into two categories depending on existing facilities. Results are given quarterly and a total performance report is issued at the end of the working year.

Results

Since inception in 1997 up to 2011, the number of participating laboratories increased from 13 to 54 mostly by voluntary participation. The participation of government hospitals increased by 70% and private laboratories by 73% with an overall increase in accurate identification of cultures from 63% to 85% and accurate ABST results from 45% to 74%.

Conducting workshops, visits to participating laboratories, periodic modifications of the NEQAS programme and presence of medical microbiologists in peripheral hospitals have contributed to the progress. Increased use of Clinical and Laboratory Standards Institute standards would have improved performance of ABST. A marked increase in both parameters identified after 2009 especially in the North and East of Sri Lanka is probably attributed to ending of civil war in May 2009.

Conclusion

The results reveal that monitoring and guidance given by the NEQAS programme have led to a greater motivation and improved diagnostic skills of the participating laboratories.

PP 6

A comparative study of 1st and 2nd waves of Pandemic Influenza A (H1N1) 2009 virus infection in Sri Lanka

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Background

The first wave of novel Influenza A (H1N1) was reported in June 2009 and the 2nd wave in October 2010 in Sri Lanka.

Objectives

To compare the clinical, seasonal pattern, epidemiological and laboratory data of the two waves.

Design and methods

Nasopharyngeal aspirates, nasal and throat swabs and lung tissues were collected in viral transport medium and real time reverse transcriptase polymerase chain reaction (rRT-PCR) was performed. The positive samples were sent to WHO collaborating centres. The incidence of cases was compared with rainfall data.

Results

In both waves community transmission occurred during October and peaked in December. 28.7% and 18.3 % were positive for pandemic influenza A respectively in both waves. Geographical distribution, seasonal pattern, age groups affected, clinical features and complications were similar in both waves. The mortality rates were 7.8% and 5.5%. Maternal deaths were 15.9% and 14.6%. Highest mortality was observed among young adults. All samples tested were sensitive to oseltamivir. No antigenic change was detected by phylogenetic analysis. The maximum number of cases was seen during the highest rain fall from October to December.

Conclusions

1. Children and young adults were predominantly affected with mild to moderate clinical illness and responded well to oseltamivir.
2. Mortality was more likely to be associated with predisposing factors and pregnant women were at a higher risk.
3. High incidence with higher rain fall during October to December was observed in both waves.
4. No antigenic variation was observed in the virus in both waves.

PP 7

Pandemic influenza A (H1N1) 2009 as a cause of myocarditis

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Acute myocarditis is a well recognized, but rare manifestation of influenza viral infection. The prevalence of myocardial involvement in influenza infection ranges from 0 to 11%.

A female with a POA of 36 weeks was admitted with fever, cough, sore throat and faintness of 3 days duration. On examination, her BP was 90/60; pulse rate was 88/min. She had bilateral crepitations and rhonchi. Four days later she became breathless. Chest X-ray was suggestive of an atypical pneumonia. Laboratory investigation of nasal and throat swabs confirmed pandemic influenza A H1N1 infection.

She was treated with IV antibiotics, nebulization, and oseltamivir 75 mg. She was given ICU care and treated with oxygen and managed with inotropes. Baby was delivered by LSCS on the same day.

Post operatively the patient was ventilated and maximum support with inotropes was given. Two days later, her electro cardiogram showed T inversion from V3 to V6 leads and tachycardia. Her BP was 110/ 60 & SPO2 was 95 %. 2D ECHO showed an akinetic septum and apex. Ventricular ejection factor was 35% .There was mild enlargement of left ventricle and atrium, but there were no pericardial effusion or vegetations. Cardiac tropinin I was elevated. Diagnosis of myocarditis secondary to pandemic influenza A (H1N1) was made. The dosage of oseltamivir was increased from 75mg to 150 mg bd. On the 10th day, 2D ECHO showed signs of improvement, with reduction of oxygen dependency. She was discharged on the 15th day.

Pandemic influenza A (H1N1) is associated with higher morbidity during pregnancy and could lead to pneumonia and myocarditis. Early detection and timely intervention would decrease morbidity and mortality.

PP 8

Controlling the spread of hepatitis B infection in a high risk population

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Introduction

National Institute of Mental Health in Angoda (NIMH) is the premier psychiatric hospital in Sri Lanka established in 1926. There are many risk factors identified among patients with psychiatric illnesses, which may lead to the rapid spread of hepatitis B viral infection.

Objectives

1. To identify the immune status of health care workers (HCW) and patients.
2. To identify the total number of hepatitis B infected patients.
3. To identify patients with active infection and direct them for further management.
4. To achieve maximum vaccination among both groups.
5. To control the spread of hepatitis B infection among the community of psychiatric patients.

Methodology

Long term inmates were screened for hepatitis B surface antigen and antibodies (from Feb to June 2011). If both were negative, a full course of vaccination was recommended. Data collection was done through a questionnaire. Hepatitis B surface antigen positive patients were managed in the medical ward. Disease transmission was minimized by using standard precautions. A decision was taken by the infection control committee to vaccinate all new admissions. High risk admissions were screened for HBs antigen as well.

Results

HBs Ag positive	long term inmates	– 14(8.5%)
HBs Ab positive	long term inmates	– 08(3.01%)
HBc Ab positive	long term inmates	– 42(15.85%)
HCW's completing a full course of Hep B vaccination		– 447(49.66%)
Defaulted vaccination HCW		– 277(30.77%)
Unvaccinated HCW		– 165(18.33%)
Newly vaccinated HCW (from April)		– 150(90.90%)
Number undergoing medical management (HBs Ag + pt's, for liver function and relative signs and symptoms)		– 14(8.53%)
New inmates vaccinated up to now (All inmates completed 1st and 2nd doses)		– 75(45.45%)

Conclusion

NIMH shows very high hepatitis B prevalence. Spread of the hepatitis B infection was contained by implemented strategies. Future management plans are already in place.

PP 9

Aspergillus aculeatinus n. sp. First report on pathogenicity in humans

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Aspergillus aculeatinus, a recently described uniseriate species within *Aspergillus* Sect *Niger*, was first reported in 2008, as a contaminant of coffee beans in Thailand. We report a case of chronic human dacryocystitis in a middle-aged female which gave positive smear appearances of a mycelial fungus in the lacrimal sac contents and a pure culture of *A. aculeatinus*. There was no mycelial invasion of the wall of the lacrimal sac which

showed a mixed cell (neutrophil and mononuclear) infiltration with fibrosis; reports of *A. niger* ocular infections do not indicate tissue invasion by this fungus. The colony morphology on Sabouraud agar resembled that of *A. japonicus*, with a dark brown-black surface, yellow pigmentation on the reverse, with septate mycelia bearing globose condiophores, uniseriate sterigmata and spherical echinulate spores. Multilocus sequence analysis of *benA, CaM* and ITS partial genes allow us to identify it as the newly described species from Thailand *A. aculeatinus*.

This is, as far as we are aware, the first report of *A. aculeatinus* in a pathogenic role.

PP 10

A ten year retrospective study to evaluate the species of *Candida* in blood cultures received at Department of Mycology, Medical Research Institute

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Introduction

Department of Mycology, Medical Research Institute being the main diagnostic and reference laboratory for fungi, receives a large number of blood cultures for identification of fungi. *Candida albicans* was the commonest species isolated, but now the change in the spectrum of isolates is a concern, as treatment is difficult. Studies done in other countries reveal this trend of change.

Objectives

To determine the species of Candida, isolated in blood from 2001-2010 and to determine the changing pattern of *Candida* species during the past 10 years.

Methodology

3137 blood samples were received during this time period. Specimens were processed using Sabouraud's Dextrose Agar supplemented with antibiotics. The species identified was plotted against time.

Results

Out of 3137 samples fungal aetiological agents were identified in 392 samples (12.49 % isolation rate). Among the 392 positive samples 366 samples yielded *Candida* species (93.6 %). *Candida tropicalis* was the commonest species isolated (187/366 - 51.09 %). 124 samples yielded *Candida glabrata* (33.87 %). There were *Candida albicans* (40), *Candida parapsilosis* (08), *Candida guillermondii* (03), *Candida famata* (01), *Candida luscitanae* (01) and speciation not done (02) isolates.

Conclusion

Candida tropicalis and *Candida glabrata* show higher isolation rates than *Candida albicans* in contrast to the past few decades. Uncommon species such as *Candida guillermondi*, *Candida famata* and *Candida luscitania* were isolated in this study.

PP 11

Non-healing chronic wound – a mycetoma

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Introduction

Mycetoma is a chronic destructive disease that affects the skin, subcutaneous tissue and sometimes adjacent bone. Causative organisms are either fungi (eumycetoma) or bacteria (actinomycetoma) which are inoculated by traumatic implantation of the organism into subcutaneous tissues. There may be many undetected mycetoma among non-healing ulcers specially with discharging sinuses.

Case report

A 13-year old girl presented to the Teaching Hospital, Kandy with chronic multiple hard nodules with discharging sinuses on the dorsum of her left foot for 4 years. Haematological investigations were normal and X-ray revealed chronic osteomyelitis of left cuneiform bone. Biopsy specimens from the lesion were sent for histology, bacterial and fungal studies. Direct microscopy of smears for Gram stain and modified Ziehl-Neelson method indicated a *Nocardia* species. Culture on blood agar and Sabouraud's dextrose agar, isolated a *Nocardia spp*.

Patient was treated with oral cotrimoxazole which is the drug of choice, combined with i.v. co-amoxyclav. After 2 weeks of treatment the sinuses healed and nodules reduced in size. Patient was discharged on long term cotrimoxazole (6 months) with regular follow up at the clinic and radiological monitoring for chronic osteomyelitis.

PP 12

Environmental mushroom type fungus, *Schizophyllum commune*, 1st case in Sri Lanka

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Introduction

Schizophyllum commune is an environmental basidiocarp fungus. It is worldwide in distribution and is found on dead trees and wood. Limited reports describe infection

among both immune suppressed and immune competent. The areas known to be affected are sinuses, lungs, central nervous system and nails.

Case report

Mrs X, a 45-year old lady presented with chronic, recurrent, bilateral maxillary and ethmoid sinusitis since November 2009. Many courses of antibiotics, antral washouts and other treatment given were ineffective. Antral washouts showed evidence of an invasive fungal infection and she was treated with antifungals. Residual fungal infection was noted 2 weeks after a course of itraconazole. Repeat antral wash outs and deep mucosal biopsies grew the same fungus on Sabourauds dextrose agar. Difficulties in obtaining sensitivities and the limited treatment options were constraints in the management. The patient underwent a clearance surgery and antral washouts. She was on voriconazole during surgery but was given itraconazole later, based on sensitivities. After 3 months of treatment the patient was asymptomatic. Complete cure was confirmed clinically and radiologically.

The fungus was identified as *Schizophyllum commune* with characteristic clamp connections and spicules with branching septate filaments. Antifungal sensitivities done by MRI showed the fungus to be sensitive to itraconazole but resistant to voriconazole.

Among the limited cases reported, treatment success was observed with antifungals combined with surgery. A similar practice was followed to manage this patient and complete cure was observed.

PP 13

Audit on the care of peripheral intravenous lines at the Lady Ridgeway Hospital

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Objectives

To describe the current practices of peripheral intravenous line care at the Lady Ridgeway Hospital.

To identify the practices where improvements are needed and to provide necessary recommendations.

Design

A two stage stratified random sampling technique was used to select the units and 7 units were selected to represent the hospital. These included 2 medical wards, 2 surgical wards, 1 medical intensive care unit, accident service and the burns unit. Data was collected by direct observation using a checklist of procedures of cannula insertion, maintenance, removal and cannula site infection.

Results

During one month, cannula insertions were observed in 23 patients, maintenance in 40, and removal in 17 patients. Use of antiseptics and method of stabilization following insertion was 100% (23/23) correct. However, only 26% (06/23) gave time for the antiseptic to dry prior to insertion, 91% (21/23) recorded the date of insertion, 65% (15/23) practiced hand hygiene prior to insertion and 60% (24/40) prior to handling the cannula. Only 60% (14/23) wore gloves, out of which 50% (07/14) used surgical gloves instead of disposables. 100% (40/40) practiced daily inspection of site, 75% (30/40) labelled the date on administration sets and 85% (34/40) changed them every 72 hours. 23% (09/40) did not cap the additional administration set. 65% (11/17) of peripheral cannula had to be removed due to cannula not being in place. Cannula site infection was not observed.

Conclusion

Many of the practices were satisfactory. Areas requiring improvement were identified.

PP 14

An audit on urine culture specimen collection and time lag from collection to processing

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Introduction

Accuracy of urine culture results depends on the collection method and the time to processing since collection. An audit was carried out to determine the method of collection and time taken to process the specimens after collection.

Objectives

To determine the accuracy of specimen collection and to determine the time lag between collection and processing.

Methodology

Data was collected by interviewing the staff and patients. Time lag was measured by reviewing laboratory data. First audit was done in 2008 and the second audit was done in 2011 after carrying out certain interventions including staff education to correct the deficiencies.

Results

Of 88 samples analyzed in 2008, 68 (77.2%) were mid-stream urine (MSU) and 20 (22.7%) were catheter samples. 33.8% of patients who collected MSU were not given instructions regarding collection. 55% of catheter specimens were collected from urine bag. 10% collected by disconnecting the tube.

In 2011, 50 specimens were analyzed, 40 (80%) MSU & 10 (20%) catheter specimens. 30% of MSU category did not receive instruction for collection. 70% catheter samples were collected from urine bag and 10% by disconnecting the tube. In 2008, 62/88 (70.5%) specimens were received after 2 hours of collection and 20/88 (22.7%) were kept in the laboratory >1 hour before processing. In 2011, 15/50 (30%) specimens were received after 2 hours of collection and the laboratory delay was minimized by quick processing and refrigerating the specimens after receiving.

Conclusion

In spite of interventions no improvement was achieved with regard to collection and transport of specimens.

PP 15

The value of the Widal agglutination test in diagnosing enteric fever

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Sri Jayawardenapura General Hospital, Nugegoda.

Objective

To determine the sensitivity, specificity and the positive and negative predictive values of the Widal agglutination test at different titres, in diagnosing enteric fever caused by *Salmonella paratyphi* A in patients suspected to have enteric fever, admitted to the Sri Jayewardenepura General Hospital, Nugegoda from 1st January to 31st December 2010.

Method

We retrospectively analyzed the results of the Widal agglutination test performed in 1291patients who were suspected to have enteric fever in our hospital. In 732 of these patients blood cultures were performed and 29 of them were positive with *Salmonella paratyphi* A.

We calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) taking various anti-AH titers as the cut off value. A positive blood culture was taken as the gold standard. Patients who had an equal or a higher titre of anti-AH of the Widal test, with blood culture being positive for *Salmonella paratyphi* A were considered as true positives for that titre. Those who had a negative or a lower titre than the particular titre with blood culture being negative or positive with some other organism were taken as true negatives.

Results

A single anti-AH titre of 1:320 or higher was the optimal indicator of enteric fever caused by *S. paratyphi* A, according to the Receiver Operating Characteristics (ROC) Curve, with the highest sensitivity of 85.18%, specificity of 93.47%. At this value the NPV was 99.39%, but PPV was only 33.33%.

Conclusions

In the Widal test, 1:320 gives the highest sensitivity and specificity for diagnosing enteric fever caused by *Salmonella paratyphi* A. A high NPV at 1:320 means that when the Widal test yields a titre below this, it is unlikely that the patient has enteric fever with *Salmonella paratyphi* A.

ERRATUM

The following corrections have been made to abstract A35 (OP 19) presented during the 18th Annual Scientific Session 2009:

Screening for IS6110 zero copy number strains of *Mycobacterium tuberculosis*

Methods: The IS6110 PCR method along with two different PCR detection methods for *M. tuberculosis* was separately performed on *M. tuberculosis* DNA extracted from **over 100 sputum samples and culture isolates.**

Results: **All the 69 culture isolates had the insertion element IS6110, 65kDa antigen gene and the 38kDa protein antigen b gene. A total of 14 sputum samples out of 92 were positive by IS6110 and 65kDa antigen screening and none of the negative samples (78/92) were positive by the 65kDa antigen PCR.** No zero copy strains were discovered.

Presidential Address delivered at the inauguration of the Annual Scientific Sessions of the Sri Lanka College of Microbiologists on 15th September 2010

Dr. Omala Wimalaratne
Consultant Virologist,
Head, Department of Rabies and Vaccinology, Medical Research Institute, Colombo 8.

PREVENTION OF HUMAN RABIES IN SRI LANKA: ACHIEVEMENTS AND CHALLENGES

The Chief Guest, Dr. Ravindra Ruberu, Secretary, Ministry of Health, Guest of Honour, Prof. Henry Wilde, Professor of Medicine, Chulalongkorn University, Bangkok, Thailand, Dr. P. G. Maheepala, Addln. Secretary, Ministry of Health, Dr. Ajith Mendis, Director General of Health Services, Deputy Directors General in the Ministry of Health, local and foreign guest speakers, members of the Council, past presidents, members of the College, distinguished invitees, ladies and gentlemen. I have selected the topic for my presidential address in keeping with the theme of this years academic sessions. "Preventing infections: ideal versus achievable".

Although rabies is a vaccine preventable disease, and safe and effective vaccines both for human and veterinary use exist, it is still a public health problem in many countries including Sri Lanka.

Dog is the main animal reservoir responsible for the transmission of rabies to humans and most often the victims are children. As the national reference laboratory for rabies, the Medical Research Institute (MRI) is responsible for rabies diagnosis, research and vaccine production.

History of anti rabies vaccine (ARV) use in Sri Lanka

In 1900, anti rabies goat brain vaccine was produced in the MRI which was known as the Bacteriological Institute and also as the Pasteur Institute at that time. This was the only anti rabies vaccine available for post exposure treatment for people who were exposed to suspected rabid animals. Goat brain vaccine was administered around the umbilicus as 14 daily injections and 3 booster doses given subsequently. Treatment failures were not uncommon due to the high drop out rate. The main reasons being the painful nature of the injections and adverse events due to post vaccinal complications.

Safe and effective Human Diploid Cell Culture Vaccine (HDCV) was introduced in Sri Lanka in 1986, but was distributed only to the main Teaching and General Hospitals. All other hospitals in the country were using the goat brain vaccine. Due to the limited supply and the high cost of the HDCV vaccine, Suckling mouse brain vaccine was also made available from 1987-1989. Purified chick embryo cell culture and purified vero cell culture vaccines were introduced in 1990 for intramuscular use.

Elimination of NTV and introduction of TCV

Equine rabies immunoglobulin was used only in Teaching Hospitals and there were many treatment failures during this period. For category II exposures 2:1:1(Zagreb) schedule was recommended (saving on one dose of vaccine) and all category III exposures equine rabies immunoglobulin plus 5 doses of anti rabies cell culture vaccines were administered intramuscularly. In 1995, the Ministry of Health took a policy decision to stop the production of nerve tissue vaccine. This was a major breakthrough for Sri Lanka. Since then all hospitals in the country are using safe and effective modern rabies cell culture vaccines. Use of equine rabies immunoglobulin was only in about 10% of patients seeking post exposure therapy. Island wide training of staff and health educational programmes were conducted and the use of equine rabies immunoglobulin was extended up to Base Hospital level in 1996.

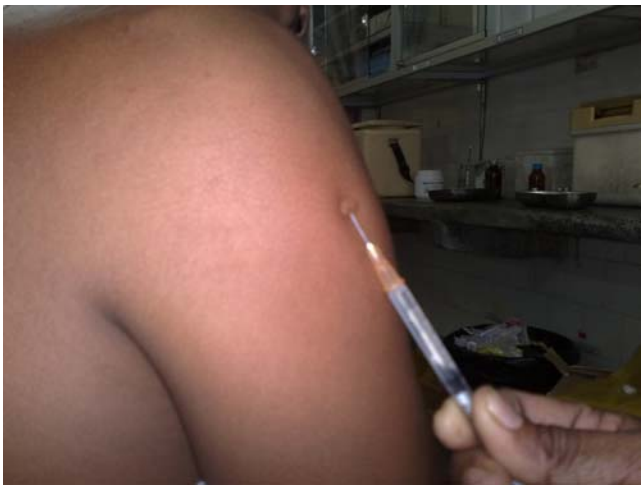
With the increase in awareness programmes, more and more patients were seeking post exposure treatment following animal bites. As a result, the Ministry of Health had to encounter with an increasing demand for anti rabies immunoglobulin and vaccine at a colossal cost. To overcome the situation, while maintaining the safety and the efficacy of the vaccine, an alternative method of treatment had to be adopted.

Introduction of ID regimen

Before switching over to the WHO recommended economical and safe intra dermal schedule for post exposure treatment in 1997, several studies were conducted on immunogenicity, safety and efficacy of

this new schedule. The results were comparable with the standard intra muscular schedule. It was one of the biggest challenges faced on how and when the new schedule should be introduced.

Following several rounds of discussions with the policy makers in the Ministry of Health and the clinicians, it was decided to introduce the new regimen in 2 Teaching Hospitals in Colombo. Close monitoring was done on patients and rabies antibodies in serum were determined in patients when necessary, by the Rapid Fluorescent Focus Inhibition Test (RFFIT). A new test which was established in the MRI.



Staff training was conducted and regular visits were made to the hospitals to give confidence to the staff while monitoring the new schedule. Gradually it was expanded to other hospitals in the country.

1998-1999 – It was introduced to other Teaching and General Hospitals.

2000-2002 – Introduced to all Base and some District hospitals where more than 6 patients attend the anti rabies clinic per day. However Northern and Eastern provinces were not included at this stage. This was because once a vaccine vial is reconstituted with the diluent, it should be used within 8 hours stored at 2-8ºC.

In 2003, ID regimen was introduced in Teaching and Base hospitals in the Northern and Eastern provinces.

2004-2010 – Currently, all hospitals in the country except a few district hospitals and peripheral units are practicing intradermal for rabies post exposure treatment.

Our experience in 2010 is as follows:-

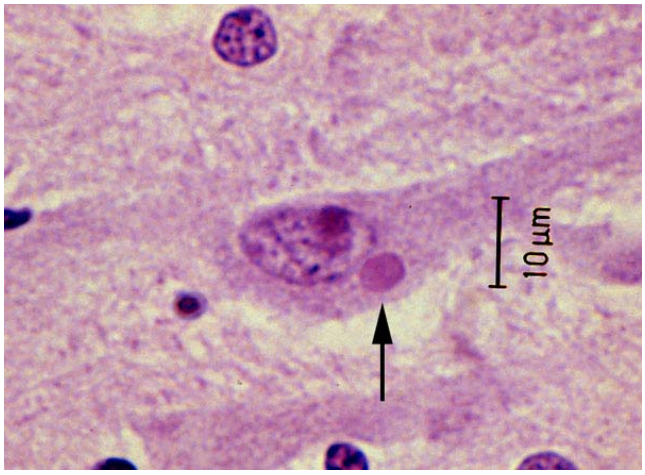
A one day training of medical officers and nurses on ID technique is conducted regularly. Regular visits to hospitals were made to monitor patients and anti rabies units were successfully established in major hospitals. A medical officer was appointed for supervision and monitoring. The Anti-Rabies Unit established in the National Hospital of Sri Lanka is considered as a model unit in the country. This is the main centre for training of medical officers and nurses on rabies post exposure treatment. In 1996, a special clinic was established in the MRI to give advice on management of patients on post exposure treatment. Expert advice is available 24 hours for doctors from any part of the country. If not for the introduction of the WHO recommended safe and effective new ID regimen, purchase of anti rabies vaccine would have created a huge burden on the country's health budget. HRIG was made available in ARV units in major hospitals since 2005 for restricted use. Special guidelines were issued to prevent wastage. Use of HRIG in hospitals is closely monitored by the MRI.

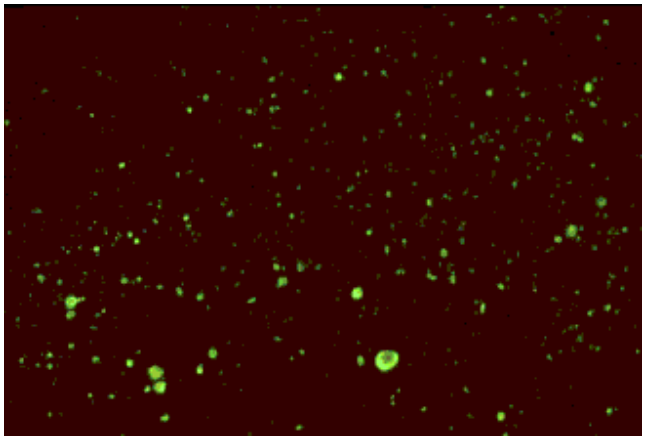
Success of ID regimen

At present, Sri Lanka is the only country in the world where more than 95% of patients seeking post exposure treatment are administered rabies vaccines intra-dermaly. I am proud to state that due to the intradermal schedule per se, no treatment failures have been reported. Credit should go to all health care personnel who worked with dedication. I am very grateful to all policy makers in the Ministry of Health for their advice, encouragement and support given to me at all times.

Rabies diagnosis in Sri Lanka

Now I would like to present the progress made with regard to rabies diagnosis.





As mentioned earlier MRI is the national reference laboratory for rabies diagnosis. Where MRI is concerned, this is considered as an essential service and has been continued without interruption up to this date. This service has never been interrupted even during crisis situations in the country. Direct microscopic examination of brain smears stained with Seller's stain. The picture shows a brain smear stained with Sellers stain. Arrow points to a negri body inside a nerve cell.

Fluorescent antibody test (FAT)

Direct fluorescent antibody test is the confirmatory test done routinely for rabies diagnosis. This picture shows rabies antigen with apple green florescence.

Mouse inoculation test was done when indicated. Several new tests were introduced during the past few years.

New tests introduced

Namely,

- Polymerase chain reaction (PCR)
- Immunochromatography test (ICT)
- Detection of rabies antigen in neuroblastoma cell culture

Rapid fluorescent focus inhibition test (RFFIT) was introduced to determine rabies neutralizing antibodies in serum and CSF. This test is done as a special test and is helpful for ante mortem diagnosis of human rabies in certain situations and also to assure protective immunity following vaccination in high risk patients.

Lab diagnosis of rabies – MRI

Due to the awareness programmes conducted throughout the country, the number of rabies suspected samples received in the MRI has increased. Dog is the main reservoir for rabies and cats come second. So far, rabies has not been reported in domestic rats in Sri Lanka. % positivity has decreased from 71.8% in 1999 to 54.3% in 2009. A decentralized rabies diagnostic laboratory which was established in 2003 in Habaraduwa was unfortunately washed away by the tsunami in 2004. But it was again re-established in Teaching Hospital, Karapitya in 2006. This has strengthened the rabies surveillance activities in the Southern Province and also reduced the unnecessary use of equine rabies immunoglobulin and vaccine in hospitals. Training of staff and quality assurance is done by the MRI on a regular basis. However, there is a great need for establishment of more rabies labs in other provinces too.

Human rabies deaths

Human rabies is a notifiable disease in Sri Lanka. In most instances a clinical diagnosis is made. It is known that clinical rabies could mimic any neurological disease and therefore it is mandatory that all deaths due to suspected rabies should be confirmed by laboratory diagnosis. Following an issue of a circular by the Ministry of Health in 2004, and training programmes conducted island wide, we have observed an increase in the number of human brain samples confirmed by laboratory diagnosis. In 1997 it was just over 10%, but in 2009 it has increased to >75%. This is clearly shown in this slide. This is considered as a major achievement in rabies diagnosis.

Challenges

In spite of all the achievements, several challenges too were encountered. The main challenge is to find ways and means of reducing unnecessary use of rabies immunoglobulin and vaccine. There have been several instances where mismanagement of patients have occurred. This could be minimized by establishing anti rabies treatment units in hospitals, which will also help in reducing unnecessary wastage of immunoglobulin and vaccine. A high turn over of staff in the hospitals is considered as a disadvantage for proper management of patients following animal bites. To overcome this, training programmes are conducted regularly for the hospital staff.

In addition a comprehensive circular on management of post exposure treatment was issued in 2008 by the Ministry of Health. To obtain a continuous supply of intradermal fixed needle syringes is a common problem in hospitals. Use of fixed needle syringes reduces the wastage of vaccine considerably due to the minimum dead space in these syringes. Spiraling cost of rabies cell culture vaccine and limited supply of rabies immunoglobulin can also be included as challenges faced by the ministry of health.

All that has been achieved was due to team work. As we go along the path of prevention of human rabies, we are sure to encounter many more challenges. However, I am confident that with the enthusiastic support of my dedicated team, we will be able to overcome these to finally achieve a rabies free Sri Lanka.

In conclusion I would like to acknowledge the following persons. I cannot forget the great debt I owe to my late parents, who nutured and guided me throughout life, giving me all the opportunities for me to be successful in my chosen career. Then, I would like to thank my teachers of Visakha Vidyalaya for moulding my character and guiding me through my formative years. I am also grateful to my clinical consultants and teachers who taught me during my undergraduate and postgraduate training periods. They helped me to be what I am today. A special mention must be made of my peers, colleagues and friends who always encouraged and supported me in numerous ways. A special word of thanks to my husband Dharshan, daughter Yashoki and son-in-law Nalinda, for the encouragement, patience and support throughout my professional life. I take this opportunity to thank Mr. Dushantha Karunanayake, for helping me in preparing this presentation. Finally, I would like to thank everyone who is present here today to grace this occasion.

Thank you!

Detection and identification and *in vitro* antibiotic susceptibility testing for first line drugs of *Mycobacterium tuberculosis*

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The important lacunae in the laboratory diagnosis of tuberculosis and drug resistant tuberculosis are: the delay in the isolation of *M. tuberculosis* using conventional culture, low sensitivity/detection limit of the direct smears and lack of technically trained personnel. The phenotypic tests in differential identification of mycobacteria in the diagnostic laboratory require minimum of 2-4 weeks for final identification and may not result in successful identification, if strain variations are encountered. Detection and Identification of *M. tuberculosis* using molecular techniques namely, Polymerase Chain Reaction (PCR), PCR based restriction fragment length polymorphism (PCR RFLP) and PCR based DNA sequencing are very rapid, more sensitive and reliable and there are several commercially available PCRs, Realtime PCRs (RTPCRs) and in house PCRs developed for rapid detection of *M. tuberculosis* genome directly from clinical specimens.

Regarding the phenotypic drug susceptibility testing for the I line antituberculous drugs there are 3 methods – absolute concentration method, resistance ratio method, proportion method and the preferred method of determination of antimycobacterial susceptibility testing is the proportion method as it allows precise determination of the proportion of resistant mutants to a particular drug. The Mycobacterial Growth Indicator Tube (MGIT) system, both in its manual and automated versions, is part of the new-generation of diagnostic techniques for the rapid detection of drug resistance based on proportion method. Many studies have now been published on the application of the MGIT system for rapid detection of resistance to first and second-line anti-TB drugs. In all these studies the MGIT system has shown very good results with a high correlation with conventional methods. The only limitation for a wide implementation of this new technique. would be its cost that can be high in many settings especially in high-endemic countries The other recently developed phenotypic drug susceptibility testing methods for the detection of drug resistance for I line drugs of *Mycobacterium tuberculosis*; microscopically observed drug susceptibility testing (MODS); nitrate reductase assay; phage-based systems for detection of drug resistance in *M. tuberculosis*.

The expectation that molecular techniques would surpass conventional methods for diagnosis of TB or phenotypic susceptibility testing has not (yet) been realized However, the clinician now has a variety of new tools to improve the diagnosis of TB and drug resistance. Most of these techniques require trained personnel and specialized equipment, hindering their application in field conditions, but they can be used in reference laboratories as part of the TB control programmes. The physician must be cautious when using results obtained by these techniques, especially when diagnosing drug resistance. Although it is not recommended, these molecular methods might be used as a complement to the standard methods in situation of difficult diagnosis, but never should be used solely to base such decisions.

Leptospirosis in the Central Province of Sri Lanka and specific management issues

Professor S. A. M. Kularatne

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In 2008, a major outbreak of leptospirosis caused 227 admissions to the Teaching Hospital, Peradeniya and 33 deaths with a case fatality rate of 14.5%. A majority of patients had fever duration of 3 days and had contact with probable sources of leptospirosis. A clinical scoring system found 132 patients (58%) with severe disease that included multiple organ involvement such as severe thrombocytopenia and bleeding, respiratory distress with fluffy shadows in chest radiographs, myocarditis, hepatic and renal failure. The pulmonary haemorrhages and respiratory distress contributed for high death rate despite being on optimal intensive care management. Administration of bolus methylprednisolone in severe cases reduced the fatal outcomes, however this benefit was negated by advanced disease. A serological and genetic analysis of a subgroup of these patients found approximately 13 serogroups and 14 serovas of leptospiral species and, of them predominant serogroups were Sejroe and Icterohaemorrhagiae. A further study in the region found a significantly higher carrier status of leptospirosis in dairy cattle and peridomestic rodents, intriguingly sharing the same serogroups of humans.

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The role of the microbiology laboratory in the elimination of parasitic diseases – malaria and leishmaniasis

Prof Renu Wickremasinghe

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In 2008, the dramatic reduction of malaria incidence over the previous 6-7 years prompted Sri Lanka to embark on a malaria elimination programme focusing on intensified surveillance, provision of early diagnosis and, prompt and effective treatment, and the use of coordinated vector control activities. With the dawn of peace in mid 2009 and infrastructure development, this programme may be more realistic as far as malaria is concerned. Leishmaniasis presents a different problem with the first detection in 1992, and the number of cases being detected rapidly increasing.

Successful elimination of any parasitic disease is dependent on early diagnosis and prompt and effective treatment. To achieve this reliable diagnostic facilities and effective treatment should be available in the most remote areas of the country where the diseases are prevalent. Strict vigilance in the form of intensified surveillance, rapid response teams to control full blown or impeding outbreaks and a well informed medical fraternity and public are also of paramount importance in an elimination target.

The microbiological laboratory is a key player in this regard giving inputs to many activities that are essential for an elimination programme. Providing a reliable and early diagnosis ensures prompt and effective treatment and feeds information into the surveillance system for further action if necessary. The microbiological services, though not necessarily high tech, should provide a reliable diagnosis using methods that are reliable, time tested and affordable to the country. For this, basic infrastructure, regular supplies and well trained personnel should be ensured. In addition, quality assurance procedures should be established that would give confidence to both the clinician and the public to ensure a concerted action for the elimination effort. Investing in the microbiological services will undoubtedly be the marker that will determine the success or failure of any parasitic disease elimination programme.

Role of the parasitologist/laboratory in the programme for elimination of lymphatic filariasis in Sri Lanka

Prof. Mirani V. Weerasooriya

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Lymphatic filariasis is a disabling disease caused by nematode parasitic worms, *Wuchereria bancrofti, Brugia malayi* and *Brugia timori* transmitted through mosquito bites. It is estimated that around 128 million people are infected in 83 countries in the tropics and subtropics.

In 1997 the World Health Assembly passed a resolution calling for the elimination of the disease. The World Health Organization established the Global Programme for the Elimination of Lymphatic Filariasis (GPELF) in 2000 aiming to achieve total elimination by 2020. The programme had two principal goals (1) to interrupt the transmission of infection in the entire 'at risk' population by treating every individual annually with a single dose of two drug regimen 6mg/kg of diethylcarbamazine or 200 mcg/kg ivermectin and 400mg albendazole (MDA) (2) to alleviate the suffering and decrease the disability of those already with the clinical disease by reducing the secondary bacterial and fungal infections of the limbs and genitals and conduct of hydrocoele surgeries.

In Sri Lanka the disease is considered to be endemic in three provinces, southern, western and north western and covering eight districts. The Ministry of Health, initiated the National Programme for the Elimination of Lymphatic Filariasis (PELF) in 2002 covering the three endemic provinces. Five rounds of mass drug administration were completed by 2006. The morbidity control programme too was continued through the years. Independent research teams have conducted many evaluation studies on drug distribution and coverage and on adverse effects of drugs. Having completed five years of surveillance after the last MDA, Sri Lanka has now entered the phase of verification. Finally the process will lead to the declaration of elimination of disease from the country. The role of the parasitologist at the present stage will be the continuation of surveillance and evaluation utilizing the recommended tools like night blood for microfilariae, tests to detect circulating antigen to *Wuchereria bancrofti* and other techniques to detect parasite DNA in humans and mosquitoes. Monitoring and epidemiological assessment of MDAs, surveys for new endemic areas and continuation of disability alleviation services on a larger scale will be discussed. Laboratory's role in this process in using the diagnostic tools in sentinel and spot check sites, conduct of transmission assessment surveys, detection of entry lesions in lymphoedema patients and their management will be highlighted.

Improving the quality of medical testing as per ISO guidelines

Rahal Widanagamage

Assistant Director - Accreditation, Sri Lanka Accreditation Board.

Improving the quality dealt with understanding the term "quality", which has been defined as the set of characteristics that a product or service should have to satisfy the needs and expectations of the customer. In medical testing the product is the test report and the customers can be the clinicians, health care bodies, health insurance companies, pharmaceutical companies or even patients. Thus the requirements of the customers could be an accurate, timely and cost effective test reports which have been issued assuring confidentiality and ethics.

In meeting the customers' needs, the ISO 15189 standard exemplifies a set of guidelines in management and technical purviews. The criteria with respect to the management requirements have been stated as to have a quality manual describing the whole quality management system with the structure of the document system, an appointed quality manager and to establish technical management, policies and procedures ensuring confidentiality, an effective document control system, guidelines on purchasing, advisory services, a system for resolution of complaints, a system for correcting nonconformance and finally conducting internal audits to verify the continuing compliance.

With respect to the technical aspects the guidelines are focused on having competent and qualified personnel with experience with the provision of adequate and continuous training. The environmental conditions should be maintained within the required limits. The laboratory equipment should be maintained and calibrated. The laboratory's specimen collection facility should have a manual with all the necessary procedures and information. ISO guidelines invoke using valid test methods with standard operating procedures and the accuracy of results should be assured by internal and external quality control programmes. Finally systematic reviewing of test results should be performed by authorized signatories before issuing the test reports.

Use of antibiotics in animal husbandry

G. A. Gunawardana

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For many years antibiotics have been widely used in food animal production both therapeutically and sub therapeutically. Improved food quality and cost reduction due to maintenance of healthier animals are identified as benefits of antibiotic application in animal husbandry. However in the past decade public attention on usage of antibiotics at sub therapeutic levels in livestock has increased along with the emergence of multi-resistant organisms. Another hazard associated with this practice is presence of antibiotic residues in food of livestock origin as well as in the environment, leading to toxicity and allergenicity. Though further studies are needed to make the link between antibiotic usage in animal husbandry and human health problems clear, considering available information responsible authorities have revised the policy and regulations on using antibiotics as feed additives. Because of the public health importance of this issue different strategies and replacements are being sought by researchers worldwide. Probiotics, organic acids, fermentable substrates, oligosaccharides, minerals and enzymes are potential substitutes of antibiotic growth promoters. Use of such substances along with changes in animal husbandry and management practices could minimize the adverse effects of antibiotic usage. By working closely, the relevant organizations, scientists and producers in Sri Lanka could ensure the safety of livestock products, protecting consumer and industry equally. To mention further, trend in organic farming and growing demand for organic meat, milk and eggs reflect the consumer preference for food free of antibiotics.

"THE ART OF SCIENTIFIC INVESTIGATION" AND "THE LOGIC OF SCIENTIFIC INFERENCE"

Prof. S. N. Arseculeratne

Emeritus Professor of Microbiology, Faculty of Medicine, University of Peradeniya.

I am borrowing those titles from W. I. B. Beveridges's and Jennifer Trusted's useful books respectively, to make general comments on (i) the role of logical inference in the pursuit of scientific investigations and then, (ii) specifically, misconceptions on a disease that result from faulty education and practice in science and an absence of elementary ideas in the philosophy of modern science. First, I will quote the opening lines from an essay in *Scientific American* (2011, May 9) titled, "Trust me, I am a scientist" by Daniel Willingham.

> "*A friend of mine has long held that a vaccination his son received as an infant triggered his child's autism. He clings to this belief despite a string of scientific studies that show no link between autism and vaccines. When the original paper on such a link was recently discredited as a fraud, my friend's reaction was that it will now be difficult to persuade people of the dangers of vaccination*".

That story illustrates the dangers that are faced by serious teachers and researchers in their attempts to establish a state of scientific literacy not only of the citizen, that I have earlier called Scientific Literacy for the Citizen - SLC, but also of Scientific Literacy for the (Scientific) Elite, SLE (Arseculeratne 1993). These dangers arise from modes of education in science that have gone awry.

The state of scientific illiteracy that results from faulty science-education is illustrated by the quagmire concerning *Rhinosporidium seeberi*, the enigmatic organism that causes rhinosporidiosis in humans and animals, that we have been researching on for the last fifteen years.

Rhinosporidiosis was first observed by Malbran in Argentina in 1892. G. R. Seeber, also in Buenos Aires, Argentina described this disease and its causative organism, for his MD thesis in 1900. *R. seeberi* has never been cultured in the laboratory *in vitro*, nor has it established rhinosporidiosis when administered as suspensions of rhinosporidial tissue, to experimental animals. These two characteristics have made research and derivation of valid conclusions on this pathogen, extremely difficult, although it is readily observed by conventional histopathology in rhinosporidial tissues and was recently explored by molecular biological techniques by in situ hybridization with primers designed on its gene sequences, and with other molecular biological tools, through which definitive conclusions on its taxonomy and natural habitat, ground waters, were made (Kaluarachchi et al. 2008). With these new techniques, *R. seeberi* was removed from the orphanage "Fungus-like organisms" (see Arseculeratne and Mendoza 2005) and placed in a new Clade, the *Mesomycetozoea*, by Herr et al. (1999), supported by the findings of an independent group of researchers, Fredricks et al. (2000).

In 1992 and in 1994, Ahluwalia and co-workers in India reported that the round body described as *R. seeberi* by earlier workers and fully described in the monograph "Rhiniosporidiosis in Man" by W. A. E. Karunaratne in 1964 is not a biological organism at all but lumps of tapioca starch in lysosomes that resulted from the excessive consumption of starch from tapioca (manioc, *Manihot utilissima* syn. *esculenta*). The same group of 'researchers', later recanted this view and then in1997, incriminated the ubiquitous cyanobacterium *Microcystis aeruginosa*, found in ground waters, as the real cause of rhinosporidiosis. Their starch-conclusion was based on (a) morphological similarities based on the "round bodies", endospores and sporangia in *R. seeberi* and lysosomes in human cells, respectively, and (b) similarities on molecular biological findings (e.g. PCR bands) from extracts of rhinosporidial polyps on the one hand and from *M. aeruginosa* (that can be cultured in the laboratory) on the other.

Refutations of Ahluwalia's *Microcystis*-thesis were made by Arseculeratne (2000) and by Mendoza et al. in the USA (2001), on the grounds of (i) the absence of control samples from normal persons living in the same habitat-area from which their water samples were collected, (ii) improper collections of samples (from contaminated sites) for molecular biological tests, (iii) the inability of *M. aeruginosa* to cause rhinosporidiosis, (iv) the absence of reactivity of *M. aeruginosa* extracts with human patients' sera and experimental antisera containing high titres of anti-rhinosporidial antibody in immunodot-blot tests, and (v) the absence of amplification in PCR tests of *M. aeruginosa* extracts with primers based on sequences of *R. seeberi*. They attributed Ahluwalia's *Microcystis*-error to contamination by this water bacterium.

The error of incriminating starch and then *M. aeruginosa* arose not from the use of faulty technology but by the use of faulty inferences from the results obtained from the misapplication of faulty logic and by being seduced by sophisticated technology without the proper application of the principles of scientific inference; indeed I would use the term, *Idola machinorum*, the uncriticial, idolatrous application of sophisticated technology, akin to *Idola quantitatis* that Peter Medawar used to describe the use of sophisticated mathematical, computer generated figures to cover the nakedness of faulty conclusions, just as much as if one looks at a leopard through a telescope, one sees just a spot and not the whole leopard. The first Ahluwalia-error is also akin to the misapplication of the Aristotelian syllogism A = B, B, = C, hence A must be equal to C (Bostock 1983); *R. seeberi* has round bodies, lysosomes containing starch are round bodies, ergo (therefore), *R. seeberi* is a starch-bearing lysosome.

This error of making faulty inferences is also exemplified by the story of the old professor-zoologist who described his work on locomotion in fleas; he fished out a flea from his box, placed it on the lecture-table and yelled at it, "jump", and the flea did jump. The professor then removed the legs of the flea, one by one, and then shouted again "jump"; but the flea did not jump, whereupon the professor announced his discovery that "the flea hears through its legs". His observation that the flea without its legs did not jump is acceptable as valid, but not his conclusion that the flea hears through its legs, as the flea does not have a hearing-organ. He apparently was not aware that a flea will respond to vibrations that his yell "Jump" would have caused, and his conclusion was therefore bedevilled by his ignorance of the fact that the flea has no ears but is sensitive to vibrations. This illustrates another requirement for valid conclusions, that of awareness of the valid facts that have already been discovered through proper scientific methodology, i.e. familiarity with the scientific literature. Ahluwalia's first misinterpretation recalls that carbohydrate molecules are ubiquitous, being shared by plants and animals and their presence in species of both Kingdoms, plants and microbes, is not proof of the identity of the species, *R. seeberi* with *M. aeruginosa*. Their *M. aeruginosa* hypothesis was born of faulty planning of investigations, in having used nasal rhinosporidial polyps with their rough, surfaces containing crypts (that are open to contamination by micro-organisms, including *M. aeruginosa*, in ground water), and not the lesions of disseminated rhinosporidiosis that remained covered by unbroken skin and which were therefore not exposed to aquatic *M. aeruginosa* in its natural habitat of ground water.

That the starch and *M. aeruginosa* theories of Ahluwalia were published in so-called prestigious western journals, opens another can of worms – the role of proper refereeing and editorship of submissions by science-journals; this topic is, however, outside the scope of this essay.

These comments bring me to consider "The place of the Philosophy of Science in Education in Science". The philosophy of science (that includes the art, and methodologies of scientific investigation, the use of proper controls, valid inferences, derivation of hypotheses, understanding the ideas of causality, the formulation of theories, the falsification or verification of theories, the formulation of scientific laws) is, as far as I am aware, not discussed in Sri Lankan undergraduate curricula, except in the Open University, Colombo, which was entirely on the personal initiative of just one man, Professor Arjuna de Zoysa. It also reinforces my conviction (Arseculeratne 2002) that it is the individual administrator of an institution rather than the institution's policy itself, that is of predominant importance, contrary to what our distinguished diplomat Mr. Jayantha Dhanapala argued; he had said: *"I have long believed that the most durable achievements in public policy are best realized through institutions rather than individuals".*

When I was given the task of administering the Centre for Research in Tropical Medicine at the Faculty of Medicine, University of Peradeniya, I intended starting with a series of lectures on The Philosophy of Science, whereupon a Senior Professor in the Peradeniya Medical Faculty asked me, *"Of what use is the Philosophy of Science?"*. I then asked ten young Ph.D's in our faculty *"Why is your degree called a Doctorate in Philosophy?"* – none knew. These young PhD's and the Senior Professor did not have a metaphorical leg to stand on, like the legless flea; I held my head in horror.

Four centuries of the growth of modern science that arose in Western Europe, that we try to propagate in our universities and in private tutories, and The Scientific Revolution which was also essentially an European phenomenon, reminded me that our efforts at the establishment of a state of scientific literacy in Sri Lanka, have been in vain.

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BLOOD STREAM INFECTIONS DUE TO CANDIDA SPECIES

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Introduction

Worldwide, there is increasing incidence of fungal blood stream infections due to *Candida* spp (1,2). This may be owing to a growing number of immunocompromised hosts, such as granucytopaenic cancer patients (3) organ transplant recipients and HIV/AIDS patients, as well as the escalating use of broad spectrum antibiotics and intravascular devices in haematological wards and intensive care units (4). Candidaemia occupies the eighth place among nosocomial blood stream infections in the UK and has reached the fourth place in the USA and Japan (5-7). Figures of Sri Lankan incidence is not yet determined and local incidence rate should not be that different from the rest of the world in the presence of widespread use of broad spectrum antibiotics and intravascular devices in most Sri Lankan hospitals.

Candida albicans remains the predominant infecting species accounting for more than 50%, in blood stream infection in most parts of the world whereas non-albicans strains have contributed to more than 59% and between 51% and 55% in Japan and USA respectively (7). This changing trend in species distribution have shown a significant impact on outcome of bloodstream infection due to the unpredictable sensitivity test profiles of non-candida albicans strains to fluconazole (8). This may have attributed to the very high crude 30 day mortality of between 26.4% and 45.8% in some series (5,8).

The clinical signs and symptoms of candidaemia are non-specific and taking a decision on the clinical significance of a positive blood culture has been problematic. Also Candida species rarely arise as skin contaminants in poorly drawn blood cultures. Hence controversies arise regarding the decision to start antifungals. This in turn will invariably lead to unacceptable delays in commencing appropriate treatment in critically important patients and will cause other management issues such as delays in replacement or removal of IVCC, and hence the undesirable outcomes .

Therefore attention must be paid to explore the standards of diagnosis and treatment of *Candida* BSI. Also it is essential to formulate local management policies considering the epidemiology of local case load focussing on species distribution and sensitivity pattern. This in turn will definitely improve the prognosis of *Candida* bloodstream infections minimizing the risk of emergence of drug resistant strains. Furthermore audits of the standards and analysis of outcome of candidaemia patients would contribute positively towards improving mortality rates of candida blood-stream infections.

What is candidaemia?

Blood stream infections (BSI) due to Candida species (candidaemia) represents a spectrum of clinical illnesses which ranges from mild, self limiting catheter related infections to severe complicated invasive disease involving one or more organs.

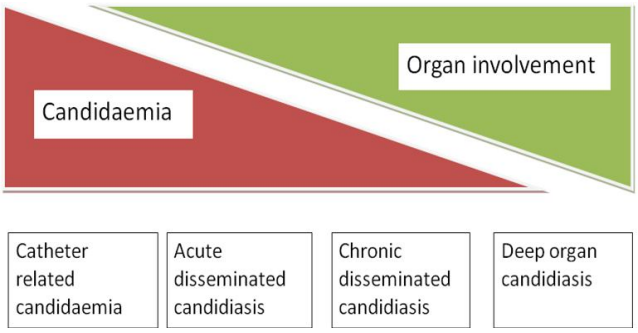

Figure 1. Candidaemia – clinical spectrum.

Majority of candidaemias indicate intra venous central catheter (IVCC) related infections (9). Also almost all IVCC related infections will have documented candidaemia. Although this clinical entity is most often limited only to the blood stream and has a relatively benign course few patients will develop complicated disease with organ involvement. Eyes, endocardium, vertebral column, meninges and joints could get involved but propensity to spread is difficult to determine clinically.

Candidaemia is also a recognized manifestation of acute disseminated candidiasis which is seen mainly in severely ill neutropenics. This is probably IVCC related or caused by translocation of gut organisms. About 50% -70% of patients will have blood cultures positive for Candida species and dissemination to deep organs is commoner than catheter related infections as they lack neutrophil defences against blood stream spread (9).

Candidaemia could rarely be attributable to chronic disseminated candidiasis. This occurs almost exclusively following prolonged episodes of bone marrow dysfunction in patients who have undergone myeloablative therapy. A Candida BSI during the initial neutropenic episode would inevitably spread to deep organs. Liver and or spleen are predominantly infected and kidneys and lungs are rarely involved. Symptoms and signs of deep seated infection appear with the recovery of host inflammatory response. Candidaemia is a rare occurrence.

In the deep organ candidiasis which results in direct inoculation of organs following trauma, surgery, via invasive devices such as peritoneal dialysis catheters and urinary catheters, candidaemia is just the tip of the ice burg.

Management of candidaemia

The following are the latest guidelines that can be used to treat the patients as well as to audit the standards of diagnosis and treatment.

- British Society for Medical Mycology guidelines – 2003 (10)
- IDSA guidelines – 2009 (6)

Diagnostic standards

- All blood and CVC tip culture isolates should be identified to the species level by referral to a specialist laboratory. As different Candida species have different susceptibility patterns this is useful in determining the local antifungal policies describing empirical treatment guidelines. Also this would help in the early detection of outbreaks and to diagnose IVCC related infections

Treatment standards

1. Antifungals

- All patients should have an appropriate systemic antifungal agent at an appropriate dose, started within 24hr of a positive blood culture unless all treatments are withdrawn. Delay of commencing treatment more than 48 hours leads to serious consequences. Although many patients of candidaemia seem to have self limited and transient infections there is no way to distinguish them from those who will develop complications. Therefore recommendation is to treat all patients.
- Flucanozole is proposed for patients with mild to moderate illness, who have no exposure to an azole at least within last 3 months and without risk factors to have infections with *C. krusei* and *C. glabrata*. Haemodynamically unstable patients with severe infections, patients with risk factors to get infections with drug resistant strains and who have had azole exposure should receive either amphotericin B or an echinocandin as an empirical therapy.
- Breakthrough candidaemias need an alternative therapy.
- It is necessary to determine the clearance of Candida from the blood stream to decide on the duration of treatment. Therefore follow up blood cultures are indicated daily or every other day until they become negative.
- Recommended duration of treatment is for at least 14 days from the last negative blood culture. However longer courses of medication is necessary for patients with persistent candidaemia, metastatic complications and prolonged neutropenia.

2. Screening for metastatic complications

   Dilated ophthalmological evaluation is essential in all patients usually within the first week of treatment, when follow up cultures show no growth and further spread to eye is unlikely. However ophthalmological examination should be delayed in neutropenics until recovery from neutropenia as without host inflammatory response, signs of endophthalmitis may not be visible.

3. IVCC care

   All patients with candidaemia should have their IVCC removed or replaced as soon as possible of the

diagnosis being made (at least within 48 hours) to lower the mortality and shorten the duration of infection. Although IVCC is not always the source of infection, it could get seeded promptly to provide a hidden focus which could cause subclinical infections. Even in neutropenics whom the gastro intestinal tract could also act as a source of dissemination replacement or removal of central venous catheters is recommended, if logistically feasible. Peripheral venous catheters are not considered as focus of infection.

Management of candidaemia at a University Hospital setting in UK

A retrospective analysis of all cases of candidaemia during the period Jan 2007 - Dec 2009 was carried out at Barking Havering Redbridge University Hospitals (NHS) Trust (BHRUT) in East London, UK. Data was compiled prospectively by treating consultant microbiologists and documented on a proforma. Subsequently they have been saved in an Excel spreadsheet incorporating the computerised laboratory results (WINPATH). This audit gives an insight to the management standards of candida BSI in the BHRUT in comparison to the UK national standards. Also this provides additional information on trend in incidence, epidemiological characteristics, antifungal resistance rates and outcomes of candida BSI in a university hospital trust in London. The overall incidence of candidaemia in the catchment area was 4.1 per 100000 population and it is consistent with the incident rates for London in 2008 (4.14 per 100000 population) (11). The incidence of 7.09 per 100000 bed days at BHRUT Hospitals is higher compared to incidence of 3 per 100000 bed days in UK hospitals reported 10 years ago (8). This difference could be explained by the constantly rising trend in candidaemia in UK over the past two decades as described in Health Protection Agency reports (13). However this incidence is nearing the reported incidence rates (7.3 episodes per 100000 patient days) from Spain in 2003 although the denominator is different (12).

Local epidemiology

Proportion of community acquired candidaemia is lower (10.3%) than studies elsewhere in the world (25% in USA and 18% in South America) (14). However this rate is comparable to the rates in some other studies (6% in UK and 10.8% in Barcelona, Spain) done in areas where there is low use of outpatient IVCC and intravenous antibiotics as in our setting (8,12). In this study population the majority of candidaemia occurred in more than 50 years age group but studies done elsewhere had two peaks in age distribution of candidaemia, in infancy and in elderly (8,11). The lack of very many infants in this audit may be explained by having no type 3 neonatal intensive care unit and no facilities for dedicated paediatric surgery in BHRUT Hospitals. The ICU stay is a major predisposing factor bringing together patients with risk factors such as IVCC, bowel surgery, broad spectrum antibiotics etc. Largest number of candida blood stream isolates were from the critical care units (40%) comparable to rates in other studies (8). However the recent studies tend to depict a lower ICU patient numbers (33% in Barcelona and 27.9% in Scotland) possibly due to management of patients with predisposing factors in relevant units which is not common in our hospitals (12,15). The high proportion of cases in the medical units is a result of inclusion of oncological patients in the category of medicine. Indwelling IVCC is the most common source of candidaemia mainly in non neutropaenic patients (9). The primary site of infection was attributed to IVCC in 39% of total episodes. This may not include all the patients with IVCC related candidaemias due to possible inadequacy of samples to define IVCC as the source. As 47% of patients did not have a defined source of infection, it highlights the difficulty in defining source of infection in most candidaemia episodes.

Mycology

C. albicans was the predominant species causing majority (47.7%) of candidaemia episodes but this is lower than the UK figures for 2008 (52%) and data from USA (56.2%) (11,14). This is a relative decrease as higher proportions of *C. glabrata* infections were isolated in the study population than reported in UK (35.6% vs. 20%) (11). The possible explanation for these differences are not clearly visible although the type of specialities available in these hospitals and antifungal policies may have played a role. The lower *C. parapsilosis* infection rate (4%) when compared to UK figures (11%) is explained by the absence of dedicated neonatal and paediatric surgical unit in these hospitals and also existing effective infection control practices in insertion and care of IVCC (11,15).

Antifungal sensitivity pattern

The proportion of blood stream isolates of Candida intermediate resistant and resistant to flucanozole (18.5%) is higher than reported studies elsewhere in UK (13%) (16). However, fluconazole resistance among *C. albicans* isolates were not seen. Flucanozole intermediate resistance and resistance among non *C. albicans* is significant accounting for 36% which is higher than that in UK studies (21.8%) (16). This result is influenced by higher proportion of *C. glabrata* infections (35.6%) and higher *C. glabrata* resistance (46%) rates in this study than UK and other countries (11,12). Possible existence of selective pressure due to first line Fluconazole therapy in all candidaemias except in neutropaenics may explain these differences.

Audit results

- Overall audit standards according to the national guidelines have been met in management of candida blood stream infections at BHRUT hospitals.
- According to the audit standard all Candida isolates from blood stream infections should be speciated and tested for sensitivity to identify changes in species

distribution and sensitivity profiles early (10). In this study majority (83%) of isolates were sent to the reference laboratory for speciation and sensitivity testing. In 2009 alone this figure has reached 100% target.

- Initiation of appropriate antifungal within 48 hours of positive blood culture is associated with reduced morbidity and mortality (10). In 92.2% (70/76) of patients appropriate antifungal treatment was started within 48 hours of positive blood culture.
- In addition to antifungal treatment removal of IVCC from all patients with candidaemia within 48 hours of positive blood culture is recommended in order to reduce the duration of candidaemia and mortality rates (10). IVCC tips were received for culture from 78% of whom candidaemia was associated with IVCC, within 48 hours of positive blood culture. However, this figure does not reflect the removal of catheters from all patients with IVCC as mentioned in the national guidelines. There was no local laboratory policy to send catheter tip isolates for speciation.
- It is recommended to repeat the blood cultures daily or every other day to confirm the clearance of candidaemia from the blood stream. In this study repeat blood cultures were done in 68% of patients reaching the audit standard.

Outcome of candidaemia in BHRUT Hospitals

- In this study 30 day mortality rate (50.2%) is slightly higher than rates reported in UK (26.4% and 45.8%) (5,8). This higher mortality rate could be partly explained by the fact that 40% of candidaemia patients audited had PITTS bacteraemia scores equal to or more than 4 which is associated with significant mortality in candidaemia patients (17). In this study a 39% mortality rate was observed in patients with PBS < 4 whereas it was 67% for patients with PBS $\geq$4 and the difference in mortality is statistically significant (p> 0.033).
- The 40% rate of critical care patients may also be contributing to this high mortality rate.
- In the patient group where antifungal therapy commenced within 48 hours of blood culture being documented, 30 day mortality was 42.8% and in those treatment was delayed it was 50%. However this difference is statistically not significant. Although audit standard was met in 92.2 %(70/76) in commencing treatment within 48hours, there is a hidden delay in treatment when time between collection and flagging of blood cultures is considered. Local laboratory turnaround time average is 2.2 days (range 1- 4 days). Therefore 46% (32/70) of correctly treated patients did actually receive treatment after 48 hours of blood culture collection date. This hidden delay may also have played a role in observed high mortality rates in candidaemia patients. Introduction of rapid methods such as real-time PCR in detecting Candida in blood cultures has an important role to play in minimizing delay in treatment in future.
- When the effect of IVCC removal is assessed, the mortality rates for the two groups, before and after 48 hours of positive blood culture were 30% and 40% respectively but it was statistically not significant (p> 0.05).
- Univariate and multi variable predisposing factors of mortality were not analysed.

Summary

Fungal blood stream infections due to Candida species is on the rise worldwide. In order to improve the prognosis of candida bloodstream infections which carries a high mortality diagnosis and treatment should be defined carefully and interventions should be prompt. Standards for diagnosis and treatment are clearly laid down in IDSA and British Society for Medical Mycology guidelines and practiced globally. It is timely to formulate management policies for candida BSI in Sri Lankan setting using those guidelines as a reference. Studies to identify local epidemiological features and sensitivity profiles of candida BSI would be required to prepare such guidelines for Sri Lanka. Audits of the implemented standards and analysis of morbidity and mortality should improve outcome of candida bloodstream infections.

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A BRIEF INTRODUCTION TO LABORATORY INFORMATION MANAGEMENT SYSTEMS (LIMS) AND ITS POTENTIAL BENEFITS FOR SRI LANKA

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Manual information management

The basic function of the laboratory is to deliver an accurate result within a reasonable time frame. This involves the sequence of transferring the sample to the lab, analysing the sample, checking/rechecking the result and issuing a report. The time that it takes for this process is the “turnaround time” for a test.

Purely by definition a laboratory information management system is a method to manage the above sequence of events. All the labs in Sri Lanka use some sort of manual method by way of record books, ledgers, files etc to manage information generated in the laboratory.

Unfortunately, manual systems are neither accurate nor timely. It is a time consuming process riddled with transcription errors. Data retrieval and retrospective data analysis is extremely difficult, sometimes impossible.

Laboratory Information Management Systems (LIMS)

In the modern context a Laboratory Information Management System (LIMS) is a computer software system designed to manage laboratory information.

The concept of a LIMS began in the 1980's and were called 1st generation LIMS. These were provided by analytical instrument manufacturers and ran on standalone computers. Their functions were limited to tracking samples and they provided no flexibility.

The modern LIMS

With the availability of powerful processors in personal computers and networking, we are now in the 4th generation of LIMS's. The present generation of LIMS have moved on from stand-alone computers to local area networks (LAN), wide area networks (WAN) and the Internet. They are highly flexible and adaptable.

The function of LIMS have moved away from purely being a tool for managing laboratory samples, into a complex system which integrates quality assurance, invoicing, billing, data analysis, system integration, enterprise management and a host of other functions.

LIMS architecture

The hardware and software framework that allows the LIMS to be operational could be defined as the LIMS architecture. There are many types of LIMS architectures.

Thick client – In this configuration the LIMS software is installed in the user’s computer, processing of data is done by the users’ computers. The data generated is stored in the central server (computer). Multiple users can connect to the central server through the network.

Web enabled – This is the same configuration as the above (thick client), but an additional software installed in the user’s computer allows it to access the LIMS network through the internet from any place with internet access.

Thin client – In this configuration the LIMS software is installed in the central server (computer). The users' computers connect to the server via the web browser. The LIMS software is not installed in the users' computers. The data is stored and processed in the server .

There are advantages and disadvantages in each type. Selection of which type of architecture to choose depends on the requirements and the resources available.

Choosing a LIMS

There are a number of basic characteristics that need to be considered when trying to implement a Laboratory Information Management System.

- The LIMS should have the functionality to manage the current and future workflows of the laboratory.
- The user interface should be user friendly, intuitive , easy to learn and pleasant to the eye.
- The system should allow the user to expand vertically (increase the samples, workflows) and horizontally (add users, departments).
- It should be flexible and configurable with the ability to be changed according to the users' needs, without being proprietary or hardcoded.
- The software should preferably be open sourced so that it can be customised easily and cheaply without being dependant on a vendor.
- The software should be easy to implement (install and run) with the existing resources, or require minimum additional resources.
- It should be easy to maintain.
- It should be a good value investment.

Finding a LIMS suited for microbiology labs in Sri Lanka

All of the software companies that develop LIMS have done so using the western health care system as the model. Most of these softwares could be modified to suite our needs. But the cost and the proprietary nature of these software would make them a poor choice for our country.

A LIMS for our country should take into account our unique health system, the resources available to us and our financial limitations. Therefore we cannot hope for a off the shelf solution. Our best option is to develop a LIMS to suite our need using the considerable human resources available to us in the field of information technology.

LIMS for central laboratories in Sri Lanka

In the Sri Lankan context, the basic function of a LIMS would be to computerise the clerical activities associated with sample processing and therefore,

- Minimise transcription errors
- Maintain an audit trail
- Allow lab manager to make informed decisions
- Shorten turnaround time of lab tests
- Allow report generation
- Provide real time and retrospective access to data
- Monitor resource utilisation
- Allow exchange of data and information
- Improve productivity

LIMS for peripheral laboratories

At the moment there are a limited number of microbiologists in Sri Lanka who are based at central locations in the country. Due to the rapid development of peripheral hospitals, there is an increased demand for diagnostic microbiology services in these areas. Maintaining proper quality controls and ensuring accurate reports from these peripheral centres is a problem.

Networking of the peripheral laboratories with the central lab and an implementation of a LIMS would allow the microbiologist at the central location to supervise multiple peripheral laboratories.

We will be able to set up workflows, implement laboratory protocols and implement quality control protocols.

During validation of reports, the Microbiologist/supervisor will be able to assess if

- Isolate matches the history
- Gauge the relevance of the isolate,
- The protocol for processing of sample and identification of the isolate has been followed,
- Sensitivities are appropriate and fits with the isolate.

If any discrepancies are found or anything has been missed the result can be withheld until the corrective measures are taken.

Reports can be issued with comments that would help clinicians to interpret the result.

Data can be analysed from the centre to detect emerging resistant patterns that could be a potential problem to the peripheral centre so that timely intervention can take place .

Conclusion

In conclusion with proper planning and implementation the introduction of a networked Laboratory Information Management System to the health sector in Sri Lanka can vastly improve the quality of the laboratory services in the country.

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BLOOD CULTURE AUDIT ON BACT/ALERT SYSTEM AT ST. GEORGES NHS TRUST

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Rationale for doing this audit

BacT/Alert blood culture system was introduced to the SGH in 2003. There were no audits conducted to detect the performance levels of the system since introduction. Further institute is curious to find out the usefulness of the anaerobic bottle. Optimising the use of anaerobic bottle may save diagnostic costs for the trust.

Objectives

- What are the performance levels of BacT/Alert blood culture system for SGH in year 2008?
- To find out the percentage of clinical significance and contamination among positive samples (with analysis of type of sepsis).
- To find out bottle wise breakdown of positive results among positive samples excluding paediatric bottle (both bottles, anaerobic bottle only, aerobic bottle only).
- How far do we need the anaerobic bottle?
- To find out the percentage of patients who got anaerobic bottle only positive with clinical significance out of total positive patients, and out of total patients.
- To further analyse the anaerobic only positive patients clinical syndrome wise, and isolated organism wise.

Data collection

Data were collected using APEX version 5.6.10015 (Release date 06/09/2005) and FOCUS 6.9.8 (created 17/10/2000).

Results

Total sets of blood cultures done for 2008 (01/01/2008 to 31/12/2008) = 14528

Total number of patients = 5300

Total sample (sets) positive (without Paed bottle) = 1992

Total number of patients with positive blood cultures = 1470

(=27.7% (CI 18.9-20.7%)

Random 100 clinically significant positives (bottle types)

Percentage of both bottles positives among clinical significant (random) = 80%(CI 71-87%)

Percentage of aerobic bottle only positive among clinical significant = 19% (CI 12-28%)

Percentage of anaerobic bottle only positive among clinical significant = 1% (CI 0-5%)

Anaerobic bottle relevance
Total blood culture sets done = 14528

Total anaerobic only positive bottles = 415

Total anaerobic bottle only positive patient number =411

Total clinically relevant AN bottle only positive patient number =88

Among those 88 patients same organism positive in other blood cultures =29

Pure anaerobic only positive patient number =59

Percentage of patients AN only positive out of total positives =4% (CI 3.1-5.1%)

Percentage of patients AN only positive out of total patients = 0.8% (CI 0.61-1%)

Clinical syndromes of 59 AN only positives

- Urosepsis = 27 (45.7%)
- Abd. Sepsis = 10 (16.9%)
- SSI = 6 (10.1%)
- Biliary sepsis = 4 (6.7%)
- Line sepsis = 4 (6.7%)
- Quincy = 2 (3.3%)
- Chest infection = 2 (3.3%)
- Others = 4 (6.7%)

(24/27 urinary sepsis urine cultures were positive)

Analysis of 59 AN only positives, organism wise.

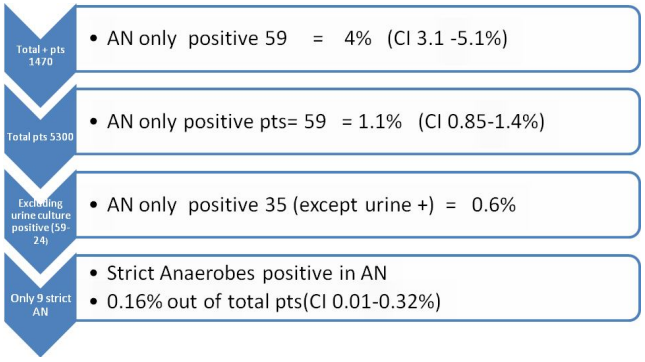
Organism types of 59 AN only

- *E.Coli* = 27 (45.7%)
- CNS = 3 (5%)
- *S. aureus* = 3 (5%)
- *Klebsiella* = 3 (5%)
- *Fusobacterium* = 3 (5%)
- *Clostridiae* = 3 (5%)
- *E. faecalis* = 3 (5%)
- *S. pneumoniae* = 2 (3.3%)
- *B. fragilis* = 2 (3.3%)

- Yeasts = 2 (3.3%)
- Others = 8 (13.5%)

(only 9 strict anaerobes from Quincy and obvious abdominal septic issues)

Actual use of AN bottle in 2008



Conclusions and actions taken

- Anaerobic blood culture bottle was used in less than 1% in diagnosis of infections in SGH.
- SGH switched to use only aerobic bottle for routine diagnosis of bacteraemia since 05/10/2009 (decide to issue anaerobic bottle only on special requests)
- Estimated cost reduction ≥ £30,000.00/y

Cost calculation

One AN bottle (marked price 3.07) (15 P for needle)	= £1.84 + 15P
Total AN bottles used	= 14528
Cost (Excluding 17.5% VAT, labour, media and other associated costs)	= £28910.70

Abbreviations

SGH	=	St. Georges Hospital
NHS	=	National Hospital Services
Paed	=	Paediatric
BC	=	Blood culture
CI	=	Confidence interval
AN	=	Anaerobic
+	=	Positive
Urosep	=	Urosepsis
Abdsep	=	Abdominal sepsis
SSI	=	Skin and soft tissue infections
Bilisep	=	Biliary sepsis
Line sep	=	Line sepsis
E. faecalis	=	*Enterococcus faecalis*
E. coli	=	*Escherichia coli*
Clostri.	=	*Clostridia*
Kleb	=	*Klebsiella*
CNS	=	Coagulase Negative Staphylococci
S. aureus	=	*Staphylococcus aureus*
S. pneumoniae	=	*Streptococcus pneumoniae*

Aknowledgements

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A STUDY TO EVALUATE TWO SAMPLING METHODS IN DETERMINING SURGICAL THEATRE AIR QUALITY

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Background

Health care-associated infections (HAIs) are an important cause of morbidity and mortality, prolongs hospital stay, increases antibiotic usage, and add costs. Surgical Site Infections (SSIs) are a very important topic of concern in the ever changing times of health care. There are countries and hospitals that have just begun to realize the importance of preventing and surveillance of SSIs while there are others who have reached heights in the implementation of protocols of Infection controls for HAIs in general and SSIs in particular, depending on the types of services the hospital renders.

Of the many inseparable contributing factors in SSIs, the air contamination in the operating theatre (OT) is an important factor that has called for the requirements of different air supply systems, cleaning protocols, use of surgical protective suites and limiting of individuals in the OTs. The use of antibiotic prophylaxis has contributed tremendously to the prevention of SSIs especially in the major surgeries and surgeries conducted in ordinary air supplies.

It is an important task for the clinical microbiology laboratory to monitor the quality of air in the surgical theatre. But controversy exists over the extent and frequency of microbiological surveillance of operation theatres. Evaluation of the quality of the air in the theatres can be performed routinely by microbiological sampling and particle counting as part of regular infection control programmes. These are especially required in the commissioning of the new operation theatres, investigation of clusters or outbreaks of infections or to validate a change in the ventilation system of the theatre.

Objectives

The objectives of the project were as follows:

1. To compare two methods of air sampling, the traditional settle plate method and a commercial slit-air-sampler.
2. To find the difference of air contamination in the theatre with laminar air flow (LAF) and the one with conventional (CV) ventilation.
3. To compare the air contamination in an empty OT before starting the list in the morning and towards the end of the morning list (during use), both in the LAF and CV theatres.
4. To determine any differences in contamination levels at different areas within an OT.
5. To determine the common bacterial species isolated from the theatre air.
6. To determine any surgical site infections in the operated patients till the time of discharge from hospital.

Study design and setting

This is a prospective study. The samples were collected from two OTs of Orthopaedic Unit of NHSL, one with LAF and the other with CV ventilation system. Sampling was done in the morning before start of the morning list (empty OT) and towards the end of the morning list. Sampling was done 8 weeks, two days a week (period sampling).

Methods and materials

Two methods of air sampling were used, the conventional settle plate method and a slit-sampler.

Settle plate method

Settle plate method was directly adopted from the method described in the Microbiology text book, Mackie and McCartney, Practical Medical Microbiology, 14th edition (1999), 15; 908, and reproduced below. The calculation of CFU/m^3 was adapted from M T parker (1978).

a) Blood agar was poured and dried of any surface moisture. All plates were marked for identification.

b) In the theatre the plates were uncovered in their chosen places and exposed by keeping 1 meter above the floor, 1 meter away from the side walls for 10 minutes, then at once the lids replaced.

c) The plates were then incubated aerobically at 37ºC overnight.

d) The colonies were counted, using hand lens when necessary. The colonies were identified using basic tests.

e) The number of colony forming units (CFU) per square meter per minute (CFU/m^2/min) is calculated to give the settling rate. Here the 90mm disposable plastic plates were used which gives a plate surface area of 63.6cm^2. From the number of CFU in a 90mm plate after 10 minutes exposure, the contamination level was calculated by using the formula of MT Parker (1978) to get the values of CFU/m^3.

Slit sampler

The slit sampler was used as per the descriptions in the 'User's Manual' for 'HiAirflow 90' of the product from HiMedia Laboratories, India described below.

a) The device was charged and cleaned with a disinfectant (70% alcohol). The head can also be sterilized, but sterilization was not done during my use.

b) From the control panel the air flow rate and the total volume to be sampled were selected. Here the flow rate chosen was 100L/min and the total volume sampled was 1000L or 1 m^3, therefore the sampling time is 10 minutes.

c) A 90 mm blood agar plate was placed into the head of the device and covered with the perforated cap.

d) The air was drawn through the perforated head at the set rate of sampling for 10 mins. The inflow air impacts on the surface of the medium and deposits the particles it carries.

e) The plates were incubated under similar conditions as for settle plate and the colonies counted and identified.

f) The most probable number of microorganisms in the volume of air sampled was calculated from the 'Conversion Table HiAirflow 90' given in the manual. (Appendix). This gives the number of CFU/m^3.

Results

The two methods of sampling show a distribution pattern in scatter plot, in a way that almost all high or low values in the sampler correlates with the settle plate values. The two methods showed moderate correlation by Pearson's correlation test but the correlation was highly significant. This indicates that, in the unavailability of a sampler the settle plate method may be a fair substitute. The OT with CV was found to be more contaminated when empty as well as during use, in both methods as expected. The differences in contamination between the CV and LAF theatre was found to be significant by the 't' test, by both the methods while empty and during use. Overall, the significance of the difference in contamination was higher when the sampling was done by the commercial sampler. This may mean that the sampler is more sensitive than the settle plate method in detecting the difference.

Difference in contamination before and after surgery is significant in both the theatres, but significance is less in LAF ($P = 0.027$ vs <0.001), which may mean that LAF theatres get less contaminated during use.

Contamination in different areas of CV theatre was more than twice that of LAF theatre. CFUs in corner was significantly higher than the table ($P < 0.05$) in both empty and end of sessions, but observed difference between corner and door, and table and door was not statistically significant ($p > 0.05$).

In both the theatres Coagulase Negative Staphylococcus (CNS) were the most common isolates followed by *Micrococci* spp, *Acinetobacter* Spp, aerobic spore bearers, Coliforms, *Staphylococcus aureus*, and *Candida* spp and *Aspergillus* spp were isolated on one occasion each.

No cases of SSIs were seen till the discharge/transfer out to other hospitals in the 48 patients operated during the air sampling in both theatres.

Conclusions

Comparing the two methods of sampling, the commercial sampler showed more consistent values but the settle plate method showed moderate correlation with the sampler values which was highly significant. This indicates that, where unavailable, settle plate can be a fair substitution for the sampler.

Both the LAF and CV theatre did not meet the requirements for empty theatre standards but met the in-use standards.

The CV is more contaminated than the LAF. The difference in contamination was more significant when sampling was done by sampler. This indicates that the sampler is a more sensitive method.

Contamination was higher after surgery in both OTs, by both methods and the differences were highly significant. But the significance is less in LAF. This indicates that LAF has lesser tendency to become contaminated during use.

Comparing contamination in different areas within an OT the corners were the most contaminated. CFUs in corners were significantly higher than the table. Observed difference between corner and door, and table and door was not statistically significant.

Limitations

One of the major limitations to this work was getting the samples in the empty theatre. Since the time between opening of the theatres and start of surgeries was very short I had very limited time to sample both the theatres within this short time. Several times, by the time I got to the theatre with my equipment, some people have already entered the theatre. Occasionally a theatre staff entered the room to drop or to pick up something urgently and this couldn't be controlled since they had to start work on time. All these could have possibly caused the failure of the theatres to meet the empty sampling criteria. The engineering specifications of the theatres used, especially of the conventional theatre was not known but I have applied the standards for the established conventional plenum ventilation systems.

Though the recommended time for duration of settle plate exposure is given as 10- 60 minutes in standard text books and guidelines, I had to choose the minimum time of 10

minutes exposure due to time constraints. I had only about 20 min in the morning before they started the surgery, so I couldn't follow the commonly used 1, 1, 1 method of settle plate. This was one of the main limitations to the study.

The follow up of patients for SSIs was done only till the time of discharge or transfer out to other hospitals. This didn't meet the definition of SSIs.

Recommendations

General

A need for classification and registry of ventilation types in all the hospitals in the country.

An intensive project involving the hospital authorities and concerned departments with an aim to develop a national guideline on theatre air monitoring with contamination standards specific to the type of ventilation systems should be carried out.

Theatre specific recommendations

Since both the OTs did not meet the empty theatre standards, both the engineering and infection control protocols may need a review, e.g. change of filters, servicing the air handling units, change in cleaning protocols etc.

Do not keep opened sterile equipments aside to the corner when not in use since the corners have been found to be the most contaminated within a theatre.

The doors of the operating theatres both in the conventional and laminar theatres were not strictly controlled during the operation. Putting this under good control can help maintain the quality of air in the rooms by maintaining positive pressure.

BOTRYOMYCOSIS

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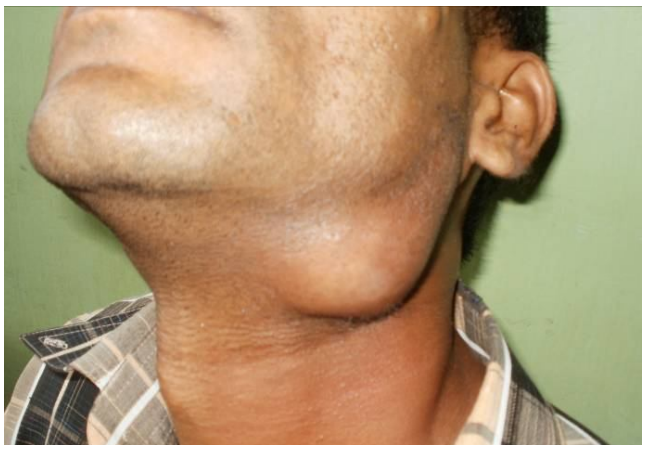
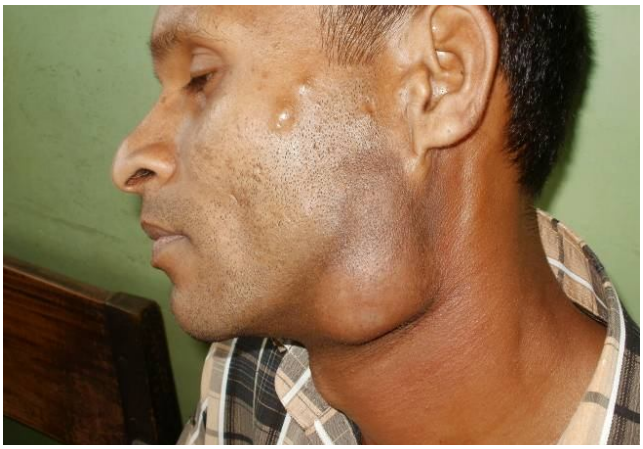
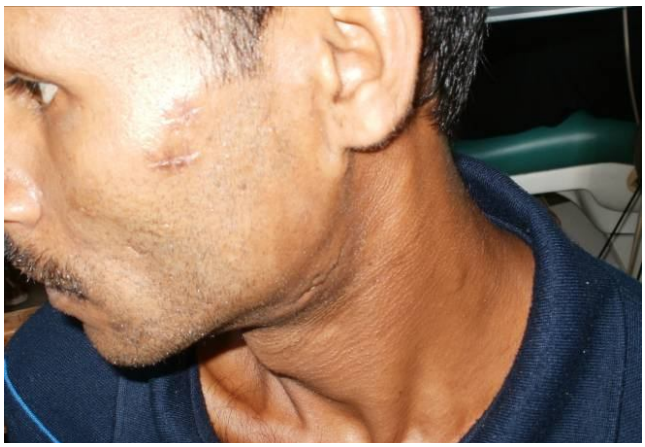
A thirty one year old soldier presented to the faciomaxilary (OMF) clinic with a painful swelling in the left submandibular region of 1 month duration. On examination it was tender, fluctuant and pointed swelling of 3 × 1.5 cm in size. There was a fracture in 1/16 of the same side with a missing molar due to a previous injury during weapon training in 2004. He had taken repeated treatment for chronic acne.

His fasting blood sugar was 5.5 mmol/L and total white cell count was 9310 /mm³ with 64% of neutrophils. He was started on amoxicillin 500mg 8h and cloxacillin 500 mg 6h. Over the next 48h his pain subsided but as the swelling persisted an incision and drainage was performed.







As the procedure resulted in drainage of pus containing brownish granules, actinomycosis was suspected and the consultant microbiologist's opinion was sought. Pus was collected in to a syringe and slides issued for crushing the granules. The granules were brownish yellow in colour, 1-2 mm diameter in size and friable. A tissue sample was sent for histology. Sample in the syringe was dispatched to MRI for anaerobic and aerobic cultures. Slides which contained crushed granules were stained with Gram stain and Kinyoun stain in the hospital lab. Gram stain showed large number of pus cells and Gram positive cocci in clusters. From the size and arrangement the cocci resembled *Staphylococci*. The Kinyoun stain was negative. Aerobic culture did not yield any growth even after enrichment.

Blood agar and Brucella blood agar plates incubated anaerobically showed growth after 7 days of incubation. Tiny, white, non haemolytic colonies, size of 0.5 mm were seen. The isolate was an anaerobic, Gm positive, non sporing, curved bacillus which showed branching and had a granular appearance. It was identified as *Propinobacterium acnes*.

A provisional diagnosis of botryomycosis due to *Staphylococci* and *P. acnes* was made and patient was discharged 3 days after incision and drainage, on oral amoxicillin and metranidazole.

The probable cause for the sterile aerobic culture was the administration of amoxicillin and cloxacillin prior to culture.

Discussion

Botryomycosis is a chronic suppurative infection which is characterized by chronic purulent and granulomatous lesions of dermis, sub dermal tissue and viscera in which the specific feature is the presence of fungus like grains or granules within the suppurative foci.

The disease was first described by Bollinger in the horse in 1870. In 1884 Rivoltar named this condition “botryomycosis” which was a misnomer thinking it was of fungal aetiology (1). The term botryomycosis is derived from the Greek word "botrys" (meaning "bunch of grapes") and "mycosis", due to the presumed fungal etiology in the early descriptions. In the 19th century more veterinary cases were reported. In 1913 Opie published the first human case which incidentally is the first visceral botryomycosis involving the liver (1,2). In the literature this condition is referred to as bacterial pseudomycosis, staphylococcal actinophytosis, granulobacteriosis and actinobacilliosis (2). Because of its rarity this condition is under diagnosed. Winslow noted in his paper which described 6 new cases of botryomycosis that these cases were initially diagnosed as mycetoma, Madura foot, actinomycosis or norcardiosis (2).

Among bacteria *Staphylococcus aureus* causes the majority of infections followed by *Pseudomonas aeruginosa* but *E. coli*, *Proteus* spp, coagulase-negative *Staphylococci, Streptococci, Micrococci* and anaerobes are reported. Among anaerobes *Propionibacterium acnes*, Peptostreptococci and variety of other organisms which cause anaerobic botryomycosis are described in the literature (3).

In this patient two organisms are involved in the etiology, namely *Staphylococci* and *P. acnes*. The co existence of chronic acne would have contributed to the onset of the disease.

P. acnes is a Gram positive non sporing anaerobic bacillus which forms part of the normal skin flora. Although it is of low virulence, it has been reported to cause botryomycosis of the liver [4]. It plays a significant role in the pathogenesis of acne and is a recognized agent of prosthetic valve endocarditis (4). As *P. acnes* may show branching filaments with a beaded appearance in the Gram stain, it may mimic the appearance of actinomyces in the direct smear.

Differential diagnosis of the patient includes actinomycosis and norcardiosis as these conditions also can give rise to chronic suppuration with discharging sinuses. The discharging pus contains characteristic granules commonly referred to as sulfur granules.

In March 2009, a diagnosis of botryomycosis was made in a 14-year old girl attending the OMF clinic at the G. H. Kegalle. Pus containing sulphur granules from a lesion in the mandibular region was sent to MRI as Actinomycosis was suspected. However the culture yielded *Peptostreptococcus prevotii* a known agent of botryomycosis.(6)

A similar case has been reported by Sivaraj et al in a 70-year old man who developed botryomycosis in the temporal region (7).

Diagnosis

High level of suspicion and awareness of this condition in chronic complaints help in diagnosis of botryo-mycosis.

Features which suggest botryomycosis include:

- identifying typical bacteria and grains in pus or biopsy specimens,
- culturing typical bacteria from biopsy specimens or swabs taken from ulcers or pus,
- identifying characteristic microscopic features of biopsy specimens.

Bacteria can be stained in tissue sections by Giemsa stain or Gram stain. Fungal stains like Gomori's silver methanamine or Girdley's stain are negative in these patients.

A nodular cellular infiltration is present in histology samples and multiple lobules or nodules are usually seen.

Within the centre of the nodules are a collection of polymorphonuclear leucocytes and possibly one or more "sulfur" granules. The biopsy classically demonstrates inflammatory nodule with a basophilic staining centre of bacteria and an eosinophilic periphery demonstrating Splendore Hoeppli phenomenon. It can be distinguished from other granular forming and Splendore Hoeppli phenomenon causing chronic infections by demonstrating that the basophilic centre is composed of bacteria which are not branching instead of filaments, broad hyphae or spores.

There are two main types, integumentry and visceral. The integumentry type can occur with or without musculo skeletal involvement. It can begin as osteomyelitis which progresses to a chronic stage with development of fistulas and sinuses. Lesions are more commonly seen in exposed areas like hands, feet and head. Friction, trauma or the presence of a foreign body can predispose to botryomycosis. Risk factors include immunosuppresion, alcoholism, HIV, chronic granulocytic disease, steroid therapy and diabetis mellitus. Most cutaneous cases lack a predisposing factor (1). Skin lesions can be multiple and may confuse with pyoderma vegetans.

The visceral type is commonly seen in the lungs and usually there is a history of immune suppression, cystic fibrosis or prior surgery. Liver is another frequent site but it is also reported from kidneys. Some of the visceral cases were fatal.

Pathogenesis of this condition is inconclusive. Some suggest that the essential factor is the delicate balance between the virulence of the infecting agent and tissue resistance of the host which has achieved a sort of symbiosis. Others have suggested relative decrease in the virulence of the organism and increase in the resistance of the host leads to this condition. Isolated organisms were of low virulence compared to their counterparts.

Management includes surgical debridement and long term antibiotic therapy for the causative bacterium. Antibiotics need to be continued for at least 6 weeks. Recently laser therapy was also reported for failure in antibiotics (8). Our patient showed improvement at the subsequent clinic visit in 2 weeks time. However amoxicillin was continued as the wound was not fully healed. After 6 weeks skin is normal with the incision mark clearly visible (picture 2).

Since botryomycosis also produce yellowish granules indistinguishable from actinomycosis or norcardiosis a high level of suspicion is necessary for diagnosis.

In conclusion, although botryomycosis is an uncommon disease, it is an entity which, microbiologists and histopathologists should be aware of so that it will not be missed.

On a personal note the authors would like to pay a tribute to their teacher and mentor, the late Dr. R. S. B. Wickramasinghe from whom they first heard of the term botryomycosis, which aroused their curiosity to learn more about this rare but interesting disease.

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ANTIBIOTIC USE IN ANIMAL HUSBANDRY AND ITS EFFECTS ON HUMAN HEALTH

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Antibiotics are used by doctors and veterinarians to treat and prevent bacterial infections in humans and animals. They have proved hugely successful in decreasing, considerably, morbidity and mortality due to communicable diseases However, with increased and often irrational use of antibiotics, antibiotic resistance has emerged and spread globally. This is due to the selection pressure exerted by antibiotics, resulting in survival of the 'fittest', i.e. survival of bacteria with wild type or acquired resistance mechanisms. Although we see the 'tip of the iceberg' in terms of antibiotic resistant, difficult to treat infections, there is also the underlying, hidden threat of increased resistance of microbes in the environment.

Antibiotics are not only used for therapeutic indications. They are also used in agriculture, industry and livestock production. In livestock production, antibiotics are used, not only to treat and prevent infections, but as growth promoters (AGP). AGP are believed to shorten finishing times, lower feed conversion rates, improve performance by promoting better condition and vitality in livestock, reduce death rates and reduce the need for therapeutic treatment. In many countries the amount of antibiotics used as AGP far exceeds that used for antibiotic therapy of humans and animals.

We are aware that bacteria rapidly develop resistance to antibiotics and that emergence of resistance is closely related to the amount of drugs used. Resistant bacteria can spread from animals to humans or resistance genes can spread from animal bacteria to human pathogens (1). Infections caused by resistance pathogens are difficult and expensive to treat.

So the question arises, "Does the use of antibiotics in agriculture and livestock production pose a threat to human health?". This threat could manifest in three different ways. Firstly, by increased resistance in well established zoonotic pathogens such as *Salmonella typhimurium*, *Escherichia coli* or *Campylobacter* sp. Secondly, by an increase in antibiotic resistance in commensal bacteria of animals, such as enterococci, that give rise to opportunistic infections in immunocompromised humans. Thirdly, by the spread of resistance genes from zoonotic flora to human bacterial commensals and pathogens.

The first scenario has already been realized with many reports of drug resistance in zoonotic pathogens, for example the emergence of fluoroquinolone resistance in Campylobacter species and, more recently, the emergence of multi-drug resistant *Salmonella* infections (2). The second scenario was exemplified by studies that contrasted the ecology of vancomycin resistant *Enterococci* (VRE) in Europe and USA (3,4). These studies demonstrated the presence of multiple clones of VRE in community and animal isolates in Europe, where a related glycopeptide, avorparcin, was used as an AGP, while in the USA, where avoparcin was not used, VRE were found almost entirely as hospital associated pathogens. Environmental studies on the ecology of VRE in Europe revealed the presence of such strains in farm animals, meat products and even sewage treatment plants (5). Avorparcin was exclusively used as a feed additive for animals and was registered as such in most countries, the USA being an exception. It appeared that the emergence of a few clones of hospital associated VRE in the USA was due to the increased use of vancomycin in US hospitals while the emergence of polyclonal VRE in animals and in community isolates in Europe was due to the use of avoparcin in livestock. This association was strengthened by studies conducted in Sweden (where the use of antibiotics as AGP was prohibited) which showed almost complete absence of VRE in the community, in contrast to almost all other countries in Europe where glycopeptides were used widely for nonmedical purposes (6). Similar associations between the use of antibiotics as AGPs and the emergence of resistance to related drugs in bacteria causing human infections were shown for enrofloxacin and fluoroquinolone resistant *Salmonella* and tylosin and erythromycin resistant *Staphylococci*. However, the evidence was not conclusive.

In view of this uncertainly a study was done that extensively reviewed the risk assessments available. It concluded that "of concern is the fact that the risk assessments reviewed in this report consider only a few narrow and specific clinical outcomes, without consideration of the more general (and probably more significant) outcome of a shift toward more resistant bacterial populations" (7).

In spite of the lack of conclusive evidence to directly implicate the use of AGP as a cause of antibiotic resistance, the WHO, in a fact sheet in 2002, stated that the "majority of the rising antimicrobial resistance problem in human medicine is due to the overuse and misuse of antimicrobials by doctors, other health personnel and patients. However, some of the newly-emerging resistant bacteria in animals are transmitted to humans; mainly via meat and other food of animal origin or through direct contact with farm animals. The best-known examples are the food-borne pathogenic bacteria *Salmonella* and *Campylobacter* and the

commensal (harmless in healthy persons and animals) bacteria *Enterococcus*. Research has shown that resistance of these bacteria to classic treatment in humans is often a consequence of the use of certain antimicrobials in agriculture……..." (8).

As a result of such reports, a complete ban on use of antibiotics as growth promoters in food producing animals was implemented in the European Union from 2006. Although there were concerns that this ban could affect the livestock industry and the food supply, subsequent studies showed that there was no effect on the efficiency of food production, animal health, food prices or food safety after implementation of the ban.

However other authorities disputed these findings. They contended that such a ban could be effective in developed countries where farmers were more educated, good practices in animal husbandry were followed and high hygienic practices were implemented. They suggested that in developing countries, such a ban could lead to worsening animal health, decreased production efficacy, and increased cost. Also there was still no conclusive evidence that such bans could lead to a decrease in the emergence and spread of antibiotic resistance. In fact they predicted that such a ban could lead to increased disease in livestock, paradoxically increasing the therapeutic use of antibiotics identical to those used in human infections, thus worsening the problem. They predicted that meat would become more expensive and that consumption of unhealthy food would lead to more infections and higher antibiotic use in humans. They also cast doubt on the contribution of AGP to antibiotic resistance, maintaining that resistance is mainly driven by increased antibiotic use in humans, that animal strains do not easily colonise humans, that colonisation does not equal infection and that adequate cooking of meat is effective in destroying bacteria.

One worker in this area was confident enough to state that, *"All the facts at our disposal persuade us that whereas resistance is undoubtedly selected in man and animals by the use of antibiotics, in organisms that are part of the normal flora as well as in pathogens, including zoonotic pathogens, and whereas some resistant organisms can be shown to reach man via the food chain, little additional harm results from resistance, even when infection supervenes. Only in the case of Salmonellae and Campylobacters do risk analyses, albeit still hampered by a lack of data, suggest that resistance possibly acquired in animals may add, albeit very little, to the burden of human disease" (9).*

In 2002 a scientific panel was convened in the USA, the Facts about Antimicrobials in Animals and the Impact on Resistance Panel (FAAIR Panel) to review all the available scientific and medical evidence (FAAIR) in relation to the subject (10). Its conclusions were as follows:

- All uses of antimicrobials in animals, agriculture, and humans contribute to the global pool of antimicrobial resistance genes in the environment.
- Antimicrobial resistance in pathogenic bacteria limits treatment options; raises health care costs; and increases the number, severity, and duration of infections.
- Commensal bacteria also contribute to the antimicrobial resistance problem by serving as reservoirs of resistance genes transferable to pathogenic bacteria.
- It is estimated that, in the United States, the amount of antimicrobials administered to animals is comparable to that used in humans. In contrast to use in humans, much of the antimicrobial use in food animals consists of administration to large groups for nontherapeutic applications, such as growth promotion and disease prevention.
- Antimicrobial use in food animal production selects for resistant strains and amplifies their persistence and dissemination in the environment.
- Transfer of bacteria from food animals to humans is a common occurrence.
- Use of antimicrobials in food animals contributes to the growing problem of antimicrobial resistance in animal and human infections.

The Committee concluded that the elimination of nontherapeutic use of antimicrobials in food animals and in agriculture would lower the burden of antimicrobial resistance in the environment, with consequent benefits to human and animal health.

The FAAIR Panel went on to recommend that antimicrobials should not be used in agriculture in the absence of disease, antibiotics should be administered to animals only when prescribed by a veterinarian, quantitative data on antimicrobial use in agriculture should be made available publicly, the ecology of antimicrobial resistance should be considered by regulatory agencies in assessing human health risk associated with antimicrobials use in agriculture and that surveillance programmes for antimicrobial resistance should be improved and expanded. It suggested that the ecology of antimicrobial resistance in agriculture should be a research priority (11).

Although USA has still failed to implement such a ban, farmers' organizations, such as the Responsible Use of Medicines in Agriculture Allliance (RUMA), uses self-regulation to limit antibiotics in animal husbandry with the slogan, "As little as possible but as much as necessary".

In Sri Lanka four antibiotics namely, avilamycin, virginiamycin, zinc bacitracin and flavophospholipol are registered for use as AGP. Many livestock farmers also admit to misusing therapeutic antibiotics such as chlorotetracycline, amoxicillin and furazolidone for this purpose. The contribution of this to the increasing rates of antibiotic resistance in bacterial isolates in this country is unknown.

It is time for Sri Lanka to think of abolishing AGP and reducing the therapeutic use of antibiotics in animals by promoting other livestock measure such as improved feeds, in-feed enzymes, probiotics and prebiotics, organic acids, improved animal husbandry, hygiene, disinfection, vaccination, nutrition, changes in stocking rates, and rational prescribing as practiced in many enlightened developed countries.

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FUNGI AND FUNGAL INFECTIONS – THE GOOD, THE BAD, THE UGLY

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The President of the Sri Lanka College of Microbiologists, Mrs. Ranganie Wickremesinghe, family members and friends of the late Dr. Wickremesinghe, members of the Council, members of the Sri Lanka College of Microbiologists and distinguished invitees.

I am honoured to be invited to deliver the 2011 Siri Wickremesinghe oration today in memory of a renowned and scholarly microbiologist, to talk on some aspects of his life and his influence on my career as an academic and a mycologist. I thank the President and the Council of the Sri Lanka College of Microbiologists for inviting me to do so.

In 1994, when Dr. Wickremesinghe was the President of the Sri Lanka College of Microbiologists, his two secretaries were Dr. Pranitha Somaratne and myself. Fate has brought the two of us together today, to commemorate the third one. Dr. Somaratne as the President of the College and I am here to deliver the Siri Wickremesinghe memorial oration.

The contents of this oration are in two parts. The first part is all about Dr. Wickremesinghe; the background information on him and my association with him as a student where he was our beloved teacher and a role model to us Diploma Trainees in Microbiology from 1986 to 1988.

In the second part of this oration, I will speak on aspects of fungi and fungal infections to which I have contributed as an academic and a mycologist. It will cover the good, the bad and the ugly aspects of fungi as well as some innovative research on fungi and the mycology diagnostic service in the Department of Microbiology, Faculty of Medicine, Galle where I work.

Rakkitha Sirimal Wickremesinghe was born on 28th November 1937 to Dr. Artie and Helen Wickremesinghe. He had his education at Royal College Colombo and obtained his MBBS in 1963 from the Faculty of Medicine, Colombo. He started his medical career in the field of dermatology which we understand was his first love and worked in the Dermatology Unit at Kandy. Thereafter he joined the Medical Research Institute (MRI) where he fell in love with microbiology, perhaps his second love. To further his postgraduate education 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.

Throughout his career he dedicated the major part of his life to the life and soul of the MRI where he always belonged. Even though he worked as a successful microbiologist in Fairfield hospital in Australia, he came back to his roots in Sri Lanka and to the MRI and worked as a consultant microbiologist in charge of the bacteriology division until his retirement. Perhaps this was his third love; love for his country and love for the MRI. He was the Director of MRI from 1996 to 1998. After retirement he took up the challenging post of Resident Pathologist and Laboratory Manager at Durdans Hospital, Colombo.

Dr. Wickremesinghe was actively involved in the Sri Lanka College of Microbiologists and was a member of the Council even at the time of his death. During his career as an eminent microbiologist he was very much interested and concerned in the ever increasing problem of antimicrobial resistance and together with the WHO was in forefront of many workshops and seminars conducted to seek remedial measures to alleviate this global problem.

Dr. Wickremesinghe was a microbiologist with wide experience, having worked in laboratories overseas that included Addenbrooks at Cambridge, a CDC-linked laboratory in Michigan and Fairfield Hospital in Melbourne. An erudite scholar he was a walking encyclopaedia with in depth knowledge on many a subject. History was his forte and he could talk on any period of history with vivid descriptions and analyze political issues with an experienced balanced view.

He was a long standing member of the Health Department Sports Club taking part in cricket, tennis and billiards. He made his mark in whatever he did. Dr. Wickremesinghe was a great entertainer too. He and Ranganie accompanied us on many College trips. His anecdotes and stories had us doubling up with laughter and delight. He had a way of saying things with a poker face which added much weightage to his remarks.

An avid nature lover his most outstanding passion was turtle conservation to which he gave his utmost by establishing the Kosgoda turtle hatchery. As a tribute to his late father, Raki Wickremesinghe, has initiated and made available the Kosgoda turtle Hatchery for ongoing undergraduate research conducted by the 4th year students of the Faculty of Fisheries and Marine Sciences and Technology, University of Ruhuna, under the guidance

and supervision of Professor Ruchira Cumaratunga. The research is in the field of turtle genetics.

There were 7 of us who enrolled for the 1st Diploma in Microbiology of the PGIM in 1986. Two of us who joined as private candidates were assigned to the Microbiology Department of the Faculty of Medicine, Colombo. All my other colleagues were being trained at the MRI by a teacher who was devoting much of his time to their training. We too wanted to be a part of this training and we walked into his class one day and that was my first glimpse of him. I was very much impressed listening to him rattle off on some topic, without any teaching aids. Many of you will have an image or an idea what their role model of a teacher is. This is my role model and these points emphasize what Dr. Wickremesinghe was to us as PG trainees. He was able to motivate me and the rest of us to do our very best. He taught us with much enthusiasm and conviction. He was stern, gave us reading assignments, questioned us at every turn and was critical when we faltered. At the same time he was not reluctant in his praise, gave positive feedback to encourage us sometimes in the jargon of cricket language.

As a university academic, I completed a course for the Accreditation of Senior Teachers in Higher Education conducted by the Staff Development Centre of the University of Colombo in 2002. This is what I wrote in my portfolio about the best teacher I ever knew. "One of the teachers in this course took a personal interest in all of us and was the driving force behind us, giving us tasks and tutorials and conducting interactive sessions and mock examinations. The teaching learning activities that he deployed were some of the most effective methods that created the learning environment for active learning to take place" (1).

I am certain that it was Dr. Wickremesinghe who influenced me to take up a career as a teacher and as a mycologist. His manner of teaching to motivate students to learn was exemplary. Dr. Wickremesinghe's interest in dermatology led him to teach us mycology since he was interested in fungi and fungal infections. He taught us and we learnt quite a lot on fungi and fungal infections. During this time he had got a teaching microscope and we had many sessions with him when he would show us interesting slides and ask us to identify them. I became fascinated with fungi which were not difficult to handle; no special media were required for isolation and they have such beautiful colours. It was then that I decided to specialize in mycology.

During my academic career, till his untimely death, I would approach Dr. Wickremesinghe many a time to seek his advice and he reciprocated with sound advice, books to refer to and tongue in the cheek statements. He had an excellent collection of books some of which he kept at MRI and were accessible to us. When I was the President of the Section A of the Sri Lanka Association for the Advancement of Science (SLAAS), his support to my Presidential Address was immense and he was gracious to be present at the occasion (2).

From now on I hope to capture your attention on the many aspects of fungi and fungal infections where I have contributed in some way to their diagnosis and management, initially as an academic in the Faculty of Medicine Colombo and later in the Southern Province when I moved to the Faculty of Medicine in Galle. It will comprise of the good aspects of fungi, the bad and the ugly and on the service commitments in mycology.

Fungi are ubiquitous – they can be found everywhere, in the air, in the swimming pool, in plants and animals, as well as in our bodies. The vast majority of them are beneficial to humans. The usage of fungi for food preservation or other purposes by humans are wide-ranging and has a rich history. For example, yeasts are used to ferment beer and wine; while *Aspergillus oryzae*, is used in the production of soy sauce. *Saccharomyces cerevisiae* – baker's yeast is used in the baking of bread and other wheat-based products such as pizza and dumplings. Fungi give cheese its distinctive flavour (3).

Fungi decompose organic matter and recycle nutrients. Soil bacteria and fungi play a central role in almost all aspects of organic nitrogen availability for plant growth, and so are a part of the "living nitrogen cycle" that supports life on earth. Scientists have found that free-living and plant fungi can, "colonise depleted uranium surfaces and transform the metal into harmless uranyl phosphate minerals". This is an important finding to clear uranium left over after usage of weapons of war. Fungi are utilized by humans to produce many industrially and medicinally important compounds. Antibiotics such as penicillin, griseofulvin, cephalosporin are produced from *Penicillium* species and other fungal species (4).

For the bad aspects of fungi and fungal infections I have taken some case reports of opportunistic fungal infections where the department of Microbiology in the Faculty of Medicine Galle was involved with the laboratory diagnosis.

The opportunistic mycoses are infections due to fungi with low inherent virulence and which are common in all environments. These fungal infections of the body occur almost exclusively in debilitated patients whose normal defense mechanisms are impaired. Important factors contributing to these infections are aggressive cancer and post-transplantation chemotherapy and the use of antibiotics, cytotoxins, immunosuppressives, cortico-steroids and other procedures that result in lowered resistance of the host.

This was a farmer from Akuressa with painful ulcers and occasional bleeding from upper alveolar region of the oral cavity for two months. A granulomatus ulcerative lesion was seen on the upper alveolar region and hard palate. An incisional biopsy was taken for histology and culture. The isolate on culture was confirmed as *Histoplasma*

capsulatum. Oral itraconazole 400mg daily was commenced. After one week of treatment, liver functions were deranged and antifungal doses were adjusted. However the patient died two weeks later in a medical ward due to liver failure (5).

A 53-year old female in ward 35 of TH Karapitiya with diabetic ketoacidosis of two months was admitted with fever and diagnosed as left upper lobe pneumonia. X ray findings showed a cavity with a halo sign. She was treated with IV antibiotics with no response. Sputum from this patient showed broad aseptate hyphae on microscopy and the isolate on culture turned out to be a mucor species which was later speciated as *Mucor hiemalis*. The patient improved when her diabetes was brought under control.

There were 10 cases of fungal sinusitis where microscopy was positive and *Aspergillus flavus* (4) and other Aspergillus species were isolated. Of these, one case especially which followed me from Colombo to Galle is noteworthy. He is a 28-year old male who underwent nasal polypoidectomy and was referred to the dermatologist for a swelling on the forehead with a discharge. The discharge showed broad aseptate hyphae and the culture was *Aspergillus flavus*. This was the start of a long history of lab investigations and treatment of fungal sinusitis with amphoterecin, itraconazole almost spanning more than 5 years. Every time we investigated him, microscopy and culture was positive for *Aspergillus flavus* and he was treated with antifungals. He underwent several surgical procedures some even in India but to no avail. For the past 3 years he has not come to our lab and I presume he is now cured.

Two case reports are shown. A 3-year old child suspected as being immune compromised was admitted to the paediatric ward for investigation. The child had an extensive superficial skin infection. The lesions were distributed over the trunk, buttocks and groin areas and were dry, circular hypo-pigmented patches with minimal erythema. Sellotape mounts and a skin scrapping was taken. Microscopy revealed hyaline, septate fungal hyphae with arthrospores. On culture a Trichosporon species was isolated. Trichosporon species was also isolated on two occasions from the urine in a patient who had bilateral stents inserted for acute renal failure due to kidney stones. This patient was followed up in TH Karapitiya, after surgery was done in NHSL. He had haematuria and fever with chills and rigors at the time of examination of urine. He was treated with itraconazole and a repeat urine specimen was clear of trichosporon species.

A 36-year old female was investigated for nasal obstruction of the right side for two months with rhinorrhoea, which was occasionally blood stained. She was a house wife and was working in a tea factory for the last two and half years. On examination a polypoidal, granulomatous growth was detected in the region of the inferior turbinate. The specimen sent to us from the growth that was removed was processed and microscopy showed broad, flat, sparsely septate, hyphae. The macroscopic and microscopic appearances of the culture were consistent with those of *Conidiobolus coronatus*. The removal of the growth temporarily relieved her nasal obstruction. She was treated with ketaconazole and was followed up in the clinic (6).

A 28-year old female from Trincomalee, who presented with a lesion on the nasal septum with pain and bleeding from the right nostril for 1 year was seen in the ENT clinic. A biopsy from the mass was sent to us. Microscopic examination with KoH showed giant sporangia filled with endospores. Surgical removal was undertaken. As expected the culture was negative since this was a case of rhinosporiodisis.

For the ugly, I have chosen some areas of research and case studies involving supeficial and subcutaneous fungal infections. Superficial fungal infections of the skin, hair or nails are restricted to keratinised tissue and no living tissue is invaded. However a variety of pathological changes occur in the host because of the presence of the infectious agent and its metabolic products. The subcutaneous infections on the other hand involve invasion of living tissue resulting in chronic, localized infections of the skin and subcutaneous tissue following the traumatic implantation of the aetiologic agent. The causative fungi are all soil saprophytes of regional epidemiology whose ability to adapt to the tissue environment and elicit disease is extremely variable.

The purpose of this study was to investigate the prevalence of *Tinea capitis* in schools in south east London and factors which might affect the spread of infection. A survey was done of all children for scalp examinations and brush sampling, in 14 nurseries, infant and junior schools in Lambeth. The accuracy of clinical diagnosis was compared with mycological findings. There were 1047 children from 4 to 14 years. The mean infection rate was 2.5%. A further 4.9% were scalp carriers of dermatophytes. All infections were caused by anthropophilic fungi mainly *Trichphyton tonsurans* and *Microsporum rivalieri* (7).

In contrast, in a similar survey done in Galle, there were no cases fitting into typical clinical diagnosis of *Tinea* capitis. The sample group included 424 children in the age group of six to twelve years. There were 99 (23.3%) children having widespread scaling with thin, white loose scales. In the study group dermatophytes were not isolated from any of the samples and 327 (77%) were culture negative, indicating neither clinical disease nor carriage of dermatophytes. This finding agrees with the previous studies done on *Tinea capitis* in Sri Lanka, from the results of this study, we can conclude that the prevalence of *Tinea capitis* in Galle Municipal area is non existent (8).

Forty specimens from suspected fungal scalp infections referred by the dermatologists in Galle were examined

in our department. Of these there were 28 cases of kerion, the type of scalp infection that may be misdiagnosed as a bacterial infection due to its boggy, suppurative appearance. They were diagnosed by direct microscopy and culture. Significant isolates were recovered from 19 specimens. All except one were from children ranging from 3 to 10 years. The exception was of a 42-year old male. *Trichophyton mentagrophytes* var mentagrophytes was the cause of 9 cases and *M. gypseum* accounted for 6 cases.

This was a prospective study which included 44 corneal scrapings from patients with long standing corneal ulcers in ward 02 of the eye Hospital, Colombo and 13 corneal buttons removed from patients undergoing keratoplasty in Teaching Hospital, Karapitiya, microscopy was positive in 21 specimens (37%). The species of fungi isolated in our study conform to those isolated world wide and in this country. One unusual isolate was *Cylindricarpon lichenicola*, reported in 2 other studies (9).

This study was undertaken to determine the prevalent species of fungi causing otomycosis in two centres: Kandy and Galle. 71 ear swabs collected from patients with clinical evidence of otitis externa were processed for evidence of fungi; 34 from Kandy and 37 from Galle. 79% were positive by microscopy and culture for fungi. *Aspergillus niger* and *A. flavus* were the predominant isolates together with *Candida albicans* and non albicans sp. Conclusions were that positive microscopy is essential for laboratory diagnosis, *Aspergillus niger* is the most common isolate in otomycosis and the positive predictive value of a clinical diagnosis in otomycosis is 77% (10).

This study was done to determine the species of fungi causing mycoses of the glabrous skin of adults visiting the dermatology clinic in NHSL. 200 patients referred from this clinic to the laboratory of the Dept. of Microbiology Faculty of Medicine, Colombo were the study population. Among the dermatophytes, *Trichphyton rubrum* accounted for 73% of the isolates. Other isolates of *E. floccosum*, *T. mentagrophytes*, *M. gypseum* and *T. simii* were seen. *Candida albicans* and other candida species were isolated from the interdigital space infections. Four of the 11 patients screened for *Pitiriasis versicoloor* were positive by microscopy for *Malassezia furfur* (11).

A multicenter prospective study was undertaken to isolate and identify fungi causing nail infections in Sri Lanka. Nail specimens were collected from patients referred to the Dept. of Microbiology, FoM, Colombo. Specimens from 5 other provinces were sent by post. One hundred samples from 6 provinces were analysed. 63% were positive by microscopy in which 44% were filaments and 19% were yeasts. All the significant isolates (60) were non dermatophyte molds and yeasts. There is a remarkable difference in the aetiology of fungal nail infections in SL when compared to temperate zones in that almost all isolates were non dermatophytes. These findings exclude the use of griseofulvin as a first line drug in the treatment of onychomycosis (12).

This was a 36-year old police officer with a recurring infection of the toe web spaces of the right foot. He has had several episodes of infection within last 2 years and had been treated with antifungal agents. On examination of the right foot, the toe web spaces were inflamed and macerated with a watery discharge. Microscopy showed septate, hyaline fungal filaments with arthrospores. The culture appeared as a dark grey floccose colony covering the agar surface and touching the lid and was identified as *Scytalidium dimidiatum*. The patient was treated with oral itraconazole 200mg BID for 7 days and was followed up in the clinic (13).

2 case reports: one of eumycetoma, the other actinomycetoma is shown. The first was of a 38-year old male from wd 22 in TH Karapitiya who had a soft tissue lump on the dorsum of his left foot. A biopsy from the swelling was sent. Microscopy was inconclusive but a black dematiaceous colony grew on SGA after 3 weeks. Since we could not identify it, the culture was sent to Public Health Lab in Bristol UK and it was identified as *Madurella grisea* and added to their collection as NCPF 7384.

The second case is of a A 65-year old man in SJGH in May 2007 with extensive ulcerative and nodular lesions around the R knee with multiple discharging sinuses. Biopsy specimens were processed but were negative. The histopathologist found a typical grain in the H&E slide and the diagnosis of mycetoma was made. He also provided us with the biopsy sections in wax on slides which were dewaxed and stained first by the Gram stain and then by the acid fast stain. The Gram stain showed Gram positive filamentous bacteria and the AF stained smear showed acid fast filamentous bacteria which was concluded as a possible Norcardia species and a diagnosis of actinomycetoma was made (14).

Not only do humans suffer from ugly fungal infections but animals do too. Infective corneal diseases are common among domesticated elephants in SL causing much morbidity such as blindness. From 140 animals of different ages and sexes, 36 had corneal opacities, ulcers and foreign bodies. Nine had lesions in both eyes (6.4%). Specimens were obtained from 26 animals with corneal ulcers for bacterial and fungal microscopy and cultures. Specimens were also taken from a control group of 20 elephants who had no eye disease. Bacteria were isolated in 60% and fungi from 40% of the specimens. (15).

Two innovative research studies conducted in the departments of Microbiology, Faculties of Medicine in Colombo and Galle are shown. The first is the evaluation of the efficacy of fresh lime juice, *Cassia alata* leaf, lime juice with CA leaf, and various soaps in inhibiting dermatophyte growth in vitro. Miconazole and econazole discs were used as positive controls. There were definite

zones of inhibition of fungal growth around lime juice, lime juice with CA leaf pulp, all the soaps tested and the control discs. There was no inhibition around the well containing leaf pulp only. We concluded that lime juice (pH 2.32) and soaps (pH 10) tested have antifungal properties against dermatophyes in vitro which may be due to the fact that dermatophytes favour a pH of 5.5 for growth. This substantiate the age old custom of cleaning toe webs with lime to prevent fungal infections of the feet (16).

Microscopic examination is an important step in the laboratory diagnosis of infections. For this, collection of a good and relevant specimen is of utmost importance. This is especially evident in the diagnosis of onychomycosis where collection of samples from dystrophic and hyperkeratotic nails is a tedious task. The conventional methods do not yield good specimens from such nails commonly seen among agricultural workers. A technique was developed to get better nail samples from dystrophic nails using discarded microtome blades used for histopatholgy sections. Holding the blade parallel to the nail, the nail was gently sliced from proximal to distal end to obtain extremely thin shavings of the nails by the technique shown. The important advantage was the increased sensitivity of the test method when compared to the conventional method (17).

After moving to Galle in 1997, and from 1998 onwards, the Department of Microbiology, Faculty of Medicine, Galle was able to establish a diagnostic service in mycology for the Southern Province. With the support of our clinical colleagues, Dr. Choolaratne and Dr. Ramya Ragunathan, Consultant Dermatologists, Dr. Ravi Ruberu, ENT Surgeon and other physicians, the laboratory processed many specimens seeking to establish a fungal aetiology for these diseases. A total of 2208 specimens for mycological diagnosis were processed till April 2011 in the laboratory of the department. An analyisis of these specimens is shown here.

Ladies and gentlemen, we are gathered here today to pay tribute to the late Dr. RSB Wickremasinghe, who was a beloved and dedicated teacher, advisor and colleague, a competent microbiologist, erudite scholar, a man of wisdom, a keen sportsperson and above all a lover of nature.

His love of nature and explorers instincts took him to many parts of the world; to England, home of Sherlock Homes in Baker Street, to Thailand, Indonesia, Africa and Egypt. He visited the golden triangle in India in 2001 with Ranganie and Raki and this was his last trip in this lifetime.

It was during my tenure as the President of the Sri Lanka College of Microbiologists in 2003 that Dr. Siri Wickremesinghe left us forever.

Ladies and gentlemen, let me finish this oration by repeating the eulogy read by me, as the then President of the College, on that fateful day in the chapel where his remains lay. It epitomizes all what he was to us his students, his dear friends and colleagues. "To Sir with love; As a long standing member, a Past President and a Council Member for many years, you have been a stalwart and a pillar of strength in the Sri Lanka College of Microbiologists. Not only have you strengthened our knowledge of microbiology and medicine, given us inspiration and motivation to achieve our goals, but you have also enlightened us many a time with your anecdotes, explicit details of history and art and the mysteries of nature and science. All your dear friends, colleagues and pupils in the College miss your presence very much, especially at our academic and social functions where you always were an active and a lively participant. May God grant you eternal rest".

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The staff of the Department of Microbiology, FoM, Galle.

The staff of the Department of Microbiology, FoM, Colombo.

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