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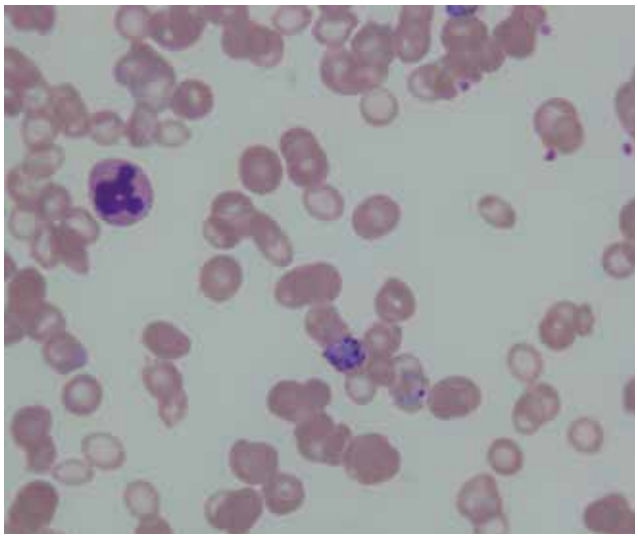

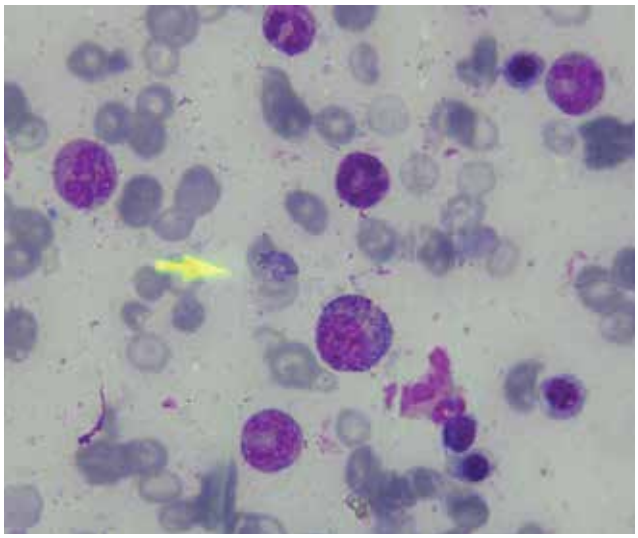

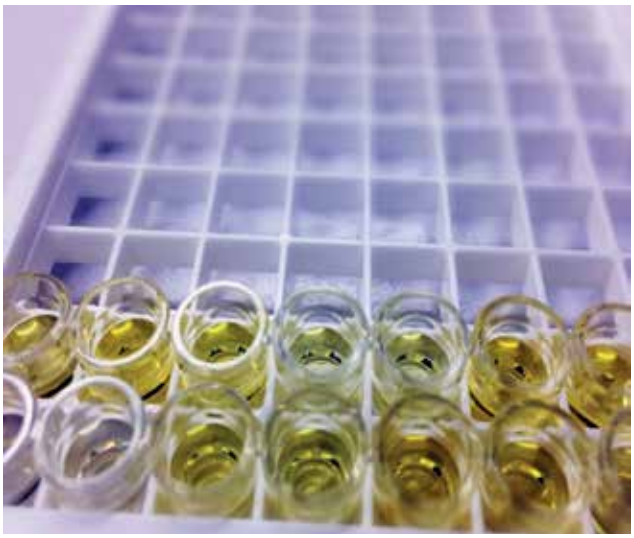

آغا خان یونیورسٹی ہسپتال، کراچی

The Aga Khan University Hospital, Karachi

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COLLEGE of AMERICAN PATHOLOGISTS

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Invited Editorial

The pressing global concern of emerging antibiotic resistance gripping masses and will affect millions in future. Among several factors, accurate disease diagnosis is an important cog in the machinery driving antimicrobial resistance.
Keeping the context in consideration, as well as the theme for this year's National Health Sciences Research Symposium, i.e. AMR, the theme for this LABRAD edition is "Accurate disease diagnosis and impact on Antimicrobial resistance (AMR)".
In both paths i.e. laboratory and imaging, diagnostic modalities are available at Aga Khan Hospital laboratory and radiology department which delineates infectious from non-infectious component, guide therapeutic response, highlight variable pathogenic process.
Our readers will find this edition very helpful by reading not only about conventional methods but also some novel diagnostic methods helpful in identifying infectious disease diagnostic process.
"Diagnosis is not the end, but the beginning of practice" – by Martin Fischer

Happy reading
Dr. Mohammad Zeeshan
Microbiology

Therapeutic Drug Monitoring for Antibiotics in Infections

Dr. Siraj Muneer
Clinical Chemistry

Despite advances in contemporary medicine, severe infections and sepsis-related mortality in critically ill patients remain a global problem. Appropriate, timely antibiotic therapy given at an adequate dose is thought to be of paramount importance in improving clinical outcome of severe sepsis. An important consideration of antimicrobial treatment failure in the critically ill is inadequate or sub-therapeutic drug exposure.

This can be a direct consequence of pharmacokinetic alterations due to the complex pathophysiologic processes associated with severe infection.
Multi-drug resistant organisms more frequently encountered in the critically ill also alter the dosing requirements for these patients. Therapeutic drug monitoring (TDM) is defined as the laboratory measurement of drug serum concentration along with adequate medical interpretation influencing the management of drug therapy in patients.

TDM, has significance in improving patient outcome and is utmost important in drugs with narrow therapeutic index, drugs with high pharmacokinetic variability, is being increasingly used for antibiotic dose optimization in the attempt to improve attainment of pharmacokinetic/pharmacodynamic (PK/PD) targets and outcomes of severe infections in the critically ill. TDM is a three step process including:

(1) The precise and reliable measure of the plasma concentration of a specific medicine;
(2) The interpretation of the obtained concentration value according to the knowledge on the concentration-effect relationship,
(3) The calculation and proposal of an individual dose adjustment for that specific patient.

The appropriate indications for TDM include:

1. Avoiding toxicity
2. T adjust dose
3. To monitor patients for ineffective drug treatment, non-compliance

In TDM, therapeutic range is used as a guide for optimal drug concentration in serum, but this range should not be considered as an absolute one. In certain patients, drug effect will be evident even in case of too low serum concentration, whereas other patients will experience toxic effects, when drug serum concentration is even in therapeutic range. Hence, dosing of drugs should not be led only by serum drug concentration, but by its clinical response as well.

Table1: Therapeutic drug monitoring for antibiotics

Drug	Therapeutic Range	Sample Timing	Sample type	Sample stability	Toxicity
Amikacin	**Once daily dose[a]** Trough = <1.0 ug/ml Peak = 56-64 ug/ml **Multiple daily dose[a]** Trough = 5-10 ug/ml	Conventional dosing Peak: 60–90 min after i.m injection 30–60 min after i.v. infusion Trough: within 30 min of next dose	Serum; EDTA plasma	2 h room temperature; freeze	Nephrotoxicity Ototoxicity (Toxic effects on the eighth cranial nerve can result in hearing loss, loss of balance, or both)
Gentamicin	**Adults Multiple daily dose[b]** Peak: 4-10 ug/ml Trough: 1-2 ug/ml **Adult Once daily dose[b]** Trough <1 ug/ml **Pediatrics trough conc[b]** <2ug/ml				
Vancomycin	**Therapeutic concentration level[c]** >10 ug/ml **Complicated & Serious Infections[c]** 15-20 ug/ml	Trough: within 30 min of next dose (should be obtained prior to the fourth or fifth dose).	Serum; EDTA plasma	2 h room temperature; freeze	Nephrotoxicity

Reference:
a: Sanford Guide to Antimicrobial Therapy (44th Edition 2014)
b: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, fifth edition
c: Therapeutic monitoring of Vancomycin in adult patients: A consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists', Am J Health-Syst Pharm. 2009; 66:82-98

Biomarkers of Sepsis

Dr Syed Bilal Hashmi and Ms Sana Arshad Mehmood
Clinical Chemistry

Sepsis is a clinical syndrome that has physiologic, biologic, and biochemical abnormalities caused by dysregulated inflammatory response to infection. It is caused by microbial invasion from a local infectious source into the bloodstream which leads to systemic inflammatory response syndrome (SIRS). The diagnosis of sepsis with high sensitivity and specificity is difficult, due to unspecific manifestations and biochemical similarities to noninfectious systemic inflammatory conditions. Early diagnosis of sepsis is necessary for earlier intervention successful management and better patient outcome.

Criteria for sepsis are considered to be met if at least two of the following four clinical findings are present:

- Temperature higher than 38°C (100.4°F) or lower than 36°C (96.8°F)
- Heart rate (HR) higher than 90 beats/min
- Respiratory rate (RR) higher than 20 breaths/min or arterial carbon dioxide tension (PaCO2) lower than 32 mm Hg
- White blood cell (WBC) count higher than 12,000/µL or lower than 4000/µL or with 10 percent immature (band) forms

Table 1: Characteristics of ideal sepsis biomarker.

Role of Biomarkers	Requisites for useful Biomarkers
Screening patients at risk of sepsis	Objectively measured
Establish early diagnosis that helps the initial management of sepsis	Have reference standard
Risk stratification to identify patients at risk of poor outcome	Have well-known kinetics
Monitoring the response of intervention	Reproducibility of test
Predict outcomes	Cost-effectiveness

Ref: Infect Chemother 2014;46(1):1-12

Biomarkers of Sepsis: A few biomarkers which are well established for their use in sepsis include CRP, ESR and Procalcitonin. Multiple studies have reported their good correlation with the prognosis of Sepsis, shown below.

1) **C-Reactive Protein (CRP):** The CRP was first discovered in a patient with lobar pneumonia in 1930 by Tillet & Francis. The CRP is a protein produced in response to infection or inflammation and it is widely used in clinical tests to diagnose and manage patients with sepsis. It is an acute phase reactant whose synthesis in the liver is upregulated by Interleukin-6. Its response is stronger in acutely ill patients and levels decrease as patients recover. It should be used cautiously in patients with sepsis, as it is also a marker of inflammation that also increases after surgery, burns, myocardial infarctions, and rheumatic diseases. The plasma half-life of CRP is 19 hours. The sensitivity and specificity of CRP as a marker for bacterial infections are 68–92 percent and 40–67 percent, respectively.

   Its inability to differentiate bacterial infections from noninfectious causes of inflammation makes CRP of limited diagnostic value. However, CRP shows promise for evaluating sepsis severity and prognosis. CRP plasma levels have been shown to correlate with the severity of infection. A rapid decrease in CRP levels has been reported to correlate with good response to initial antimicrobial therapy in septic patients.

2) **Procalcitonin (PCT):** The PCT is a prohormone of calcitonin and it is released by parenchymal cells (liver, kidney, adipocytes, muscle cells) in response to bacterial toxins, leading to increased levels within two-four hours. In contrast, it is down regulated in patients with viral infections. It is a 116 amino acid protein with a molecular weight of 14.5 KDa. The biological half-life of PCT is 22 to 26 hours. The normal level of PCT in the serum is low, less than, 0.1 ng/mL. When bacterial infections occur, PCT is further produced as a response to proinflammatory stimulation. At present, PCT levels have been used to guide empirical antibacterial therapy in patients with acute exacerbations of chronic bronchitis, community-acquired pneumonia and sepsis. In a systematic review and meta-analysis, PCT was found to be more specific (specificity 81 percent [95 percent CI: 67–90 percent]) than CRP (67 percent [95 percent CI: 56–77 percent]) for differentiating bacterial infection among hospitalized patients.

   This serum Procalcitonin is performed at Aga Khan clinical laboratory and published data from our center have shown that it is a useful biomarker for monitoring prognosis of sepsis patients, as well as identifying bacterial infection.

3) **Lactate:** Lactate is a metabolite of glucose produced by tissues in the body under conditions of insufficient oxygen supply. Serum lactate levels can reflect tissue hypoperfusion and anaerobic metabolism in severe sepsis and septic shock. Several studies have demonstrated that elevated lactate levels are related to mortality in patients with sepsis. In a large study of 1,278 patients with infections, those with lactate levels above four mmol/L had higher in-hospital mortality rates than patients with lactate levels less than 2.5 mmol/L (28.4 percent vs. 4.9 percent). Recently, data from a retrospective study by the Vasopressin Septic Shock Trial and a single center septic shock cohort (St. Paul's Hospital cohort) have suggested that even minimal increases in arterial lactate concentration within the reference range (1.4-2.3 mmol/L) may predict 28-day mortality (sensitivity and specificity of 86 percent and 27 percent, respectively). A study done at our centre have reported that in comparison to PCT and CRP, high plasma lactic acid levels are associated with the development of all-cause multiple organ dysfunction syndrome and worse outcome in critically ill children admitted in Pediatric intensive care unit.

4) **New Markers of Sepsis:** Apart from the aforementioned biomarkers newer sepsis related biomarkers are under research for clinical use, including:
   - Soluble triggering receptor expressed on myeloid cell one (sTREM-1) is a soluble form of TREM-1, a glycopeptide receptor expressed on the surface of myeloid cells such as PMNs, mature monocytes, and macrophages. TREM-1 expression increases in bacterial or fungal infections.
   - Soluble urokinase plasminogen activator receptor (suPAR) is a surface signaling receptor expressed on most leukocytes.
   - Pro-Adrenomedullin (Pro-ADM) is a 52-amino-acid peptide produced by the adrenal medulla. ADM is produced during

physiological stress and has various functions including vasodilation and anti-inflammatory and antimicrobial effects.

The diagnostic accuracy of these markers is shown in Table 2.

Table 2: Diagnostic Accuracy of Newer Biomarkers of Sepsis:

Biomarkers	cutoff/Range	Sensitivity	Specificity	AUC	NPV	PPV	Type
sTREM-1 (pg/mL)	40-755*	79	80	0.87	70	86	Diagnostic
Pro-ADM (nmol/L)	4.86	53	84	0.72	77	64	Prognostic
suPAR (ng/mL)	10 8.9	80 66	77 64	0.79 0.73	95 76	42 50	Diagnostic Diagnostic

Where AUC stands for 'Area under curve', PPV is 'Positive predictive value',and NPV is 'Negative predictive value'
**Cut off point based in a meta-analysis of two studies.*

Serum Procalcitonin Level - Biomarker of Bacterial Infection

Zaib-un-Nisa
Clinical Chemistry

Procalcitonin (PCT) is the peptide precursor of calcitonin, a hormone that is synthesized by the parafollicular C cells of the thyroid and involved in calcium homeostasis. Procalcitonin arises from endopeptidase-cleaved preprocalcitonin. It is also produced by the neuroendocrine cells of the lung and intestine and is released as an acute-phase reactant in response to inflammatory stimuli, especially those of bacterial origin. It is a biomarker that exhibit greater specificity than other pro-inflammatory markers (eg, cytokines) in identifying sepsis and can be used in the diagnosis of bacterial infections.

The raised procalcitonin level during inflammation is associated with bacterial end toxin and inflammatory cytokines. Increased levels of serum procalcitonin in response to viral infections and noninfectious inflammatory stimuli such as autoimmune disease and chronic inflammatory processes are much less pronounced, rarely exceeding 0.5 ng/mL. The reference cutoffs are shown in Table 1.

Table 1: Interpretation of Procalcitonin Levels

Test Name	Reference Cutoffs	Conditions Associated
Pro Calcitonin	< 0.5 ng/mL represents a low risk of severe sepsis and/or septic shock	• Localized mild-to-moderate bacterial infection • Noninfectious systemic inflammatory response • Untreated end-stage renal failure
	2.0 ng/mL represent a high risk of severe sepsis and/or septic shock.	• Bacterial sepsis • Severe noninfectious inflammatory stimuli (e.g., major burns, severe trauma, acute multiorgan failure, major abdominal or cardiothoracic surgery). • Medullary thyroid carcinoma (may exceed 10,000 ng/mL).

PCT levels intensify within two–four hours of infection and the maximum level is reached by six–eight hours and with continued infection or sepsis the elevated level persists and decreases once infection is controlled. PCT half-life is about 20–24 hours. PCT levels persist as long as the inflammatory process continues and the level correlate with the severity of sepsis. The applications or indications of testing PCT include to:

- Aid in the diagnosis and risk stratification of bacterial sepsis.
- Aid in distinguishing bacterial from viral infections, including meningitis.
- Screen therapeutic response to antibacterial therapy and reduce antibiotic exposure.
- Aid the preference and timing of the initiation of antibiotic treatment

(Procalcitonin Algorithm) for improved antibiotic stewardship. The figure 1 shows the algorithm of PCT which can be utilized in antibiotic therapy.

- Aid in the diagnosis of systemic secondary infection after surgery and in severe trauma, burns, and multiorgan failure

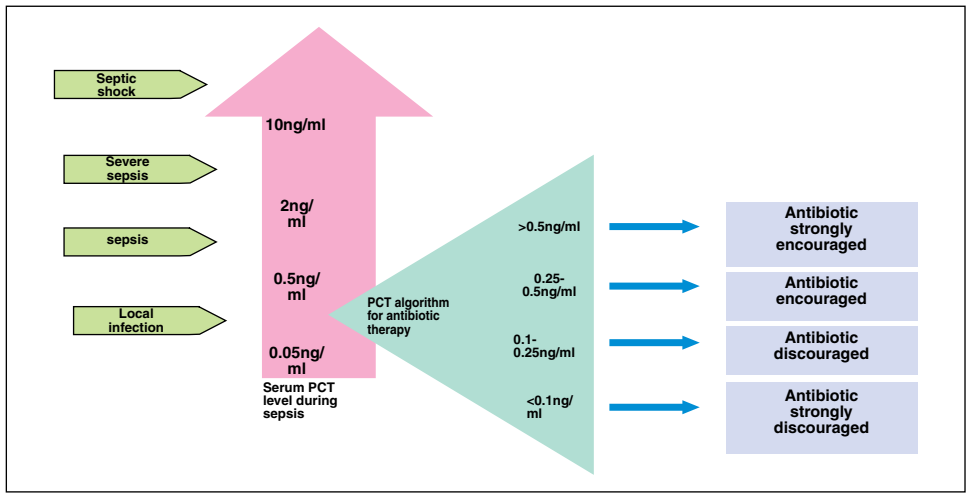


Figure 1: Algorithm for use of PCT for antibiotic Therapy. (taken from Vijayan et al. Journal of Intensive Care (2017) 5:51; DOI 10.1186/s40560-017-0246-8)

Bone Marrow Findings in Malaria

Dr. Sana Brohi
Resident Haematology

Case: A 10-year-old boy presented with history of fever, generalized weakness, weight loss and recurrent nose bleed since 20 days. Physical examination revealed pallor and palpable spleen one-two cm. Bone marrow examination is done for diagnostic workup.

CBC: Hb: 6.8 g/dL, WBC: 4.24 x 10E9/L, platelets: 16 x 10E9/L. Peripheral blood smear showed, trophozoites, gametocytes and schizonts of plasmodium vivax with parasitic index of 1.5 percent (Figure 1). Bone marrow aspirate showed the gametocytes in the background of trilineage hematopoiesis with some dysplastic features (Figure 2).

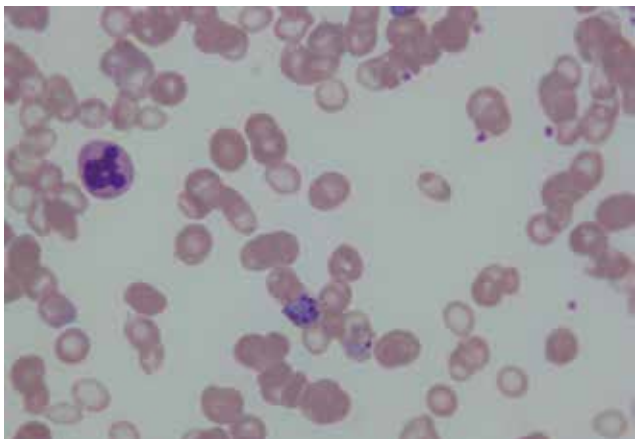


Figure 1: Peripheral blood smear

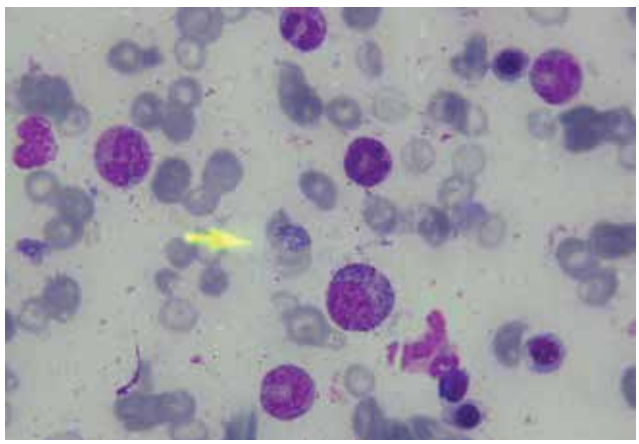


Figure 2: Bone marrow aspirate

Bone Marrow Findings in Malaria

Peripheral smear: In malaria, parasites are seen within red cells. Malaria is associated with hemolytic anemia, leucocytosis and thrombocytopenia. The reticulocyte count may be inappropriately low as a result of bone marrow suppression.

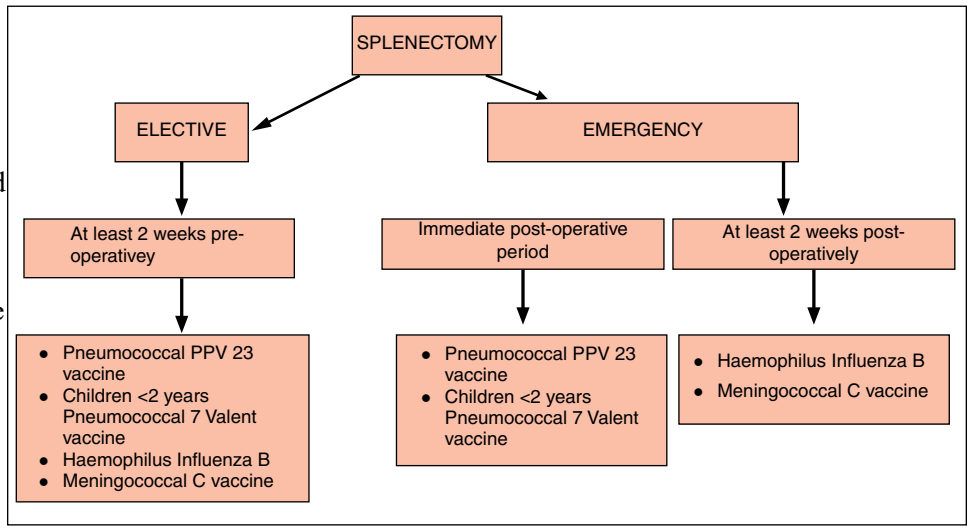
Bone marrow aspirate: Malaria parasites are sometimes detected in red blood cells and neutrophils, in bone marrow aspirate. In ***acute falciparum malaria***, the bone marrow may be hypocellular, normocelullar, or mildly hypercellular. Erythropoiesis is usually suppressed. In ***chronic falciparum malaria***, there is hypercellularity with erythroid hyperplasia, increased lymphocytes, plasma cells and macrophages. Haemophagocytosis and macrophages can also contain malarial pigments. Bone marrow in ***Plasmodium vivax malaria*** is also characterized by dyserythropoiesis, increased macrophages (some showing haemophagocytosis) and plasma cells. In hyper-reactive malarial splenomegaly, there may be a marked increase in bone marrow lymphocytes.

Bone marrow trephine histology: Bone marrow biopsy in malaria usually shows increased cellularity and increased macrophage activity, often with haemophagocytosis. During acute attacks of malaria, sinusoids may be packed with parasitized red cells. In patients who have suffered recurrent attacks of malaria, the bone marrow may be slate grey or black due to deposition of malaria pigments. It is important to distinguish malaria pigment (haemozoin) from formalin pigment. ***Haemazoin*** is found not only in macrophages but also within erythroid and granulocytic precursors, possibly contributing to dyserythropoiesis and erythroid suppression.

Post-Splenectomy Care and Anti-Microbial Prophylaxis

Dr Hareem Alam
Hematology

Spleen mediates important immunologic and hematologic functions as well as contribute to numerous pathological processes. Surgical removal of the spleen results in reduced clearance of particulate antigens [extracellular (e.g. bacteria) or intracellular (e.g. malaria)], diminished response to new antigens, particularly polysaccharides and impaired phagocytosis of un-opsonized and opsonized bacteria and cells. Asplenic individuals are particularly vulnerable to sepsis caused by bacteria and, occasionally, protozoa. Such infections are often fulminant, with high mortality. Splenectomy usually is performed in two major clinical scenarios: trauma and hematologic diseases including hereditary hemolytic anemias and autoimmune cytopenias. Auto-splenectomy may occur in sickle cell anemia.



Infections: Overwhelming post-splenectomy infection is usually caused by the encapsulated bacteria Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis. Other pathogens may include Escherichia coli, Pseudomonas aeruginosa, Capnocytophagia canimorsus, group B streptococci, Enterococcus species, Ehrlichia species and protozoa such as Plasmodium species leading to malaria.

Predisposing risk factors: The main risk factors for infections include: the age at which splenectomy is performed, children are particularly at risk, the reason for splenectomy, and the time interval from splenectomy (most infections occur within two years).

Post-splenectomy care: Guidelines published by the British Committee for Standards in Haematology emphasized that most infections after splenectomy could be avoided through measures that include offering patients appropriate and timely immunization, antibiotic prophylaxis, education, and prompt treatment of infection.

Time of Vaccination: For patients undergoing splenectomy, vaccines should be given at least 14 days prior to the procedure when feasible. When vaccines cannot be given in this time frame, they should be given 14 days after splenectomy.

Antibiotic prophylaxis: Lifelong prophylactic antibiotics should be offered to patients considered at continued high risk of pneumococcal infection either with penicillin V or amoxicillin. Factors associated with high risk of invasive pneumococcal disease in hyposplenism include: age less than 16 years or greater than 50 years, inadequate serological response to pneumococcal vaccination, a history of previous invasive pneumococcal disease, and splenectomy for underlying hematological malignancy particularly in the context of on-going immunosuppression.

Patient education: It is important that the patient should be well informed and educated about infectious risks associated with history of splenectomy, and understands the steps that must be taken prior to splenectomy.

Invasive Fungal Infections in Hematological Malignancies

Dr Jyoti Mohan Lal
Hematology

The control of bacterial infections in patients with hematological malignancies has been significantly improved with broad-spectrum antibiotics in the past decades however, treating invasive fungal infections (IFIs) is still a major problem in these patients, especially in patients with prolonged neutropenia after chemotherapy. The incidence of invasive fungal infections has dramatically increased over the past several decades, and the majority of these infections are in patients with hematologic malignancies (HMs). They remain a leading cause of morbidity and mortality in this population, especially among patients with acute myeloid leukemia (AML) and those who have undergone allogeneic hematopoietic stem cell transplantation.

Common organisms: Candida and Aspergillus species are the most common recognized causes of IFIs in patients with hematological malignancies. However, other typically less common yeasts and filamentous fungi e.g., Blastomyces spp., Cryptococcus spp., Trichosporon spp. and Zygomycetes) are emerging in patients with HMs as significant human pathogens, frequently as breakthrough infections in patients receiving empirical antifungal therapy or antifungal prophylaxis.

Risk factors: Among the most important patient-related factors is the underlying fitness or functionality of the patient. Many studies have shown that older patients (≥60 years) with AML or myelodysplastic syndrome (MDS) with preexisting cytopenias have an increased risk of fungal infections because their innate immune system is weakened. Frail patients, who are more likely to be older are thought to have a higher risk. Another risk factor is the type of therapy administered for the underlying leukemia. Compared with patients with acute leukemia and hematopoietic stem cell transplant (HCT) recipients, the risk for invasive fungal infections is lower in patients with chronic leukemias, lymphoma, and multiple myeloma, but invasive fungal infections do occur in such patients.

Prognosis: Studies have shown that the mortality associated with invasive fungal infections in patients with AML undergoing induction therapy, autologous stem cell transplant, or allogeneic stem cell transplant may be as high as 40 percent to 60 percent, as compared with approximately 20 percent among patients treated with less-intensive or less-myelosuppressive therapy.

Following factors have been identified that negatively influence the outcome of IFI:

- old age,
- use of corticosteroid
- an absolute neutrophil count (ANC) of less than 0.1X109/L at the time of IFI diagnosis
- lack of recovery from aplasia and
- multiple pulmonary localizations of infection

Clinical presentation: The most common presentation of a leukemia patient with a fungal infection is a neutropenic fever of unexplained etiology. Typically, patients who develop a neutropenic fever will initially begin treatment with broad-spectrum antibiotics. A fever that persists without a clear source should prompt consideration of a systemic fungal infection, with the appropriate diagnostic workup. The second most common presentation of a fungal infection is pulmonary infection, such as pneumonia, and sinusitis. The third most common presentation is disseminated infection, manifested commonly as skin lesions or soft tissue involvement, particularly in patients with systemic Candida infections or disseminated candidiasis. Lastly, infections may also be identified through a routine blood culture.

Diagnostic tool: Monitoring patients during aplasia includes history and physical examination, daily blood counts, serum C-reactive protein (CRP) and serum Galactomannan index (s-GMI) thrice-weekly. For febrile neutropenia (FN), an infectious disease workup is performed and intravenous broad-spectrum antibiotics are started. Additional workup is obtained for antibiotic-refractory FN, including chest CT even in the absence of pulmonary findings. Patients with negative serum fungal biomarkers but with radiologic findings suggestive of IFI should undergo bronchoalveolar lavage (BAL), with testing of BAL fluids for fungal biomarkers.

Blood cultures have moderate sensitivity for hematogenous infections with yeasts and molds with adventitious sporulation but have no sensitivity for invasive aspergillosis (IA). Cultures, stains, and histopathology with direct examination of involved sites can be diagnostic (e.g., metastatic skin lesions of candidiasis, trichosporonosis and fusariosis).

Detecting circulating serum 1,3-β-D glucan (s-BDG) may be useful in diagnosing various IFIs, including candidiasis, aspergillosis, fusariosis and others.

Antibiogram: A Clinicians' Reference for Antibiotic Selection

Noureen Saeed
Instructor Microbiology

An antibiogram is a summary of antimicrobial susceptibilities of bacterial pathogens that are reported by a clinical microbiology laboratory in an institute during a specific time period. Due to the emerging resistance patterns, this important tool guide clinicians in deciding empiric therapy in view of prevailing susceptibility trends against a specific pathogen. Apart from this it can be used to track the evolving antimicrobial resistant trends across different units of the hospital.

Antibiograms usually represent a cumulative report of an institution, however, it can further be segregated according to different important clinical units e.g. Intensive care units (MICU, SICU, NICU, PICU). Therefore, it could also be affective in evaluating the infection prevention practices by monitoring specific drug resistance pattern in any surveillant hospital unit. It could also be use as an important antibiotic stewardship tool. This important document is institute specific data and cannot be generalizable to other institution.

Antibiogram Preparation

1. **Responsibility:** Clinical microbiologist are responsible for preparing the antibiogram based on antimicrobial Susceptility testing data generated from microbiology laboratory

2. **Reliability of Antibiogram:** An institution antibiogram is not a true reflection of its resistance trends unless reporting of microbiology culture results are accurate

according to international guidelines. Clinical Laboratories Standards Institute (CLSI) and European Committee for Antimicrobial Susceptibility Testing (EUCAST) provide guidelines about performance methods, incubation, quality control and recommended antimicrobial agents against each organism group. Guideline document for antibiogram preparation M39-A4 by CLSI is also available and gives specific recommendations on different variables included in preparation of antibiogram, these includes minimum number of isolates to be tested for reporting in an antibiogram, frequency of reporting, exclusion of screening isolates from antibiogram, and which antimicrobials to test.

3. **Frequency of antibiogram preparation:** Generally, it is recommended to have it at least annually or it could be biannually depending upon institutions' workload.

4. **Organisms' selection criteria:** Bacteria which have significant available data and frequently isolated from a laboratory must be selected and reported in an antibiogram, otherwise data may be skewed and would not be representative. Data filtration and duplicate removal is an uphill task and must be done meticulously.

5. **Using Antibiogram:** It is better to have uniformity in reporting the final data every year. CLSI has recommendation for reporting it in percentage susceptible. Segregation of data according to the units can be very effective in antibiotic selection in view of available data of the specific area.

6. **Accessibility:** Pocket size printed version of antibiogram should be available at every ward, nursing stations. It could be uploaded in hospital's websites for ready reference.

Limitations

1. Quality of antibiogram is directly related to the quality of patient's reports. If colonizers or other organisms are reported which are not true pathogens this may yield a low quality antibiogram with misleading information.
2. It shouldn't be used solely to make clinical decisions about antimicrobial therapy
3. Antibiograms do not reflect the synergistic effects of antibiotics which are used in combinations

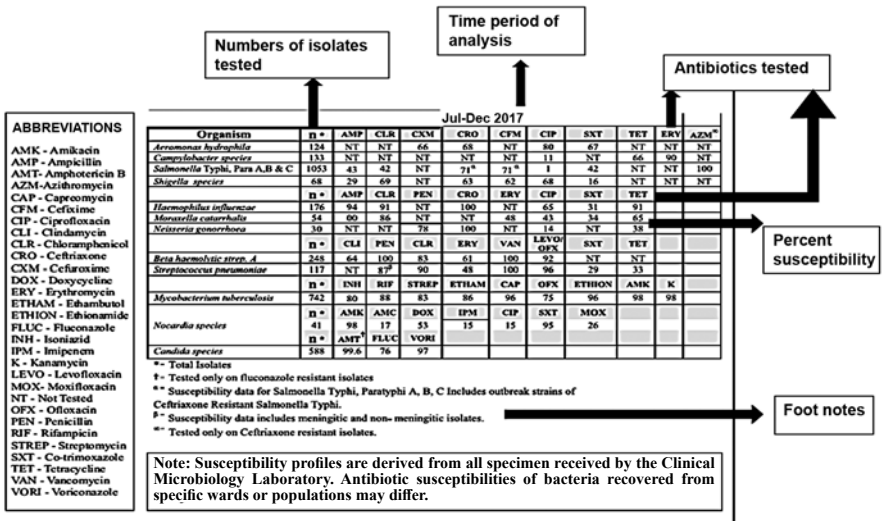


Figure 1: Template of an antibiogram

Pakistan Antimicrobial Resistance Network (PARN)

A detailed module on antibiogram, preparation and its use is available at Pakistan antimicrobial resistance network (PARN) website which is available at http://parn.org.pk/course-list/Antibiograms of various institutions is also available at PARN website http://parn.org.pk/antimicrobial-data/

Antimicrobial Susceptibility Testing (AST): Best Practices, Better Outcome

Shireen Shahid
Microbiology

Adversity of antimicrobial resistance has started to affect the mankind since its inception and now it has become a brute force against the humanity. It is substantive responsibility of treating physicians to confirm susceptibility of chosen empirical antimicrobial agents. There is also important obligation of clinical microbiology laboratories to detect resistance in individual microbes using methods which are reproducible and reliably.

Empirical therapy continues to be effective for some bacterial pathogens because resistance mechanisms have not been observed e.g., continued penicillin susceptibility of Streptococcus pyogenes. Susceptibility testing is important with microorganisms that may possess acquired resistance mechanisms (e.g. members of the Enterobacteriaceae, Pseudomonas species, Staphylococcus species, Enterococcus species, and Streptococcus pneumoniae).

The phenotypic methods for AST in a clinical microbiology laboratory are:

1. Disc diffusion (DD) method
2. Minimal inhibitory concentration (MIC) methods
   i. Microbroth dilution (MBD)
   ii. Gradient strip (e-test)
   iii. Automated instrument systems (e.g. Vitek, MicroScan).

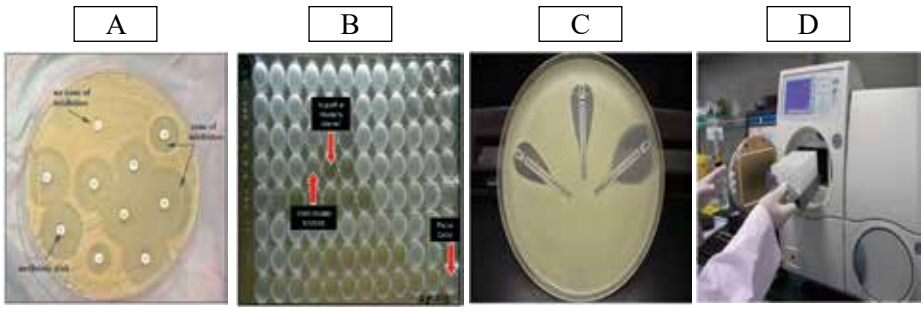


Figure 1: AST Methods A: Disc diffusion. B: Broth microdilution. C: Gradient strip D: Automated instrument

Getting the reliable results in a laboratory is conditional to the adherence with quality management and quality assurance practices. Quality assurance process not only describe step of the specific laboratory method but also ensure its validation and documentation. In this way the retrospective tracing of an incidence that cause an erroneous result become feasible for a laboratory manager.

Quality Management (QM) in AST

A microbiology laboratory manager, who is responsible for a reliable AST results, can only deliver by vigilant monitoring of essential quality management components which are elaborate below.

1. **Staff:** a motivated staff is the back bone of quality process. Competency can be achieved with the nexus between motivation and proper training. Learning objectives and methods of training must be clear. Evaluation of staff performance done by regular competency assessments, internal audits and external proficiency testing. Feedback process to the staff is a vital for bringing the improvement.

2. **Standard operating procedures (SOPs):** It is a compilation of step-by-step instruction that help workers to carry out complex routine operations. For AST, the SOP must adhere to international guidelines e.g. Clinical Laboratory Standardization Institute (CLSI) or European Union Chemotherapy and Antimicrobial Susceptibility Testing (EUCAST). Department committee for AST procedure must monitor any recommended changes in the aforementioned documents and also ensure to update and implement it in the laboratory SOP.

3. **Policy:** It is a plan of what to do in a particular situation that has been agreed officially by a group of people. For AST, manager must form a committee comprises of senior technical staff members who responsibilities would be development and implementation of policies in view of recent therapeutic and diagnostic recommendation. For smooth running of process this committee must

keep in their consideration inventory management policies. Manager ensure that the policies must disseminate, read and followed by all technical staff.

4. Documentation: "Not documented, Not done". This canonical saying is the basis in quality management. It's not enough that they do it well but they should also have to show it in the documents e.g. maintenance forms. They have to document instrument performance and maintenance, daily good laboratory practices, results of quality control activities. Technical staff must be sensitized and trained in documenting what has happened in laboratory and the activities performed by them. This can help to trace back the information whenever if required. These documents must have some schedule reviewing process by the manager.
They must ensure daily prompt and proper documentation of quality control activities of AST and "root cause analysis" of errors must have done properly in order to avoid the repetition of that specific incidence.

5. Validation: Before implementing new method of susceptibility testing (e.g. disc diffusion to broth microdilution) it must be validated for the intended results.

6. Verification: Reagents, media and antibiotics use in AST must be verified before use at time of different shipments or after receiving a new lot.

7. External quality assessment: this can help in assessing the technical skills of technologist and also evaluate the processes of any laboratory test. For example, proficiency tests by College of American Pathologist (CAP) and UK NEQAS microbiology.

Quality Assurance (QA) in AST:
The objective of QA in AST is to maintain the standardization and quality of the data. Any deviation could cause faulty outcomes in the form of patient's morbidity and mortality and promotion in antimicrobial resistance.

QA can be achieved through quality control (QC) and the goals of QC are to monitor:

a. The precision and accuracy of AST procedure
b. The performance of reagents in the test
c. The performance of technical staff who perform and read the test

The goals are best achieve-but not limited to- by testing the quality control strain. QC strains are well characterized organisms with defined susceptibility or resistance mechanisms to the antimicrobial agent(s) tested e.g. American Type Culture Collection (ATCC). Maintenance, storage and logging of these strain is critical and therefore senior staff must be responsible for this activity.

Standardization in performance is vital and factors that can influence the results of AST are:

i. Size of inoculum
ii. Content of the acidity (pH) of the media
iii. Cation content (Ca^{2+}, Mg^{2+})
iv. Incubation time and temperature
v. Reading procedure

Moreover, for diffusion methods:

vi. Diffusion rate of antimicrobial into the agar
vii. Depth and dryness of the agar
viii. Microbial growth rate

Reliable and reproduceable results => standardize method + quality assurance.

Following are the examples of International guideline that describes the methods (DD, MIC) in detail about media, inoculation, incubation etc.

- ISO (International organization for standardization)
- CLSI (Clinical and Laboratory Standards Institute- www.clsi.org)
- EUCAST (The European Committee on Antimicrobial Susceptibility Testing – www. eucast.org)

For training of a new staff in AST and its quality control, laboratory manager can refer to www.parn.org.pk. This website provides free online course of AST, quality control, specimen processing and biosafety in 7 different modules. After module completion they will be assessed and graded.
If good laboratory practices (GLP) and compliance with quality management for AST is vigilantly followed by laboratory personnel, reliable antimicrobial susceptibility result can be generated and that help clinician in selecting right antibiotic and hence contribute in reducing antimicrobial resistance (AMR).

Parasitological Diagnosis of Malaria Using Rapid Diagnostic Test (RDT)

Dr Moiz Khan
Microbiology

The theme for World Malaria Day 2019, "Zero malaria starts with me", suggests this hope and emphasises country ownership and community empowerment for malaria prevention and care in Pakistan.

Malaria is a life-threatening disease. According to an estimate 6.8 million people are died with malaria globally since 2001. The situation in Pakistan is also grave and roughly 60 percent of Pakistan's population, live in malaria endemic regions.
In a recent study, it is noticed that Khyber Pakhtunkhwa is the most malaria-affected province of Pakistan as compared to the other three provinces i.e., Punjab (2.4 percent), Sindh (10.8 percent) and Baluchistan (3.8 percent).

Figure 1: Sample sites and distribution of Plasmodium species across Pakistan.

According to WHO guidelines, parasitological diagnosis is required for confirmation of the clinical diagnosis of malaria. It is recommended for all suspected malaria cases in all transmission settings. The advantages of parasitological diagnosis are:

a) improved care of parasite-positive patients owing to greater certainty that the cause of the present illness is malaria;
b) identification of parasite-negative patients for whom another diagnosis must be sought;
c) prevention of unnecessary use of anti-malarial, thereby reducing the risk of adverse side effects and drug interactions;
d) confirmation of treatment failures; and
e) improved malaria case detection and reporting

Parasitological diagnosis should be available within a short time (less than two hours) of clinical examination of the patient. If this is not possible, the patient should be treated on the basis of a clinical diagnosis.

The two main methods in routine use for parasitological confirmation of malaria are light microscopy and **rapid diagnostic tests (RDTs)**.

For the management of a new fever episode, quality-assured microscopy and RDTs are equivalent in terms of performance for the diagnosis of uncomplicated malaria. In addition, molecular diagnosis (e.g. polymerase chain reaction / PCR) is usually applied in research settings, and in surveillance in areas where elimination of malaria is in progress. Serological tests for malaria have no place in the management of febrile patients.

In settings of low to moderate and/or unstable transmission and the low transmission season in areas with seasonal malaria, health workers should be trained to identify, through the case history, patients who have been exposed to malaria risk before a parasitological test is ordered.

Malaria species identification: in areas where two or more malaria species are common, either malaria microscopy or certain RDTs can provide a species-specific diagnosis. Where P. falciparum and non-falciparum malaria are both prevalent and commonly occurring as single-species infections, if microscopy is not available, it is recommended to use combination RDTs that detect all species and distinguish P. falciparum and non-falciparum malaria.

In epidemic-prone zones, good quality malaria diagnosis must be available, not only for case management but also for surveillance, i.e. investigation of an unexpected increase in cases of fever.

During outbreak investigations and field surveys, RDTs have the advantage of allowing both detection and immediate treatment of malaria-positive cases. When the positivity rate is very low, it becomes difficult to maintain the interest and skills of microscopist and their capacity to implement an effective quality management system based on slide cross-checking (because of problems of sensitivity). In complex emergency situations, there are several logistic and practical advantages to using RDTs rather than microscopy: rapid deployment in the field, less training needed than for microscopy, no need for an electricity supply, immediate availability of results for treatment of positive cases in the field, and potential for testing more cases. However, if the patient has received previous antimalarial treatment, a microscopic examination of blood film for malaria is recommended to investigate possible treatment failure.

Rapid diagnostic tests (RDTs) that are accurate, relatively inexpensive and can be used in field conditions make this possible. Use of antigen detecting rapid diagnostic tests (RDTs) is a vital part of this strategy, forming the basis for extending access to malaria diagnosis by providing parasite-based diagnosis in areas where good-quality microscopy cannot be maintained. The number of RDTs available and the scale of their use have increased rapidly over the past few years; however, limitations of field trials and the heterogeneous nature of malaria transmission have limited the availability of the good-quality data on performance that national malaria programmes require to make informed decisions on procurement and implementation.

Therefore, in 2006, WHO special programme for research and training in tropical diseases (TDR) and the foundation for innovative new diagnostics (FIND) launched a programme to systematically evaluate and compare the performance of commercially available malaria RDTs. The RDT product testing has undergone 6 rounds of testing from 2008 to 2015 and the results have been published annually since 2009.

In each round of testing, products were evaluated against geographically diverse, cryopreserved plasmodium falciparum and plasmodium vivax clinical samples diluted to 200 and 2000 parasites/µL with consistently comparable concentration ranges of histidine-rich protein ii (HRP-2), plasmodium lactate dehydrogenase (pLDH) and aldolase determined by quantitative enzyme-linked Immunosorbant assay (ELISA). The former is well below the mean parasite density found in many populations with endemic malaria and is considered close to the threshold that must be detected in order to reliably identify clinical malaria in many settings. The aim of the evaluation is to provide comparative data on the performance of the submitted production lots of each product.

The results in this report show the comparative performance of RDTs and indicate which products are likely to be more sensitive in the field, particularly in populations with low-density infections. The performance of the products varied widely at low parasite density (200 parasites / µL) and all products had a high rate of detection of falciparum at 2000 or 5000 parasites/µL, as did the majority of products for vivax at 2000 parasites/ µL. However as stated previously a low parasite density (200 parasites/µL) is considered close to the threshold that must be detected in order to reliably identify clinical malaria in many settings.

Figure 2: Rapid Diagnostic Kits (RDTs) for diagnosis of malaria

Laboratory Diagnosis of Meningitis

Dr Salima Rattani
Microbiology

In clinical practices suspicion of meningitis requires laboratory evaluation and confirmation. This in essential as it can influence the therapeutic management by selecting appropriate antimicrobial agents. Impact of inadequate laboratory diagnosis of meningitis is multifaceted as it increases the morbidity and mortality, promote antimicrobial resistance and increase financial cost on patient.

In community acquired meningitis the etiological agents could be bacterial, fungal, mycobacterial, viral and parasitic. These could be endogenous flora of susceptible host but could also be from exogenous sources e.g. environmental, water and animals especially in case of immunocompromise scenario. In health care setting it is usually from exogenous sources e.g. of hospital environment and transmit through indwelling devices.

Aseptic meningitis is the presence of clinical and laboratory evidence for meningeal inflammation with negative routine bacterial cultures. Enterovirus is the commonest cause of aseptic meningitis. Other viral causes include Herpes simplex type I (HSV I), Herpes simplex type 2 (HSV 2), Epstein-Barr virus (EBV), Varicella-zoster virus (VZV), Measles, Human immunodeficiency virus (HIV), Cytomegalovirus (CMV), Adenovirus, Arboviruses, Coxsackieviruses types A and B, Echoviruses, Influenza A and B.

Aseptic meningitis has a self-limited course that will resolve without specific therapy whereas bacterial meningitis is life threatening and requires prompt administration of antibiotics. Whenever a patient is suspected of having meningitis, it is advised to obtain cerebrospinal fluid and blood for cultures and susceptibility and start empiric treatment according to common causative agents in that particular age group. However, specimen collection after initiating antibiotic therapy may cause sterilization of CSF and lead to negative culture results. This leads to an inability to properly tailor therapy in view of culture and susceptibility findings.

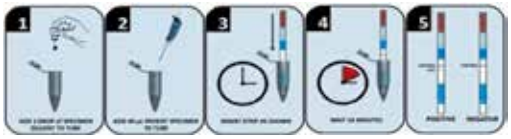
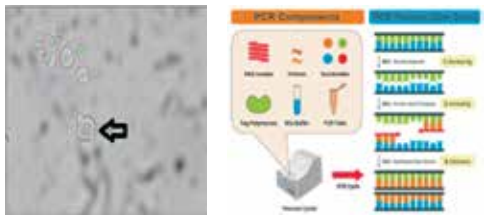
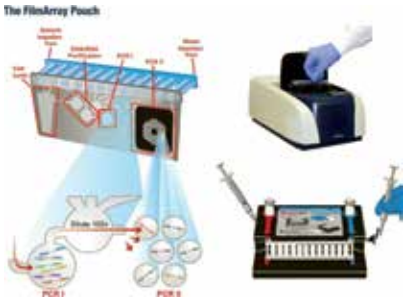
The following table represents CSF findings in different kinds of meningitis.

Table 1: Typical CSF Parameters in Patients with Meningitis

Pathogen	*White blood cells per µl (× 10^9 per l)*	*Percentage of neutrophils*	*Glucose level*	*Protein level in mg per dl (g per l)*	*Likelihood of observing organism on CSF stain*
Aseptic, often viral	10 to 1,000 (0.01 to1.00)	Early: > 50 Late: < 20	Normal	< 200 (2.00)	Not applicable
Pyogenic	> 500 (0.50)	> 80	Low	> 100 (1.00)	~70%
L. monocytogenes	> 100 (0.10)	~50	Normal	> 50 (0.50)	~30%
Partially treated pyogenic	> 100	~50	Normal	> 70 (0.70)	~60%
Tuberculous	50 to 500 (0.05 to 0.50)	< 30	Low	> 100	Rare
Fungal	50 to 500	< 30	Low	Varies	high in cryptococcus

Apart from CSF analysis and culture and sensitivity testing, serological and molecular techniques against etiological agents of community associated meningitis.

Table 2: Serological and molecular diagnostic techniques against agents of community associated meningitis:

TEST	ORGANISMS
Latex Particle Agglutination (LPA) 	• Streptococcus group B • Haemophillus influenzae type b • Streptococcus pneumonia • Neisseria meningitidis (groups A, B, C, Y, W135) • Escherichia coli K1
Cryptococcal Antigen Test (CAT) 	• Cryptococcus neoformans
CSF GeneXpert for TB and TB culture 	• Mycobacterium tuberculosis complex
CSF PCR for Primary amoebic meningoencephalitis (PAM) 	• Naegleria fowleri
CSF FilmArray (PCR based test) Meningitis/ Encephalitis Panel (BFM)	Bacteria • Streptococcus agalactiae (GBS) • Haemophillus influenzae • Streptococcus pneumonia • Neisseria meningitides (encapsulated) • Escherichia coli K1 • Listeria monocytogenes Viruses • Cytomegalovirus (CMV) • Enterovirus • Herpes simplex type I (HSV I) • Herpes simplex type 2 (HSV 2)
	• Varicella-zoster virus (VZV) • Human parechoviruses • Human Herpesvirus 6 Yeast • Cryptococcus neoformans/ gattii

The utility of the above-mentioned diagnostic tests is essential for better patient's outcome as it not only strengthened the diagnostic and prognostic capabilities and but it can also be utilized for implementing antibiotic stewardship.

Methods of Diagnosing *Entamoeba histolytica, Giardia lamblia,* and *Echinococcus granulosus* in A Routine Diagnostic Laboratory

Nadia Zehra
Clinical Microbiology

Global burden of parasitic disease affecting human species is significant. Using the disability-adjusted life year (DALY) as a metric, human parasite also cause an enormous disease burden with more than 100 million DALYs annually by some estimates. This value exceeds the global disease burden from HIV-AIDS. Despite their public health importance, most of the human parasitic diseases have been neglected by the scientific, medical, and public health communities. Their neglect stems from the observations that parasitic diseases generally occur among the poor, and more often than not occur in remote and rural areas where they are hidden from view. Moreover, parasitic diseases not only occur predominantly in the setting of poverty, but often because they result in chronic disability, they are themselves poverty-promoting.

The aforementioned crucial situation necessitates clinical microbiology laboratories to have diagnostic options in their repertoire against parasitic infections. This will help to get the best of both worlds by minimizing the antibiotic use and initiating the targeted anti-parasitic drugs.

Entamoeba histolytica and *Giardia lamblia* are the most important diarrhea-causing parasitic protozoa. *Echinococcus granulosus* is a tissue invasive parasites (larval Cestodes) that invade the major tissues and organs of the human body and cause a major disease called Echinococcosis (cystic hydatid) disease. Humans are accidental dead-end hosts.

For the diagnosis of diarrheagenic protozoal parasites *(E. histolytica, G. lamblia)*, microscopic examination of fecal specimen in saline and iodine preparation using concentration technique is still the most commonly used method in resources limited diagnostic settings. Permanent stain smear (e.g. Giemsa stain preparation) can improve the sensitivity of microscopic method. Due to its dependence on the technical skill of laboratory personnel, the probability of misdiagnosis is prevailing. Apart from technical skills, presence of a smaller number of pathogens in the specimen is also an important limitation for early diagnosis of enteric protozoa.

Echinococcal disease manifest as mass lesion affecting multiple body sites. Radiological evidence followed by excystation and visualizing hooklets under microscope.
Diagnostic limitations necessitate the need of alternative methods which could be affective, reliable and early diagnose these important parasitic diseases. Available modalities include serological methods e.g. Enzyme-Linked Immunosorbant Assay for antibody and antigen (ELISA), Direct Fluorescent-Antibody Assay (DFA), immunochromatography test (ICT) and molecular methods e.g. PCR.
The diagnostic facilities available in microbiology section of Aga Khan Hospital Laboratory for the detection of E. histolytica, G. lamblia and E. granulosus are as follows:

- Fecal wet mount and concentration methods with trichrome stain for confirmation of both the Giardia lamblia and E. histolytica are classical tests, popular due to their standing as gold standards.
- **Antigen detection by ELISA** is highly sensitive and specific for Giardia lamblia in

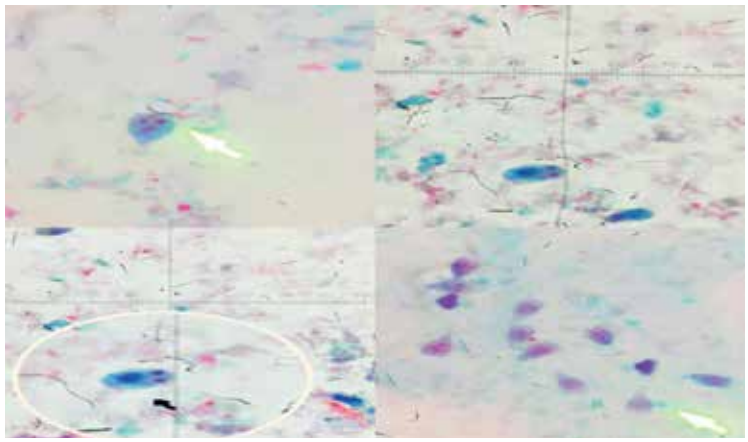
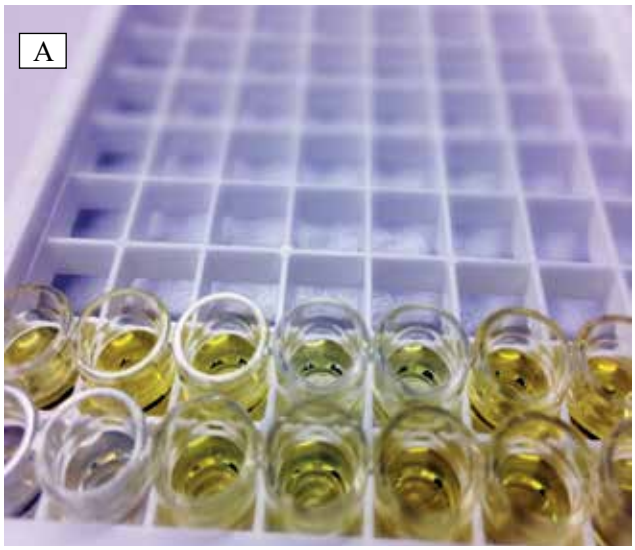


Figure 1: Giardia lamblia, cyst and trophozoite, stained with trichrome.

(Picture courtesy: Mr. Ferozuddin Zaheeruddin, senior technologist- microbiology

a single fecal sample. While microscopic method for the detection of organism need at least 3 samples to be examined.

- Serologic tests such as **IgG antibody** detection by ***ELISA*** against ***E. histolytica*** and is important to distinguish amoebic from bacterial liver abscess. For **Echinococcus** it is intended for non-invasive diagnosis of hydatid cyst.



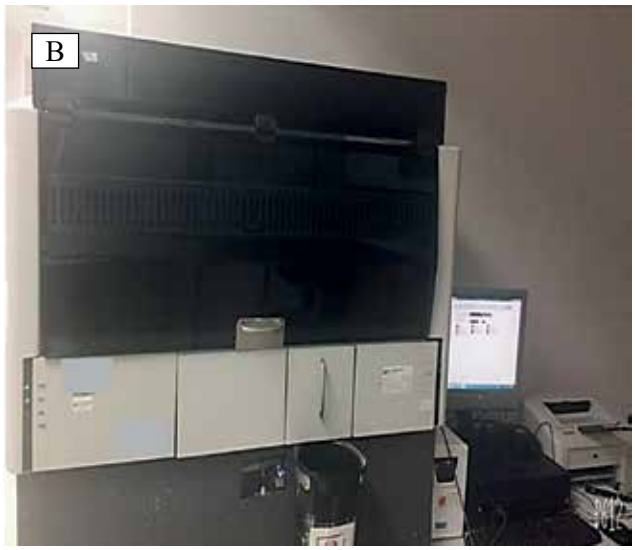


Figure 2: A: ELISA plate with microplate; B: ELISA reader

Detection of *Pneumocystis jirovecii* by PCR

Dr Safia Moin
Microbiology

Pneumocystis pneumonia (PCP) is an opportunistic infection in immunocompromised patients, particularly those with HIV infection, in patients with transplants, those receiving corticosteroids and other immunosuppressive agents. The causative agent, *Pneumocystis jirovecii*, previously misnamed as *Pneumocystis carinii* is an atypical fungus. PCP is still a major cause of morbidity and mortality among HIV/AIDS patients and constitutes a worldwide problem. While the incidence of PCP among HIV-infected individuals has decreased in developed countries, the prevalence of AIDS related PCP in developing countries remains high and poorly controlled.

P. jirovecii replicates asexually by binary fission of trophozoites and sexually, resulting in the formation of a cyst. Since *P. jirovecii* is not cultivable in vitro, the diagnosis of PCP has long relied on microscopic detection of trophic forms and cysts using different stains [Giemsa, toluidine blue, calcofluor white and Gomori methenamine silver]. Overall, the diagnostic performance of conventional stains is comparable. These staining techniques are less sensitive and dependent on the skill of the technologist and patient's immunosuppression. These stains were supplanted in the mid-1990s by direct (DFA) or indirect immunofluorescence assays (IFA) using anti-*P. jirovecii* monoclonal antibodies. The sensitivity of the immunofluorescence assays, irrespective of the technique used (direct or indirect),was better than conventional staining. These staining techniques offer the advantage of easy and rapid identification of the organism. Preparation time varies from one to two h. The appearance of the organism on the smear varies according to the specificity of the monoclonal antibody. DFA detects both trophozoites and cysts, whereas IFA does not detect trophozoites. (Figure 1) Because of the variability in the appearance of the organism

and the presence of background fluorescence, interpretation of these assays also requires a certain degree of technical expertise and familiarity with the morphology of the organism.

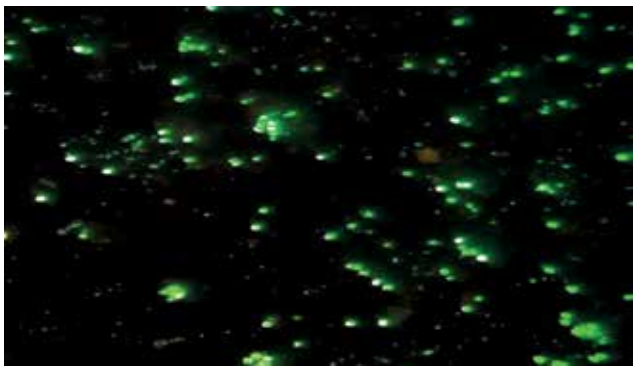


Figure 1: Detection of P. jirovecii cysts by Indirect immunofluorescence assay (IFA) (courtesy; Microbiology Section, Department of Pathology and Laboratory medicine, AKU).

Microscopic diagnosis is particularly difficult when fungal burdens are low, and thus may be false negative. Additionally, immuno-fluorescent stains are limited by reliable quality control material as the species available as control is *Pneumocystis carinii* (animal pathogen), while the target is *Pneumocystis jirovecii,* the human pathogen.

Recently polymerase chain reaction (PCR) based assays for *Pneumocystis* have been reported to be of high sensitivity and specificity and are advocated as new test of choice. Various PCR targets and methods have been developed , and real-time PCR has progressively supplanted conventional PCR. PCR has been reported to enhance PCP diagnosis with a fourfold increase in case detection compared to staining techniques.

Owing to the non-specificity of imaging and clinical signs and the frequent co-infections and non-infectious causes of lung infiltrates, together with the possible toxicity and the long duration of PCP therapy, making a PCP diagnosis on clinical criteria or imaging alone is not recommended and guidelines recommend that clinical, radiologic, and other laboratory findings should be considered together with the PCR results.

Thus in keeping with the improved diagnostic standards as per international evidence-based recommendations, the Aga Khan University Hospital Laboratories have decided to discontinue Pneumocystis jirovecii monoclonal fluorescent antigen test and replace it with *Pneumocystis jirovecii* PCR effective from August 1, 2018. This is a real-time PCR assay for specific detection of *Pneumocystis jirovecii* in respiratory samples specimens including: bronchoalveolar lavage (BAL), tracheal aspirates and sputum. DNA is extracted from specimen and is tested by PCR using primers and probe specific to *Pneumocystis jirovecii.*(Figure 2)

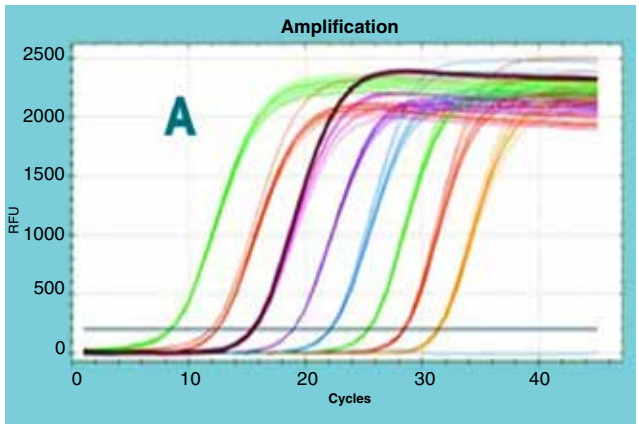


Figure 2: Detection P. jirovecii specific DNA by PCR (courtesy; Molecular Pathology Section, Department of Pathology and Laboratory medicine, AKU).

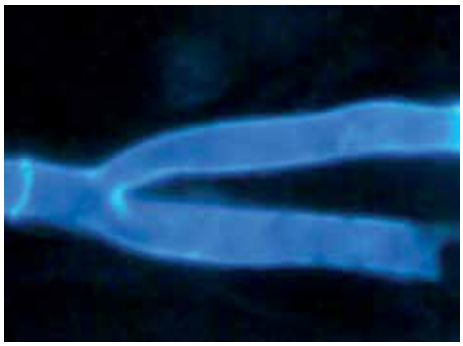
Appropriate internal controls and positive controls are included in the assay. Result interpretation is based on detection of fluorescent signals.

Thus timely accurate diagnosis will lead to appropriate management and avoid unnecessary extra antibiotics leading to prevent antimicrobial resistance.

Microbiology Quiz

Faheem Naqvi
Clinical Microbiology

A 25-years-old male patient who underwent renal transplant surgery a year before and is on prednisone 10mg/d dose and Mycophenolate mofetil 500 mg per oral dose twice daily presented with altered mentation and severe headache for last five days.



On radiologic evaluation a space occupying lesion of low attenuation was found in frontal area. He underwent craniotomy and perioperative sample send for microbiological assessment. Direct microscopic assessment at X 40 magnification showed following structure.

Questions:

1. What is the possible microscopic identification and diagnosis?
2. What is the staining technique?
3. What is the possibility of culture positivity in this situation?
4. What incubation temperature, culture plates and time required for growth?

Histologic Features Of Bacterial Lymphadenitis Other Than Mycobacteria

Dr Alka Rani
Histopathology

Inflammatory processes of lymph nodes that occur in response to a variety of pathogenic agents are viruses, bacteria, fungi, and protozoa. They may be specific or nonspecific, featuring necrosis, abscesses, granulomas, and fibrosis in various combinations. The cellular reactions in each category are characteristic and contributory to the histologic diagnosis. Adjuvant diagnostic methods such as special stains, immunohistochemistry, and in situ hybridization analysis, and polymerase chain reaction (PCR) techniques can be used in the identification of the etiologic microorganisms.

Suppurative Lymphadenitis

Opportunistic bacterial infections can involve regional lymph nodes and lead to acute lymphadenitis that may become suppurative. Infections are often caused by Staphylococcus aureus and, less often, ‘group A streptococcus’. In early phases of bacterial lymphadenitis, the nodal architecture is intact and sinuses are distended by a pale eosinophilic proteinaceous fluid with numerous admixed neutrophils and macrophages (sinus catarrh). Neutrophils eventually form microabscesses that may enlarge and coalesce to form suppurative lymphadenitis leading to abscess formation or a draining sinus. In a later stage, the acute inflammatory process subsides, and polymorphonuclear leukocytes are progressively replaced by lymphocytes, plasma cells, and particularly macrophages containing ingested cellular debris. Rarely, healed, late-stage suppurative lymphadenitis appears xanthogranulomatous. Bacterial colonies may be seen on histologic sections.

Cat Scratch Disease

Necrotizing granulomatous lymphadenitis caused by Bartonella henselae. A cat scratch, bite, or lick

introduces the bacteria at the site of cutaneous inoculation, characterized by granulomatous inflammation on histological examination of the lymph nodes. The skin lesion demonstrates a circumscribed focus of necrosis, surrounded by histiocytes, often accompanied by multinucleated giant cells, lymphocytes, and eosinophils.
The regional lymph nodes demonstrate follicular hyperplasia with central stellate necrosis with neutrophils, surrounded by palisading histiocytes (suppurative granulomas) and sinuses packed with monocytoid B cells, usually without perifollicular and intrafollicular epithelioid cells.

Lymphogranuloma Venereum Lymphadenitis (LGV)

Lymphadenitis caused by the sexually transmitted bacterium Chlamydia trachomatis. The early lesions in the lymph nodes consist of accumulations of neutrophils and small, necrotic foci. Shortly thereafter, lymphocytes and plasma cells surround the necrotic foci, which coalesce to form the typical abscesses of LGV (stellate or geographic) and they comprise a central area of necrosis and polymorphonuclear leukocytes surrounded by a zone of palisaded epithelioid cells, macrophages, and multinucleated giant cells . In tissue sections, the microorganisms stain red to violet (gram-negative, Brown-Hopp), light blue (Giemsa or hematoxylin and eosin), and black (Warthin-Starry). The suppurative lesions may form sinus tracts, which often reach the skin. Electron microscopy reveals elementary and reticulated bodies and intermediate forms characteristic of the genus Chlamydia.

Syphilitic Lymphadenitis

Lymphadenitis in the course of syphilis caused by infection with Treponema pallidum. Histopathologic features include marked hyperplasia of the lymphoid follicle. The lymph node capsule is substantially broadened by chronic inflammation and fibrosis. Arteritis and phlebitis affect the numerous, newly formed vessels of the capsule and pericapsular tissues, which show wide, perivascular cuffs of plasma cells and lymphocytes. Proliferation of plasma cells is extensive, particularly in the medulla, sometimes resulting in solid cellular sheets. Scattered, small, usually noncaseating epithelioid granulomas are sometimes present, whereas at other times single, multinucleated giant cells, completely isolated (“naked giant cells”) among the lymphocytes of the parenchyma, can be seen. Silver staining with the Warthin-Starry technique or the Levaditi technique reveals the presence of spirochetes in the lymph node parenchyma and more often in the walls of postcapillary venules and capsular vessel. Immunofluorescent and immunoperoxidase staining with specific antisera directed against T. pallidum has successfully demonstrated spirochetes.

Lymphadenitis of Whipple Disease

Lymphadenitis caused by infection with the bacterium Tropheryma whipplei. The enlarged lymph nodes comprise large numbers of foamy mononuclear cells, singly and in loose aggregates, in the sinuses and lymphoid areas. The cells are histiocytes containing a PAS-positive, diastase-resistant mucopolysaccharide substance. Tissue necrosis is absent, and the result of Ziehl-Neelsen acid-fast staining is negative.

Radiology Pathology Correlation- Orthopedic Pathology

Dr Nasir Ud Din and Dr Dawar Khan
Department of Pathology and Laboratory Medicine and
Department of Radiology

A 5 year old child presented with history of pain on wrist of several months duration. Mother noticed son having low grade fever as well. Complete blood count (CBC) showed a raised white blood count and a raised erythrocyte sedimentation rate (ESR). An x-ray of wrist showed soft tissue swelling at the wrist joint with some erosion and sclerosis of the carpal bones. Distal radius also shows lytic changes.

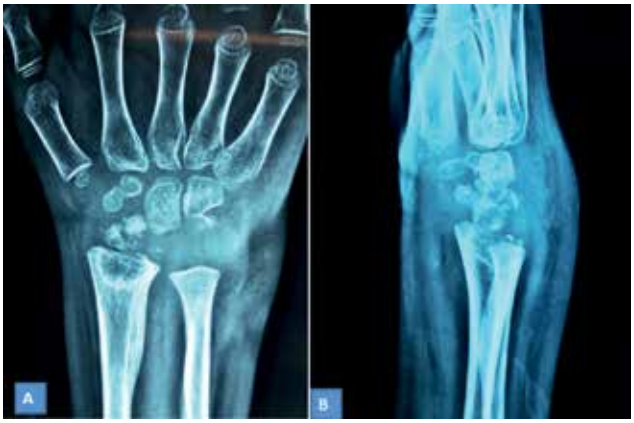


Figure 1: X-ray of AP & Lateral view shows soft tissue swelling with some erosion and sclerosis of the carpal bones. Distal radius also shows lytic changes.

A biopsy was performed for primary diagnosis and to rule out any neoplastic process. Histological examination of biopsy showed intertrabecular areas filled with scattered granulomas (Figure 2A). These were composed of collection of epithelioid histiocytes and few multinucleated giant cells. Focal necrosis was seen in the center of granulomas as well. Rare Acid Fast Bacilli were identified on Ziehl-Neelsen stain (ZN stain) stain. Fungus stain was negative. Based on radiological and histological features, a diagnosis of Tuberculous osteomyelitis was made.

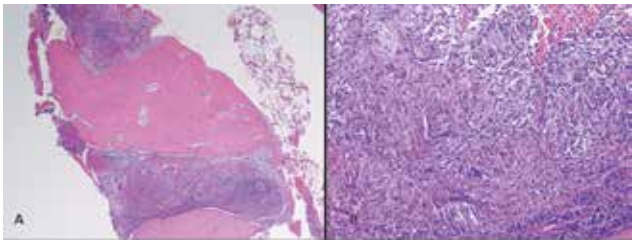


Figure 2: Low & high power magnification showed granulomas composed of palisaded collection of epithelioid histiocytes (H&E stain)

Discussion

Pakistan is one of the countries in Asia with high incidence of tuberculosis. Tuberculous osteomyelitis is one of the common causes of bone infection seen in our practice. Bone tuberculosis is usually spread from active visceral disease. Spine is most commonly involved followed by hip and knee joints. Vast majority of spinal tuberculous infection is centered in the anterior column of T6-L3 leading to the formation of a gibbous or kyphosis. Any joint can be involved and females are most affected than males. Mid-tarsal joints are commonly involved in foot bones and calcaneum is frequently involved in tuberculosis of ankle.
Poor socioeconomic status, concurrent respiratory or intestinal tuberculosis and contact with person of active disease are predisposing factors. In a local study, Montoux test was positive in approximately 2/3rd of patients. A raised ESR is typically seen, although CBC can be normal. Sensitivity of ZN stain varies as well as those of AFB culture. Diagnosis is usually made on histological examination of the affected tissue.

Tuberculous osteomyelitis is usually hematogenous in origin and is most commonly seen in bones of the extremities, including the small bones of the hands and feet. In long, tubular bones, tuberculosis often involves the epiphyses. In children, metaphyseal foci can involve the growth plate. This feature differentiates tuberculosis from pyogenic infection. The initial radiologic appearance of tuberculous osteomyelitis is similar to that of other types of osteomyelitis and includes foci of osteolysis with varying degrees of eburnation and periostitis.

Neuro-Radiology and Pathology Correlation Rhinocerebral Invasive Aspergillosis

Dr Umaima Ayesha Jilani, Dr Shayan Sirat and Dr Sidra Arshad
Department of Pathology and Laboratory Medicine and
Department of Radiology

26 year old gentleman, with no known co-morbid, presented to the consulting clinic with complain of headache and reduced vision for the past two months, which was progressively worsening. On clinical examination, he was vitally stable and well oriented.

An MRI was performed which demonstrated a

large infiltrative abnormal signal intensity lesion arising from the floor of the anterior cranial fossa. It infiltrated bilateral frontal lobes and genu of the corpus callosum. There was significant associated perilesional vasogenic oedema and mass effect with subfalcine herniation and midline shift. It also resulted in mass effect on the brain stem along with effacement of the frontal horns of bilateral lateral ventricles.
It also infiltrated the cribriform plates and showed extension into the right ethmoid sinus and the right lamina papyracea, with displacement of the right medial rectus muscle. Features were supportive of aggressive mass lesion on imaging.(Figure 1 A and B)

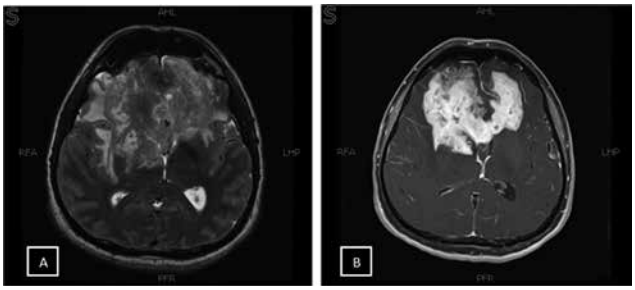


Figure 1:
Tumor like Invasive CNS Aspergillosis: (A) Axial T2WI showing heterogenous low signal intensity lesion in bi-frontal lobe showing (B) intense enhancement on post contrast T1WI

A neuro-navigation guided craniotomy and resection of the frontal space occupying lesion was done. Initially frozen section was done which was reported as granulomatous inflammation with a few suspicious filamentous organism, thus ruling out malignancy. Histological examination of the frozen and permanent sections showed glial tissue exhibiting multiple well-formed granulomas; which were composed of giant cells (Figure 2 A). The usual differential diagnosis in such cases are Tuberculous granulomas and fungal infections. Special stain PAS+/- D was performed and highlighted multiple branching septate fungal hyphae (Figure 2 B). Thus a diagnosis of asperigellosis was favoured with microbiological correlation. Culture also showed Aspergillus Flavus colonies.

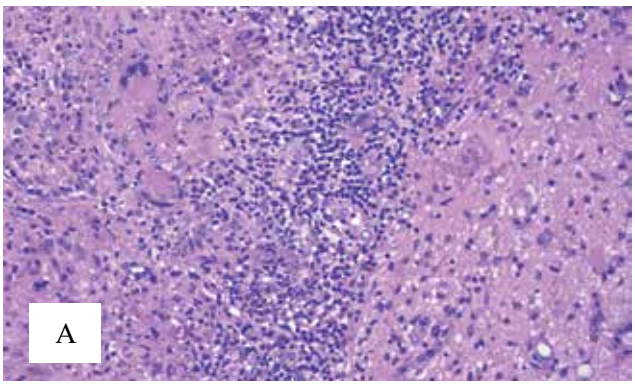


Figure 2

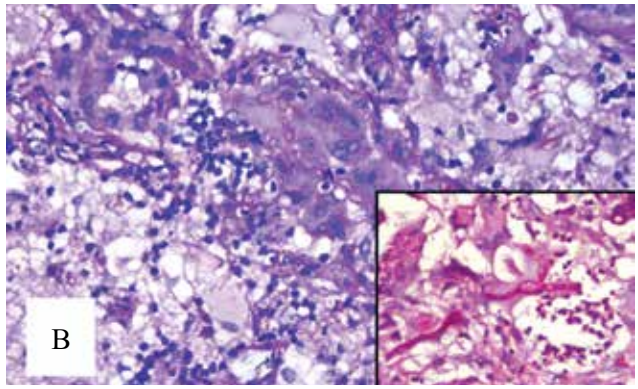


Figure 2

Discussion
Aspergillous derives its name from “aspergillum’ a perforated ball for sprinkling holy water, due to similarity of its fruiting heads with aspergillum. Aspergillosis of paranasal sinuses affects healthy and immunocompromised individuals alike. It is not contagious and source of infection is usually endogenous.
It has four basic clinic-pathological presentations. Two of these are saprophytic (Aspergilloma and allergic aspergillous sinusitis) and two are infectious (chronic indolent and invasive fulminant sinusitis). CNS aspergillosis results from angioinvasive infection of the central nervous system by the fungus Aspergillus spp. It is one of the most common fungal opportunistic infections of the central nervous system, after CNS cryptococcosis.
Tissue invasive and angioinvasive aspergillosis can be a rapidly fatal diseases.

Epidemiology
The disease predominates in immunocompromised individuals.

Clinical presentation
Symptoms of cerebral aspergillosis are non-specific and akin to other CNS infections. These include, fever, headache, meningeal irritation, nausea/ vomiting, focal neurological deficits, seizures, mental alteration or lethargy.

Pathology
There are two mechanisms of spread of Aspergillus spp to the CNS. Firstly, haematogenous spread to central nervous system. Secondly, direct spread to the CNS via the paranasal sinuses, where it may manifest as invasive fungal rhinocerebritis.

Radiographic features
The disease can have a variety of radiographic presentations, but the main three findings on **MRI** are:

Aspergillus abscess and invasive fungal rhinosinusitis
Cerebritis followed by abscess formation, are often multiple, present in a random distribution, and appear radiologically as classic ring-enhancing lesions with central diffusion restriction, identical to other brain abscesses. However, aspergillus abscesses may also have peripheral low signal intensity on T2-weighted, GRE or SWI images, and is secondary to surrounding hemorrhage.
In cases where CNS aspergillosis is secondary to paranasal sinus disease, associated invasive rhinosinusitis (either acute or chronic), osteomyelitis, local dural enhancement and subdural empyema may also be present.

Aspergillus infarction
Infarcts are often multiple, present in a random distribution, and are radiologically identical to other ischemic strokes, especially those of perforating artery territories. Hemorrhage is present in up to 25 percent, and mycotic aneurysms may or may not be identified. Infarction and associated hemorrhage are best ascertained on DWI and SWI sequences.

Tumor-like lesion
Rarely, CNS aspergillosis can present as a granulomatous tumor-like lesion. These are often hypo-to-isointense on T1-weighted images, hypointense on T2-weighted images, and demonstrate significant contrast enhancement on post-gadolinium T1-weighted images, but there is thought to be considerable variation.

Treatment and prognosis
CNS aspergillosis is treated with intravenous antifungal. Neurosurgical opinion and intervention may also be sought. In immunocompromised hosts, which encompasses nearly all cases, it has a mortality approaching 100 percent if left untreated.

Differential diagnosis
CNS toxoplasmosis, CNS tuberculosis, pyogenic abscesses, CNS lymphoma, cerebral metastases.

Role of Next Generation Sequencing in Antimicrobial Drug Resistance Detection and Monitoring

Dr Najia Ghanchi
Molecular Pathology and Microbiology

With emergence of rapid sequencing technologies, referred as next-generation sequencing (NGS), have considerably transformed the way we look at microbes. NGS enabled us to analyses pathogenic bacterial genomes, human microbiome and the environmental microbiota. Microbial whole genome sequencing (WGS) offers the potential to predict antimicrobial susceptibility from a single assay. NGS not only identifies known resistance genes but also predicts of novel genes linked with resistance, genomic origin antimicrobial resistance (AMR) and information on new markers for AMR surveillance.

The introduction of massively parallel DNA sequencing, NGS revolutionized the field and allowed hundreds of samples to be analysed simultaneously. A typical NGS workflow includes DNA extraction and fragmentation, ligation of adaptors, amplification and sequencing. There are several platforms available such as illumina Hiseq and Miseq, Thermo Ion torrent and Roche GS-FLX to name few. (Figure 1)

Figure.1 Next generation sequencing Platforms using variable chemistries.

Third-generation sequencing platforms are also introduced in market such as Oxford nanopore MinION which is the only portable, real-time device for DNA and RNA sequencing. It can easily plugged to laptops and data processing can be monitored in real time.

After the first bacterial genome sequencing in mid 90's, whole bacterial genome sequencing (WGS)

has been implemented as routine diagnostic technique in developed countries. Bacterial WGS has emerged as the ultimate technique for bacterial outbreak analysis, difficult to cultivate bacteria or the previous use of antimicrobial agents which hinders bacterial growth.

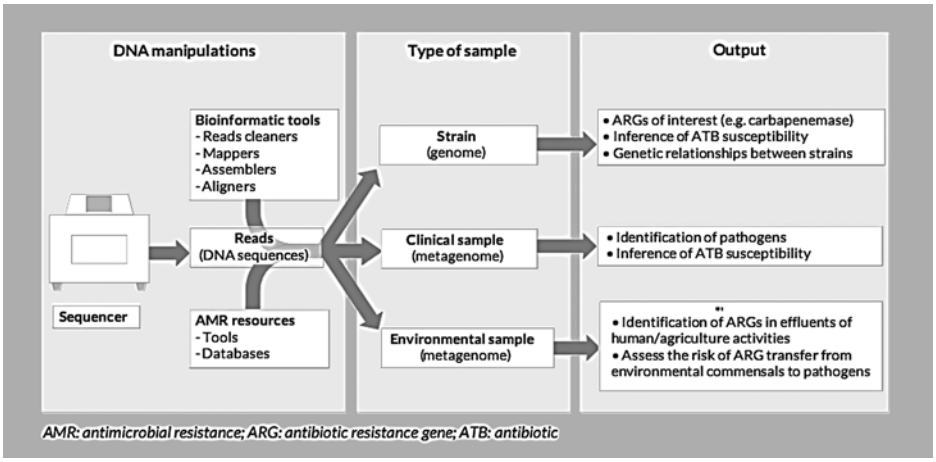


Figure 2: Workflow of the application of next-generation sequencing (NGS) in the field of antimicrobial resistance (Adapted from reference 4)

Besides WGS of microbes the important utility of next generation sequencing is clinical metagenomics. It refers to the application of NGS on clinical samples to generate information on clinical relevance, such as the identification of pathogens and prediction antimicrobial susceptibility.

The prediction of antimicrobial susceptibility for Mycobacterium tuberculosis has now become routine and it was made possible due to the highly clonal nature of the organism, the absence of horizontal gene transfers and the very large number of strains sequenced providing actionable results much faster. Indeed, Metagenomics offers the potential to directly detect all microorganisms present in a sample or even detect RNA viruses. This approach could therefore provide unbiased detection of all microorganisms present in the sample however this require lot of data mining and supporting clinical information.

Clinical metagenomics can address the AMR challenge by the rapid identification of pathogens and parallel prediction of susceptibility to antimicrobials reducing the reporting time from 48 hours to six–eight hours.

Role of Imaging in the Diagnosis and Management of Hepatic Abscess

Dr Shaista Afzal
Radiology

The most common type of visceral abscess is the liver abscess and its predominant clinical symptoms include fever and right upper quadrant abdominal pain. Liver parenchymal infection resulting in abscess formation can occur through vessels, biliary channels or by contiguous spread from adjacent structures. The multiple etiologies include bile duct lithiasis, ischemia, cholecystitis, cholangitis, liver transplant / trauma, intraabdominal collections e.g. secondary to appendicitis etc. The infection is commonly bacterial, sometimes parasitic and rarely fungal .In Africa and South East Asia, the most frequent cause of abscess is amebic infection while in the Western countries pyogenic/bacterial abscesses are more prevalent.

Imaging plays a crucial role in the diagnosis of liver abscess, with the sensitivity of ultrasound (US) and CT reported as 85 percent and 97 percent respectively. The micro abscess ie less than two cm appear as hypoechoic nodule or as an ill-defined area of hepatic parenchyma on US. The larger abscesses appear as hypo / hyper echoic lesions due to presence internal echoes resulting from of debris and thickened septae. Thus, the abscess may appear as a solid lesion; however the presence of acoustic through transmission and absence of Doppler flow in an abscess can help to exclude a solid lesion. (Figure 1a) Though, a solid tumor with extensive necrosis may be difficult to exclude.

On CT, the hepatic abscess appears as a well-defined round mass of low attenuation and enhancing peripheral rim. These may be single non loculated / single multiloculated lesions, solid phlegmonous or may be multiple lesions (Figure 2). The typical imaging feature of abscess on contrast enhanced CT is the "double target sign" which represents the central fluid as low attenuation area surrounded by high attenuation inner ring representing the pyogenic membrane and a low attenuation outer ring due to edema of liver parenchyma (Figure 1b). The other imaging signs described with liver abscess include the "cluster sign" i.e. multiple small abscesses coalesce into one large abscess and the "transient hepatic hyper enhancement" which occurs due to compression of small portal venules by the abscess and compensatory increased arterial flow.

Gas in the form of bubbles or as an air fluid level may be seen in up to 20 percent of liver abscesses. On US, the gas within abscess appears as hyper echoic foci with posterior acoustic shadowing and reverberations.

The complications of liver abscess include vascular thrombosis (i.e. of portal vein, hepatic vein, IVC), rupture (into sub phrenic space, abdominal cavity etc.) and sepsis.

The mainstay of treatment of liver abscess is anti-microbial therapy and radiological intervention. Nowadays, the operative intervention is rarely needed and image guided percutaneous drainage has been a vital advancement in management.

Image Guided Percutaneous Liver Abscess Drainage

The reported success rate of this procedure ranges from 70 to 100 percent and two methods are employed i.e. percutaneous deployment of indwelling catheter and needle aspiration. However better success rate as evident by clinical improvement and decrease in abscess cavity volume is reported with catheter drainage especially in abscess with large volume, partially liquefied or having thick pus. The pre-requisites for the procedure includes informed consent and determination of coagulation factors and in presence of coagulopathy, correction to acceptable levels is required. The procedure is performed after all aseptic measures, under local anaesthesia ie two percent lidocaine with intravenous analgesics and sedation when needed. The procedure is performed using freehand technique under real time ultrasound guidance, an 18 gauge needle is advanced in liver abscess and contents aspirated to completely evacuate the cavity if the abscess is less than three cm in largest dimension.

Percutaneous catheter drainage in indicated for larger abscesses (Figure 3 a, b, c). An, appropriate sized catheter i.e. eight to 12 French pigtail can be introduced in the abscess using the Seldinger technique and secured to the skin and connected to a closed collecting system. Catheter removal is warranted when the patient shows clinical improvement with relief of symptoms and normalization of total leucocyte count and when the follow up ultrasound shows negligible abscess cavity.

The advantages of needle aspirations are being less expensive, problems related to catheter and long term hospital care can be avoided and aspiration of multiple abscesses can be performed in the same sitting. However, its success rate is lower than catheter drainage which may lead to repeated aspirations in a short time period of one to two weeks. The catheter drainage of abscess provides continuous drainage of pus and obviates the need for repeated procedures.

The percutaneous drainage of liver abscess is a safe technique and few complications are reported like hemorrhage, persistent drainage of bile, hollow organ injury, displacement/blockage of catheter, sepsis, pleural effusion/empyema etc. The possibility of secondary bacterial infection has been infrequently reported. Failure of procedure due to thick pus or premature catheter removal can be avoided by using adequate gauge catheters and strict protocol should be followed with respect to flushing and removal of catheters.

Conclusion

Image guided percutaneous drainage is a minimally invasive, safe, effective and widely preferred treatment of liver abscess

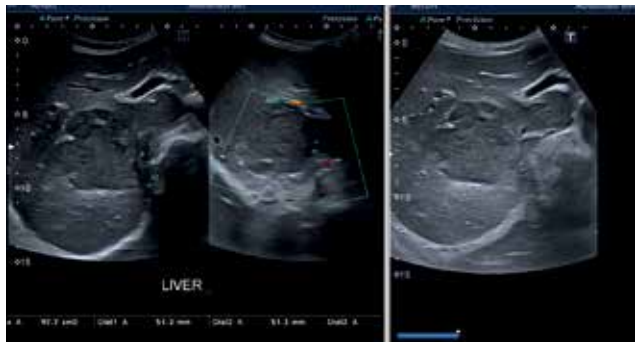


Figure 1a: US image shows liver abscess in right lobe. It appears as hypoechoic lesion with posterior enhancement. No vascularity seen within it on Doppler.

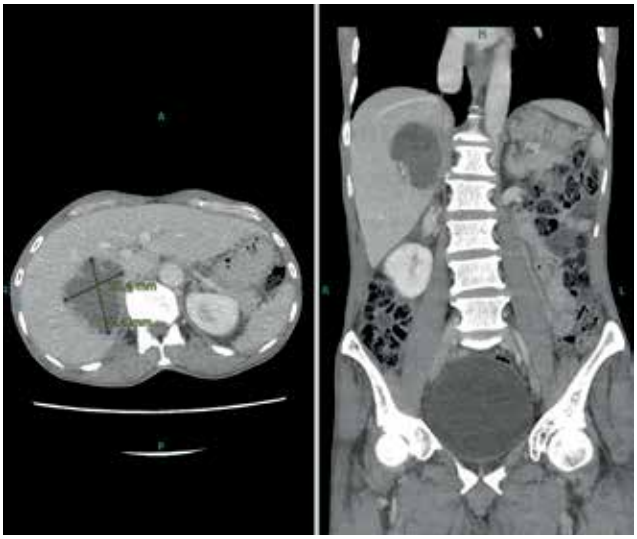


Figure 1b :CT scan of same patient shows a low attenuation lesion demonstrating "double target sign" ie central fluid as low attenuation area surrounded by high attenuation inner ring and a low attenuation outer ring

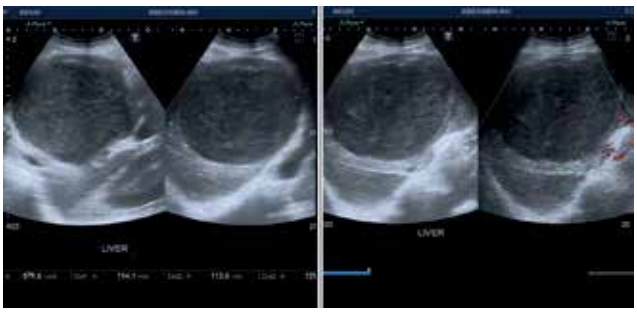


Figure 3 a:US shows a large well-defined heterogenous predominantly hypoechoic lesion without internal vascularity in liver, occupying segment IV, VIII and part of left lobe. Findings are consistent with liver abscess

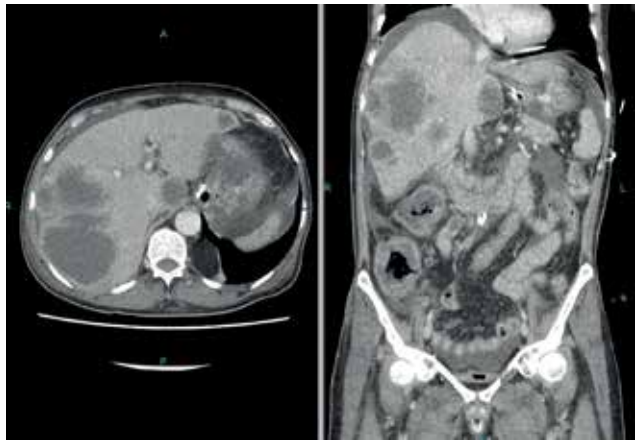


Figure 2 :Multiple well-circumscribed low attenuation lesions scattered in both lobes of liver. Severely oedematous thick-walled right hemicolon and cecum.Appearances are suggestive of amoebic colitis with multiple liver abscesses .The perihepatic fluid represent fluid due to spontaneous abscess perforation

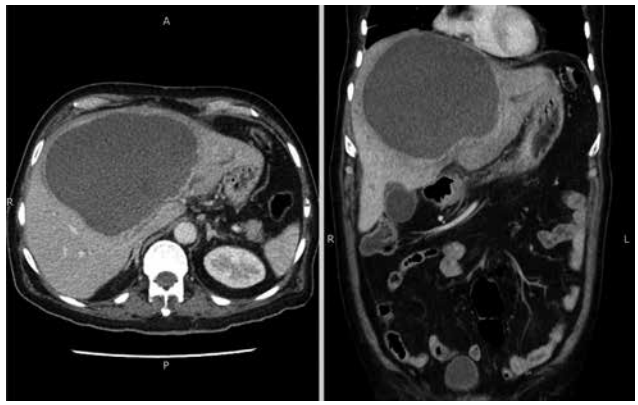


Figure 3b: CT of the same patient shows large well-defined low-attenuation lesion with peripherally enhancing wall consistent with liver abscess

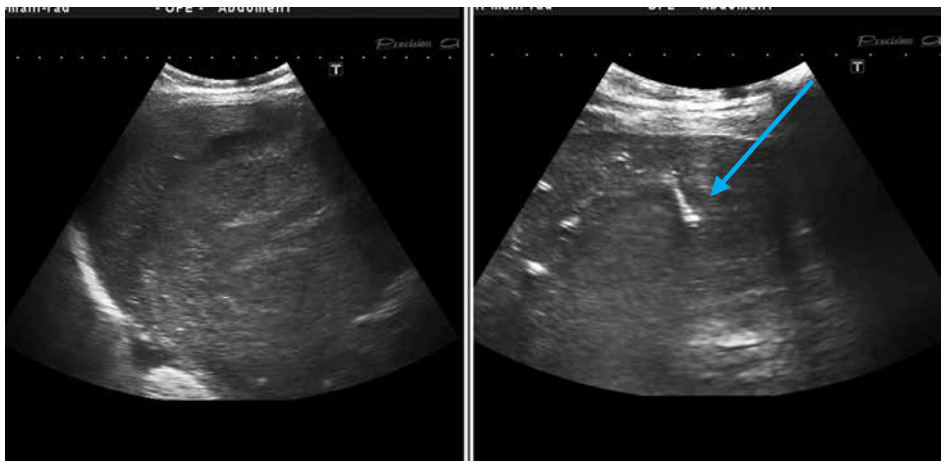


Figure 3c:US image shows the liver abscess, accessed with needle (arrow) for catheter placement

The Best of the Past

Radiologist #Vascularintervention# Followtheirlead

Interview recorded by
Dr. Shayan Anwer

Q. Considering your entire time as an Intervention radiologist at your organization, can you recall a time (any AHAA moment) when you felt most alive or most excited about your involvement in the organization?

Prof Tanveer ul Haq: I felt a similar emotion at several occasions. In my recollection, certain moments stand out like when I first started Neurointervention (first case of brain AVM in 1999) as a first case in Pakistan, a while back when I received the best section head award which, in my department, was the first of its kind, and, when I started the IR fellowship program in 2005 which was the foremost fellowship program in radiology held anywhere in the country. Most importantly, in 2014, mainly because of my considerable attributes and achievements in IR, I was promoted to Professor, which, to me, is the one occasion I consider to be the real fruit of all my previous efforts.

Q. Please briefly share your initial phase of journey i.e. from medical graduate to consultant.

Prof Tanveer ul Haq: Initially after the completion of my house job (1988), I lacked a sense of clarity regarding my future. Unresolved and uncertain about what lay ahead, I relied completely on Allah. I delved into a few peripheral private practices during which I cleared my FCPS Part 1 exam. Immediately after, I sprung into internal medicine residency at AKU. Amidst second year, a realization dawn upon me that my instincts did not match well with medicine. Luckily, while I was still in internal medicine, I got greatly inspired by radiology. This resulted in my concluding transfer to radiology, following which the direction was pretty straight forward, with successes one after the other (Alhamdolillah). I also started taking a keen interest in IR during my radiology residency, an area which was very underdeveloped during that period of time. Due to this, I was sent for fellowship training in IR to Halifax, Canada and later the entire journey moved around IR.

Q. Let's consider for a moment the things you value deeply. Specifically, the things you value about yourself and the nature of your work, what is the single most important thing your work has contributed to your life?

To "Pursue Excellence" in the field of my choice

Q. As a senior most Intervention radiologist of the country, please share your experience of development of Intervention radiology field in Pakistan and its future in next 10 years.

Prof Tanveer ul Haq: When I first began (1993), IR was quite in premature shape and we were considerably lagging behind in this modern era. I am deeply thankful to AKU and my seniors for their support and for providing the much needed facilities and a platform through which advance IR could be practiced. We therefore, pioneered almost all the advanced IR techniques and procedures at AKU within the country, and gradually came at par with the rest of the world. Our fellowship program has made a massive impact and now many centers in Karachi are being led by our ex fellows. The growth of IR, within the country, was further accelerated by the launch of national IR society (IRSP), in 2015, which could not have been possible without AKU's team effort. I became the first president of the society. More and more centers are now being introduced for advance IR practices, and this current rising pace is indicative of a very bright future for IR in the next 10 years.

Q. Any advice for Junior Radiologists?

Prof Tanveer ul Haq: Work with honesty, dedication, passion and pray to Allah, your probability for success will be much higher. Keep on striving and never loose heart. Your journey will be full of achievements if you set targets at the upper extremes.

The Best of the Past

#Microbiologist #Dedication Followtheirlead

Interview recorded by
Dr Mohammad Zeeshan

Q. How long you have been associated with AKUH

Mr Shahid Ahmed: It feels like yesterday but 31 years have passed since I joined this prestigious institution

Q. If you could change one thing about AKUH, what would I be?

Mr Shahid Ahmed: In my opinion workload distribution and hiring process is not what it used to be in past, this can make a huge difference in overall productivity

Q. One thing you like most, about working at AKUH

Mr Shahid Ahmed: Unlike other organizations AKUH has a strong system in place, so it is not a single person but the entire system that works this feature is unique to AKU

Q. Any memorable event at work from past, that you still recall?

Mr Shahid Ahmed: There are many, but I can't forget the day when entire hospital was full of armored persons when one of the prominent political person was bought to hospital after being shot, we were confined in hospital premises for many days

Q. What do you like most about your job at AKUH?

Mr Shahid Ahmed: A family environment where everyone cares for each other

Q. What is the difference between past and present AKUH environment?

Mr Shahid Ahmed: I miss all the greenery and beautiful landscape that has changed a lot over time

Microbiology Quiz's Answer

1. Thick-walled, refractile broad ribbon like aseptate hyphae 6–15 µm in diameter. Possibility of mucormycosis. Sparse septa can be seen in mucoreous mold
2. Calcofluor White that bind to chitin present in the cell wall and fluoresce in ultraviolet light
3. Sensitivity of culture is not optimal, as only approximately one third of all microscopically positive specimens reveal positive cultures.
4. All Mucorales grow rapidly (3–5 days) on most fungal culture media, such as Sabouraud agar and potato dextrose agar incubated at 25–30°C

Polaroid

17th Health Asia – 5th Annual Seminar on Radiology

Polaroid

Workshop on "Standardized Antimicrobial Susceptibility Testing" conducted in collaboration at Aga Khan University Hospital in collaboration with "Health Security Partner -HSP" USA, engaging Microbiology technical staff and faculty of private and public sector from selected institutes across Pakistan.

Dr. Muhammad Shariq Shaikh completed training in multicolour flow cytometry at the Laboratory Medicine Program,
University Health Network, Toronto. (Back row: third from right)

Polaroid

Section of Molecular Pathology, Department of Pathology and Laboratory Medicine in collaboration with the Department of Pediatrics and Child Health held a CME seminar on "Molecular diagnostics of genetic disorders" on Friday, October 11, 2019 at AKU. The purpose of the CME was to update on the new molecular diagnostic technologies available for clinical genetic testing at AKUH. These included genome testing using chromosomal microarrays, sequencing for thalassemia and other platforms for the first time in Pakistan. Importance of pre-test counselling and genetic carrier screening were also highlighted. This seminar was attended by over 200 participants which included physicians, medical students, and laboratory staff from AKU as well as from other medical institutions and hospitals. National coverage for participants from AKU Lahore lab, Shaukat Khanumm hospital and AFIP hospital was also available".

Polaroid

A Pre-Conference Half Day Workshop titled 'Aminoacidopathies from basics to diagnostic caveats' was conducted by Section of Chemical Pathology on September 20th, 2019.

A Pre-Conference Half Day Workshop titled 'Fracture Liasion service' was conducted by Section of Chemical Pathology on September 20th, 2019.

THE AGA KHAN UNIVERSITY HOSPITAL CLINICAL LABORATORIES

UPDATE
Test Update: Film Array Multiplex PCR RTI Panel (Viruses and Atypical Pathogens)

VOL. XXV No. 7, 2019

INTRODUCTION:
Acute respiratory tract infections (RTIs) are frequent causes of acute illness requiring hospitalization in both children and adults, with significant impact on patient morbidity and mortality. Early and rapid diagnosis of RTIs improves patient outcomes through identification of correct antiviral or antibacterial therapy, with potential to avoid overuse and abuse of antibiotics. More importantly, early diagnosis of highly contagious pathogens, such as *Bordetella pertussis* (*B. pertussis*) and Influenza viruses, enables early isolation of patients. Conventional microscopy/ culture and serological methods are limited due to inability to culture for atypical and viral pathogens and lower sensitivity, respectively.

The Clinical Laboratory of Aga Khan University Hospital is introducing a sensitive, **Film Array based** test on ***nasopharyngeal samples*** for patients with acute respiratory tract infections. The Film Array RTI Panel tests for 18 viral and 4 atypical respiratory pathogens which can cause acute respiratory tract infections from a single sample**, with a turnaround time of one hour**.

Organisms tested include:

Viruses:
Adenovirus, influenza A viruses H1, 2009H1, H3 (FluA-H1, FluA-2009H1, FluA-H3), influenza B virus (FluB), parainfluenza virus types 1 to 4 (Para 1–4), coronaviruses 229E, HKU1, OC43, and NL63 (CoV-HKU1, NL63, 229E, OC43), MERS-CoV (MERS coronavirus), human metapneumovirus (hMPV), Respiratory Syncytial Virus (RSV), human rhinovirus/enterovirus (Rhino/Entero)

Bacteria:
Chlamydia pneumoniae, *Mycoplasma pneumoniae*, *Bordetella pertussis,* and *Bordetella parapertussis*.

PRINCIPLE:
Multiplex PCR technique for amplification of multiple targets in a single reaction mixture.

SPECIMEN COLLECTION, STORAGE, and TRANSPORT:
Nasopharyngeal swab in viral transport media (VTM). The swab must be placed in VTM, making sure that the cap is securely tightened. If not transported within 4 hours to laboratory, store at 2°C to 8°C for up to 3 days.

REJECTION CRITERIA:
- Duplicate samples- Only one sample per patient will be tested. Specimens received in duplicate, whether same day or later follow up, will be rejected.
- Without VTM swab
- Leaking container

SCHEDULE:
The assay will be performed daily (Monday to Sunday) and reported same day by 7 pm (Cutoff time 11:00 am).

PLEASE FILE FOR QUICK REFERENCE

hospitals.aku.edu/Karachi/clinical-laboratories