Editorial Committee

Chief Editor:

Dr. Raman Sardana
Chairperson and Head, Infection Prevention and Control
Senior Consultant Microbiology Indraprastha Apollo Hospitals, New Delhi
ramansardana@apollohospitals.com

Assistant Editors:

Dr. Vikas Manchanda
Chacha Nehru Bal Chikitsalaya
Geeta Colony, Delhi
microcnbc@gmail.com

Dr. Reetika Dawar
Indraprastha Apollo Hospitals,
New Delhi
reetika_d@apollohospitals.com

Scientific Committee

Dr. Anita Chakravarty
Maulana Azad Medical College
New Delhi

Dr. Chand Wattal
Sir Ganga Ram Hospital
Delhi

Dr. N P Singh
University College of Medical Sciences
Delhi

Dr. Raman Sardana
Indraprastha Apollo Hospitals,
New Delhi

Dr. Reetika Dawar
Indraprastha Apollo Hospitals,
New Delhi

Dr. Sarman Singh
All India Institute of Medical Sciences
New Delhi

Dr. Sonal Saxena
Lady Hardinge Medical College
New Delhi

Dr. Vikas Manchanda
Chacha Nehru Bal Chikitsalaya
Delhi

Image on cover page: Dirty business: gut transplants give bacteria and scientists new choices.
Contents

Microbiology and Solid Organ Transplant .................................................................................................................. 4
  Vikas Manchanda. Clinical Microbiology and Infectious Diseases Division, Chacha Nehru Bal Chikitsalaya and associated Maulana Azad Medical College, Delhi

Time for new antibiotics-Tigecycline and Polymyxin Derivatives ........................................................................... 6
  Shivani Satia, Sonal Saxena, Renu Dutta. Department of Microbiology, Lady Hardinge Medical College, New Delhi

Probiotics and Their Current Status in India ................................................................................................................. 9
  Vidhi Jain, S. Krishna Prakash. Department of Microbiology, Maulana Azad Medical College, Delhi

Fungal Vaccines ................................................................................................................................................................. 12
  Urvashi Tiwari, Mukta Tandon, Shukla Das, Rumpa Saha, I.R. Kaur. Department Microbiology, UCMS & GTBH, Delhi

Infectious Disease Challenges in Transplant Recipients .............................................................................................. 14
  Suraiya Ansari, Sonal Saxena, VS Randhawa, Renu Dutta. Department of Microbiology, Lady Hardinge Medical College, New Delhi

Toll like receptors ............................................................................................................................................................... 18
  Charu Nayyar, Sonal Saxena, Renu Dutta. Department of Microbiology, Lady Hardinge Medical College, New Delhi

Non Tubercular Mycobacteria (NTM) with special emphasis on drug resistance mechanisms, susceptibility testing and therapy .................................................................................................................. 22
  Vrushali Patwardhan and Anita Chakravarti. Department of Microbiology, Maulana Azad Medical College, Delhi

Crossword Puzzle 0314 ..................................................................................................................................................... 26
  Reetika Dawar, Indraprastha Apollo Hospitals, New Delhi
Microbiology and Solid Organ Transplant

Vikas Manchanda
Clinical Microbiology and Infectious Diseases Division, Chacha Nehru Bal Chikitsalaya and associated Maulana Azad Medical College, Delhi

Solid organ transplantation has increased worldwide since the first successful human kidney transplant was performed in 1954. Worldwide, 40,000 organ transplants are performed annually, with very high success rates (90% 1-year graft survival). Among the solid organ transplantations (SOT) renal transplants are the most common, followed by those of the liver, heart, lung, and others, including dual organ, pancreatic, and intestinal transplantation. As immunosuppressive agents and graft survival have improved, infection and malignancy have become the main barriers to disease-free survival after organ transplantation. Over the last few decades, the field of SOT has advanced significantly, only to be continually challenged by the risks for infection in SOT recipients. Guidelines are constantly being refined to outline the most practical and appropriate screening processes to minimize donor-related infections. Conversely, attention to implementing preventive measures such as pretransplant vaccination in SOT recipients also represents an important step in optimizing safe organ donation and retention.

Management of challenges, such as healthcare-acquired infections, represent integral part of successful infection prevention strategies. Thus, clinical microbiologists can play key role in the success of transplant programs. These microbiologists must be supported by advance laboratory support.

Clinicians caring for SOT recipients have been able to guide infection-prevention and control management strategies based on the classic timeline for infections originally proposed by Rubin et al. Although newer immunosuppressive and antimicrobial prophylactic regimens have affected the pattern and timing of specific infections posttransplantation, certain general observations still hold true. One of the most important consequences of an episode of organ rejection or increased immunosuppression (from regimen modifications) is that the overall timeframe tends to get reset to an initial period of vulnerability comparable to the transplantation itself. Within the first 30 days after transplantation, the patient is at greatest risk for healthcare-associated infections, often due to antibiotic-resistant organisms and often polymicrobial in etiology. Superinfections may even develop, and these may carry a poor prognosis. Various infection risks have been dealt in great details in an article by et al in this issue of Jeevanu Times.

Remaining vigilant to the concept of net state of immunosuppression, the clinician is advised to approach solid organ transplantation (SOT)–related infections using a framework of infection risk assessment based on exposure to organisms potentially acquired through the following six different paths:

- Community-acquired pathogens
- Reactivation of previous infections (either from donor or recipient)
- Specific epidemiologic exposures, including hobbies, food and water, work, recreational activities, pets, zoonotic infections, or sexual activity
- Infection specific to donor organ
- Iatrogenic or healthcare-associated infections
- Specific travel-associated pathogens, including a range of tropical diseases

This framework will hopefully enable the clinician to relate organism category (bacteria, viruses, fungi, parasites) to each of these 6 potential sources of exposure and infection.

Approach to diagnosis of infection in solid organ transplant recipients

Summary of initial diagnostic evaluations recommended is described in Table 1.

Infection Prevention Strategies

The key infection-prevention strategies in solid organ transplantation (SOT) recipients include (1) improving adherence to the eligibility criteria for deceased donor organ donation, (2) primary or preemptive prophylaxis with antimicrobials and vaccination, and (3) promoting healthy behaviors and risk reduction in various settings post-SOT (eg, adherence to pretravel consultation for SOT recipients, infection-control recommendations in healthcare or home settings). Additionally, guidelines for reducing human immunodeficiency virus, hepatitis B virus and Hepatitis C virus transmission through organ transplantation have been released recently by CDC. Infection-control practices must be optimized for SOT recipients and providers.

Conclusions

The laboratory must make a extra-ordinary efforts to implement rapid methods that can respond to the broad spectrum of potential pathogens in solid organ transplant patients. The integration of microbiologists in multidisciplinary teams is highly recommended, and is the only method to obtain the highest quality and efficiency in the diagnostic process and clinical outcome. The role of the microbiologist is also crucial in the pretransplant period, as good microbiological evaluation at this time strongly conditions the success of the transplantation program.
### Table 1. Summary of initial diagnostic evaluations recommended for patients with Solid Organ Transplantation.12-13

<table>
<thead>
<tr>
<th>Clinical Condition</th>
<th>Initial diagnostic Evaluation</th>
</tr>
</thead>
</table>
| Fever without localizing findings | • Urinalysis and urine culture  
• Chest radiography  
• Blood cultures  
• CMV PCR  
• Purified protein derivative (PPD) (Consider QuantiFERON testing using interferon gamma assays.)  
• Antigen detection/PCR tests for adenovirus, influenza A, respiratory syncytial virus, and rotavirus |
| Pulmonary infiltrates (alveolar pattern) | • PPD (consider QuantiFERON testing using interferon gamma assays)  
• Blood cultures  
• Sputum Gram stain and culture  
• Urine Legionella and pneumococcal antigens  
• Sputum acid-fast bacillus (AFB) smear and culture (DNA probes if available)  
• Urine Histoplasma antigen in endemic areas or suggestive travel  
• Bronchoscopy if fever and infiltrates persist |
| Pulmonary infiltrates (interstitial pattern) | • Workup for pulmonary infiltrates (alveolar pattern) plus CMV PCR  
• Coccidioides serology, if warranted  
• Bronchoscopy with transbronchial biopsy if fever and infiltrates persist  
• Bronchoalveolar lavage (BAL) fluid for bacterial, viral, fungal, and AFB stains and cultures; direct fluorescent antibody (DFA) and culture for Legionella; DFA for P jiroveci; CMV PCR; cytology; modified AFB smear and culture to identify Nocardia |
| CNS symptoms | • Brain MRI (with gadolinium)  
• Lumbar puncture for CSF analysis: usual studies (cell count and differential); glucose, protein; bacterial, viral, fungal, AFB cultures, cryptococcal antigen, and several PCR probes (eg, herpes simplex virus) that may be available and relevant; and cytology  
• Consider PCR and serology for other pathogens (CMV, EBV, WNV), arboviral testing  
• Biopsy of mass lesions and/or leptomeninges (especially to identify granulomatous meningitis) |
| Diarrhea | • Stool for WBC and cultures (for enteric [Salmonella, Shigella, Campylobacter]  
• At least 2 separate stool specimens for C difficile testing (enzyme immunoassay [EIA] acceptable)  
• Three separate stool specimens for ova and parasites  
• CMV PCR (blood)  
• If stool studies unrevealing and diarrhea persists, endoscopic evaluation warranted (with mucosal biopsy); immunohistochemical staining for CMV should be performed |
| Lymphadenopathy | • EBV PCR, CMV PCR (blood)  
• Bartonella (catscratch disease) serology  
• T gondii serology  
• PPD (consider QuantiFERON testing using interferon gamma assays)  
• Biopsy of involved lymph node is often diagnostic and performed quickly to exclude PTLD and occult infections (eg, tuberculosis); node tissue should be submitted for histologic examination to look for atypical cells, granulomata, and cultures (aerobic, anaerobic, AFB, fungal and modified AFB)  
• CT scanning of neck, chest, abdomen, and pelvis might be useful to demonstrate the extent of nodal involvement. |

### References

Introduction
Emergence of Gram-negative bacteria with extended spectrum β-lactamases and metallo-β-lactamas have become major health threats that affect patient outcomes especially in intensive care unit. Acinetobacter baumannii, Pseudomonas aeruginosa and Klebsiella pneumoniae have acquired resistance to almost all available antibiotics in hospital acquired infections in critically ill patients. Due to lack of development of new antimicrobial agents and depletion of most of available therapeutic options for MDR bacterial infections, Polymyxins and Tigecycline have emerged as the last therapeutic options for treatment of patients with these infections

Polymyxins
Polymyxins discovered in 1947, are group of cationic polypeptide antibiotics consisting of 5 chemically different compounds, polymyxins A–E. Only polymyxin B and polymyxin E (colistin) are used in clinical practice. Polymyxin A, C and D are not used because of toxicity.

Structure of Polymyxins
Polymyxins consist of a polycationic peptide ring and a tripeptide side chain with a fatty acid tail. There is only one amino acid difference between polymyxin B and colistin.

Colistin was discovered in 1949 and was nonribosomally synthesized by Bacillus polymyxa subspecies colistinus. Colistin was initially used therapeutically in Japan and in Europe during the 1950s. However, the intravenous formulations of colistin and polymyxin B were gradually abandoned in most parts of the world in the early 1980s because of the reported high incidence of nephrotoxicity. Two forms of colistin are commercially available, colistin sulfate and colistimethate sodium (also called colistin methanesulfate, pentasodium colistimethanesulfate, and colistin sulfonylethylate).

Mechanism of action
Both polymyxin B and colistin are rapidly acting bactericidal agents. Polymyxins interact with lipopolysaccharide (LPS) of the outer membrane of Gram-negative bacteria and subsequently taken up via the ‘self-promoted uptake’ pathway. The polycationic peptide ring binds to the outer membrane displacing the calcium and magnesium bridges that stabilize the LPS. This results in increase in permeability of the cell envelope, leakage of cell contents and cell death. Colistin also has potent anti-endotoxin activity. The endotoxin of gram negative bacteria is the lipid A portion of LPS molecules, and drug binds and neutralizes LPS.

Polymyxin B and colistin have no activity against Gram-positive bacteria and anaerobes.

Antimicrobial Susceptibility testing
The CLSI recommends breakpoints of 2 μg/ml as susceptible, 4 μg/ml as intermediate, and 8 μg/ml as resistant to P. aeruginosa and 2 μg/ml as susceptible and 4 μg/ml as being resistant for Acinetobacter spp. No recommendation is made for Enterobacteriaceae. These recommendations apply for both polymyxin B and colistin. The BSAC recommends breakpoints of 4 μg/ml as susceptible and ≥4 μg/ml as resistant for P. aeruginosa and Enterobacteriaceae. These recommendations are for colistin only.

Figure 1. Mechanisms of action of polycationic polypeptide antibiotics on lipopolysaccharide surface of bacteria.

Spectrum of Activity
Both polymyxin B and colistin have excellent bactericidal activity against a wide variety of gram-negative pathogens.

The great majority of isolates of Escherichia coli, Klebsiella spp., Enterobacter spp., Pseudomonas aeruginosa, and Acinetobacter spp., all important nosocomial pathogens, are susceptible to polymyxins. In addition, considerable activity exists against Salmonella spp., Shigella spp., Pasteurella spp., and Haemophilus spp. Several pathogens possess intrinsic resistance to the polymyxins: Proteus spp., Providencia spp., and most isolates of Serratia spp. In addition, isolates of Brucella spp., Neisseria spp., Helicobacter pylori, Chromobacterium spp., and Burkholderia spp. are resistant. Polymyxin B and colistin have no activity against Gram-positive bacteria and anaerobes.
For disk susceptibility methodology, CLSI recommends a 10-µg colistin disk or 300-µg polymyxin B disk, Mueller-Hinton agar, a solution with a 0.5 McFarland standard for the inoculum, and a 16- to 18-h incubation time. Recommended breakpoints are ≤ 10 mm and ≥11 mm for colistin and ≤ 11 mm and ≥12 mm for polymyxin B.

**Indications for use**
Colistin and Polymyxin B should be considered for the treatment of infections caused by gram-negative bacteria resistant to other available antimicrobial agents. Apart from the intravenous route, colistin has been administered by 2 other parenteral routes (aerosolized and intraventricular).

- There is extensive experience with the use of aerosolized colistin in treating patients with cystic fibrosis. It has been observed that combination of colistin with antipseudomonal agent (azlocillin, piperacillin, aztreonam,) more effective than colistin alone.
- Synergistic activity of colistin with ceftazidime for MDR P. aeruginosa strains
- combination of colistin, rifampin, and amikacin has been used in immunosuppressed patients with multiple abscesses of the lungs, perineum, and gluteus
- Rifampin/colistin have synergistic bactericidal activity against MDR P. aeruginosa strains
- Polymyxin B is used in the treatment of infections of the urinary tract, meninges, and bloodstream caused by multidrug-resistant Gram-negative bacteria.
- Polymyxin B used topically and subconjunctivally in treatment of infections of the eye caused by Pseudomonas aeruginosa.
- In ICU settings colistin is used for treatment of Ventilator associated Pneumonia(VAP), bacteremia/ sepsis and Meningitis
- Intrathecally or intraventricularly- colistin is used as an alternative intervention for treatment of multidrug-resistant Gram-negative central nervous system infections

**Adverse Effects**
The most common adverse effects of colistin therapy are nephrotoxicity and neurotoxicity. Renal toxicity mainly includes acute tubular necrosis manifested as decreased creatinine clearance and increased serum urea and creatinine levels. Neurological toxicity is associated with dizziness, weakness, facial and peripheral paresthesia, vertigo, visual disturbances, confusion, ataxia, and neuromuscular blockade, which can lead to respiratory failure or apnea. Other adverse reactions include hypersensitivity reactions, skin rash, urticaria, generalized itching, fever, and mild gastrointestinal disorders.

**Polymyxin Derivatives**
Polymyxins are nephrotoxic, and this feature may complicate therapy or even require its discontinuation. The nephrotoxicity of polymyxins is related to the highly cationic nature of the molecule. The novel polymyxin derivatives NAB739, NAB7061 and NAB741 have their cyclic part identical to that of polymyxin B, but their side chain consists of uncharged octanoyl-threonyl-d-serinyl, octanoyl-threonyl-aminobutyryl, and acetyl-threonyl-d-serinyl respectively. Novel polymyxin derivatives carrying only three positive charges are effective antibacterial agents. NAB739 has a cyclic peptide portion identical to that of polymyxin B, but in the linear portion of the peptide, it carries the threonyl-d-serinyl residue (no cationic charges) instead of the diaminobutyryl-threonyl-diaminobutyryl residue (two cationic charges). NAB739 was effective against other polymyxin-susceptible strains of Enterobacteriaceae and against Acinetobacter baumannii. NAB740 is most active derivative against Pseudomonas aeruginosa. In various studies it has been shown that NAB7061 (linear portion of the peptide, threonyl-aminobutyryl) lacked direct antibacterial activity but sensitized the targets to hydrophobic antibiotics by factors up to 2,000

**Tigecycline**
Tigecycline, the first of new class of broad spectrum antibiotics, glycylcyclines, was recently(2005) licensed in South Africa for parenteral treatment of patients with complicated intra-abdominal infections (cIAIs) and complicated skin and soft-tissue infections (cSSTIs). Glycylcyclines are modified antimicrobial agents that possess central 4-ring carbocyclic skeleton (antibacterial activity). Substitution of an N-alkyl-glycylamido group at the 9 position on the D ring is responsible for broader spectrum of activity and evasion of resistance to tetracycline

**Figure 2. Structure of Tigecycline**

**Mechanism of action**
Tigecycline binds to the 30S ribosomal subunit, inhibiting protein synthesis .It is generally bacteriostatic- except Streptococcus pneumoniae and Legionella spp. (bactericidal).

**Figure 3. Mechanism of Action of Tigecycline**
Tigecycline overcomes two major determinants of tetracycline resistance, active efflux of drug from inside the bacterial cell and protection of ribosomes owing to steric hindrance produced by the large substituent at position 9.

**Spectrum of activity**
Tigecycline is highly active against gram-positive pathogens including MRSA, Methicillin-resistant *S.epidermidis* (MRSE), *Streptococcus agalactiae*, *streptococcus anginosus*, *streptococcus pyogenes* *Enterococci-including vancomycin resistant enterococci* (VRE). It is also highly active against *Enterobacteriaceae-Citrobacter freundii, Enterobacter cloacaee, E. coli*, *Klebsiella pneumoniae, oxytoca*, anaerobes and atypical pathogens. Reduced activity of tigecycline has been observed for *Proteus spp, Providencia spp, Morganella spp, Pseudomonas aeruginosa*.

**Tigecycline susceptibility testing**
Most published studies have a provisional breakpoint of ≤2 mg/l. *British Society for Antimicrobial Chemotherapy* (BSAC) recommends breakpoint of susceptibility of ≤1 mg/l. Testing for *Acinetobacter baumannii* is complicated by presence of manganese in Mueller-Hinton media, influence susceptibility With regard to Enterobacteriaceae owing to poor correlation between MIC and zone diameters for species other than *E. coli*, disc diffusion should not be used and MIC be determined by E-test.

**Appropriate use**
Tigecycline is used for treatment of patients with cIAI and cSSTI.

**Empiric monotherapy**
- In the elderly or patients with significant co-morbidity who have received frequent antibiotic therapy or are from long-term care facilities and are at risk for resistant bacteria such as ESBL-producing strains polymicrobial MDR infections.
- Serious and complicated infection due to MRSA and or ESBL-producing infections in patients with renal dysfunction.
- Where there has been treatment failure with other broad spectrum agents.
- Infections with organisms likely to be susceptible to tigecycline in patients with β-lactam allergy.

**Directed monotherapy**
- Polymicrobial Infections with MDR organisms, serious and complicated infection due to mixed infections of MRSA or ESBL-producing organisms.
- MRSA Infections in presence of renal dysfunction - alternative to Linezolid.

**Directed combination therapy**
- Utilised as Salvage Therapy in combination with other agents.
- Combinations with Polymyxin and/or fosfomycin and/or rifampicin have been reported.

**Inappropriate use**
- Patients with cIAI who are at risk of infection with *P. aeruginosa*, and in particularly those with recurrent infection.
- Patients with cSSTI where *P. aeruginosa* is a predominant organism, chronic diabetic foot infection.
- Patients who are not at risk of resistant infections.
- To avoid development of resistance it should not be used for infections caused by gram-positive organisms only, unless other agents have failed to work.

**Conclusions**
Polymyxins and Tigecycline promises as new antimicrobial agents can be administered as monotherapy to patients with certain serious bacterial infections. Clinicians should prescribe these drugs appropriately to avoid the emergence of resistant strains. Tigecycline confers broad antibiotic coverage for vancomycin-resistant enterococci, methicillin-resistant *S. aureus*, and many species of multidrug-resistant gram-negative bacteria and polymyxins are highly effective for multidrug-resistant gram-negative bacteria.

**References**
2. Nickie D. Greer, Pharm D. Tigecycline (Tygacil): the first in the glycycline class of antibiotics; Proc (Bayl Univ Med Cent)200; 19:155-161.
What are probiotics?
The story of probiotics started with the description of lactic acid producing bacteria by Louis Pasteur in 1857 and their isolation from rancid milk by Joseph Lister in 1878. Elie Metchnikoff observed that some bacteria were beneficial to health and postulated that they could be administered to humans to replace harmful bacteria in the gut. For his foresight and pioneering work in the infancy of the study of probiotics, he has been named the “father of probiotics”. Henry Tissier (1908) isolated Bifidobacterium from the faeces of breast fed infants and called it “Bacillus bifidus communis”. Alfred Nissle (1917) Isolated E.coli from faeces of a healthy soldier and used the strain in the prevention of Shigellosis during an outbreak. Minoru Shirota (1930) cultured a strain called Lactobacillus casei “shirota”. A drink incorporating it called “Yakult” was commercially released in 1935 and the concept of consuming beneficial microorganisms orally for the promotion of health started becoming widely acceptable. The term “probiotic” meaning “for life” was first proposed by Lilly and Stillwell (1965), refined by Fuller (1989) and finally given the form of a scientific definition by WHO and FAO to mean “Live microorganisms which when administered in adequate amounts, confer a beneficial effect on the health of the host”.

What are the biological effects of probiotics?
The biological effects of a probiotic are strain-specific. This means that the beneficial effects attributed to one strain cannot be assumed to be provided by another strain, even when it belongs to the same species. The main feature of beneficial strains is “competitive exclusion”. This means, the probiotic strain is able to colonize the gut and to thereby reduce the binding sites available for pathogens to bind. Additionally they aid the host in short chain fatty acid metabolism which promotes the integrity of the gut mucosal barrier and also smoothens enterohepatic circulation. Some probiotics can produce Vit B12, Vit K and beta galactosidase enzymes to aid in the digestion of lactose, stimulate immune system to promote mucosal immunity and suppress inflammation. Many traditional Indian foods already contain helpful bacteria.

Do probiotics offer anything more?
Many traditional food items like curd, lassi, chhachh, idli, dosa, uthapam, vada etc contain fermenting bacteria that have long been assumed to have health-promoting effects. However, there is no knowledge regarding the exact strain(s) present in the food product. It varies from preparation to preparation and even between households! Also, there is no method available for the quantification of the amount of probiotic present per unit of the food product. If the quantity is insufficient, the food item may not have any therapeutic potential despite containing an efficacious probiotic strain. Commercially available preparations usually contain a combination of probiotic strains in quantities >10^9 CFU/unit to ensure target site availability and are thus considered superior to traditional preparations.

What criteria must be met for the approval of a strain as a probiotic?:
1. The strain should be of human origin
2. It should be able to survive gastric acid and bile to ensure viability at target site
3. It should be able to adhere, multiply, aggregate and colonize the intestinal mucosa
4. It should be safe for human use : Non-invasive, non toxigenic, non-pathogenic, and its genome should be free of resistance genes
5. It should not be associated with infectivity in immunocompromised animal models
6. It should be able to withstand processing conditions
7. It should have a scientifically proven health claim

Some examples of probiotics:
- Lactobacillus spp : L. acidophilus, L. casei (rhamnosus),L. reuteri, L. bulgaricus, L. plantarum, L. johnsonii, L. lactis
- Bifidobacterium spp : B. bifidum, B. longum, B. breve, B. infantis, B. lactis, B. adolescentis
- Saccharomyces spp: S.boulardii, S.cerevisiae
- Streptococcus spp : S.cremoris, S.diacetylactis, S.intermedius, S.thermophilus, S.lactis and Enterococcus faecium SF68
- Aspergillus oryzae has also been shown to have some beneficial affects

Figure 1 : Functional classification of the currently available probiotics
Table 1. Summary of Common clinical conditions and respective probiotic use.

<table>
<thead>
<tr>
<th>Clinical condition</th>
<th>Probiotic microbe(s) which may be used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose maldigestion</td>
<td>Lactic acid bacteria and <em>Streptococcus salivarius</em> subsp. <em>Thermophilus</em></td>
</tr>
<tr>
<td>Gastroenteritis acute diarrhea</td>
<td>Lactic acid bacteria, <em>Bifidobacterium</em> species, or <em>Saccharomyces boulardii</em></td>
</tr>
<tr>
<td>Antibiotic-associated diarrhea</td>
<td>Lactic acid bacteria or <em>S. boulardii</em></td>
</tr>
<tr>
<td>Traveller’s diarrhea, allergies, dental caries, <em>Clostridium difficile</em> induced colitis</td>
<td>Lactobacillus casei GG strain</td>
</tr>
<tr>
<td>Inflammatory bowel disease or irritable bowel syndrome</td>
<td>Lactic acid bacteria and <em>Bifidobacterium</em> species, <em>S. boulardii</em></td>
</tr>
<tr>
<td>Acute infectious diarrhea in children</td>
<td>Lactic acid bacteria specifically <em>L. rhamnosus</em></td>
</tr>
<tr>
<td>Rotavirus nosocomial infection in children</td>
<td><em>L. casei, L. bulgaricus, S. thermophilus, L. acidophilus</em></td>
</tr>
<tr>
<td><em>Helicobacter pylori</em> eradication</td>
<td><em>L. rhamnosus, L. johnsonii</em></td>
</tr>
<tr>
<td>Pouchitis</td>
<td><em>B. longum, B. infantis, B. breve, L. acidophilus, L. casei, L. delbrueckii subsp. bulgaricus, L. plantarum, S. salivarius subsp thermophilus</em></td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td><em>B. infantis, S. salivarius subsp. thermophilus, B. bifidum</em></td>
</tr>
</tbody>
</table>

What are the health claims associated with probiotics?
It has been claimed that specific probiotics can be used to treat certain conditions summarized in table 1.

What are Designer probiotics?
Designer probiotics have been genetically engineered to express structures that mimic receptors on their surface. The receptor mimics could be either epithelial receptors for attachment of the pathogen or binding sites for toxins. In the former case, the probiotic is designed to express receptors for pathogen attachment and compete with receptors on gut epithelial cells for the same. As a result, the pathogens binds to the designer probiotic and the gut epithelium is protected. In the latter case, probiotics express receptors for toxin binding and neutralize toxins in the gut lumen. Additionally, the designer probiotic may secrete cytotoxins that kill the pathogen in the gut lumen. The designer probiotic available in India is ViBact which is made up of genetically modified *Bacillus mesentericus*. This acts as an alternate to B-complex capsules.

What is the status of probiotics in India?
Currently probiotics are only approved as “functional foods” in India. Functional foods are defined as products that are similar in appearance to conventional foods that are consumed as part of a normal diet and have demonstrated physiological benefits, and/or have the potential to reduce the risk of chronic disease beyond nutritive function, i.e. they contain bioactive compounds.

Probiotics are not formally approved as “drugs” in India as yet though many market formulations containing Lactobacilli in conjunction with antibiotics are available for treating diarrhea in paediatric population.

Which agencies regulate probiotics in India?
Functional foods are regulated by PFA (Prevention of Food Adulteration Act) as per The Food Safety and Standards Act of (FSSA) and Drugs are regulated by FDA. Evaluation of probiotics in food is carried out by ICMR.

Are there any Indian guidelines regarding probiotics?
ICMR-DBT guidelines have been issued in 2011 to regulate probiotics in food and major stress is laid on the identification of probiotic strain, health claims and labelling claims.

ICMR-DST Guidelines for evaluation of probiotics in food
Probiotics should be evaluated for:
1. Genus, species and strain identification
   • Phenotypic identification
   • Genotypic identification
2. *In vitro* tests to screen potential probiotic strains
   • Resistance to gastric acidity.
   • Bile acid resistance.
   • Antimicrobial activity against potentially pathogenic bacteria (acid and bacteriocin production).
   • Ability to reduce pathogen adhesion to surfaces.
   • Bile salt hydrolase activity.
3. *In vivo* safety studies in animal models
4. *In vivo* efficacy studies in animal models
5. Evaluation of safety of probiotics for human use
   • Determination of antibiotic resistance patterns.
   • Assessment of undesirable side-effects.
   • Tests for toxin production and hemolytic activity respectively if strain belongs to similar category.
6. Evaluation of efficacy studies in humans
7. Effective dosage of probiotic strain / strains
8. Labeling requirements
   • Genus, species and strain designation following the standard international nomenclature.
   • The minimum viable numbers of each probiotic strain should be specified at the level at which efficacy is claimed and at the end of shelf- life.
   • Evidence-based health claim(s) should be clearly stated.
   • The suggested serving size to deliver the minimum effective quantity of the probiotic related to the health claim.
   • Proper storage conditions to be mentioned.
9. Good Manufacturing Practices
   • Hazard analysis
   • Critical control point

Which are the probiotics currently available in India?
Probiotic food market in India is currently dominated by Amul, Mother Dairy and Nestle. Some probiotic based pharmaceutical formulations named Sporolac, ViBact, Darolac, Biglac, Bifilac etc are also available.

Where does India stand in the global probiotics market?
Most probiotic products across the world are available as milk formulations or fermented milk products. While India presently accounts for less than 1% of the total world market turnover, the Indian probiotic industry is evolving at a steady pace. The Indian probiotic industry is expected to show tremendous growth in the near future because India has the highest population of cattle and is the world’s largest producer of milk. The Indian probiotic industry is valued at more than $2 million at present and is expected to reach $10 million in the next 2-3 years. Major pharmaceutical companies have become active and are trying to formulate newer drugs and products. Packaged products like probiotic-based nutritional supplements for special conditions like pregnancy, lactation, immunodeficiency etc and products especially formulated for pediatric and geriatric patients are in the pipeline.

References:
Fungal Vaccines

Urvashi Tiwari, Mukta Tandon, Shukla Das, Rumpa Saha, I.R. Kaur
Department Microbiology, UCMS & GTBH, Delhi -110095

Introduction
The incidence of invasive fungal infections has been on a rise. The morbidity and mortality due to such infections remains high leading to increase in healthcare costs. Fungal vaccines are being developed to enhance the ability of the immune system to clear fungal pathogens. In cases where total extermination of the fungal elements is not possible, vaccines can prevent reactivation of already dormant fungi. Till date, none of the vaccines have been approved by the US-FDA. This could be attributed to the high cost of antigen production and high cost of conducting Phase-1 clinical trials and compromised immune status of the patient.

Vaccine Candidates for fungal pathogens

Yeasts : Candida albicans, Cryptococcus neoformans
Candidemia is the fourth most common noscomial bloodstream infection in US and Europe having a high mortality rate, despite antifungal treatment. Many vaccine trials for Candida have been undertaken, two of which have entered Phase-1 clinical trials. Of them, the first utilizes Agglutinin like sequence (Als) proteins. It protected mice from disseminated candidiasis. Phase-1 clinical trial in human subjects was performed by Novadigm Therapeutics. The vaccine was well tolerated and stimulated both humoral and cell mediated immunity. The other vaccine, Sap2p (secretory aspartyl proteinase) has gone through Phase-1 trials in 2010 and had shown tolerability and efficacy at low doses against candidal vaginitis.

Vaccines in preclinical trials include live attenuated vaccines such as Candida albicans tet-NRG1 strain and protein conjugate vaccines utilizing antigens such as laminaran linked to diphtheria toxoid, mannoprotein fraction (MP-F2), candidal heat shock proteins, surface proteins such as HYR1 and enolase. Cryptococcus causes life threatening infections in patients with substantially compromised cell mediated immunity. One of the vaccine trials which had advanced into Phase 1 was based on glucoronoxylomannan (GXM) capsule which is an important virulence factor. On conjugation with tetanus toxoid, this vaccine was found to be antigenic and appeared to be safe. Other antigens included in vaccine are yeast-mannosylated ovalbumin and protein conjugated laminaran vaccine.

Molds : Aspergillus spp
Aspergillus is the second most common cause of nosocomial invasive fungal infection after Candida. However, the chief hindrance to effective vaccination in these patients is the depressed cellular and humoral immunity. Recombinant antigen such as Aspf3 and more recently Asp16f are studied as vaccine candidates in murine models. They were found to improve survival in these models. Other vaccine models include mouse dendritic cells pulsed with Aspergillus or dendritic cells transfected with IL-12 expressing adenoviral vector caused stimulation of type 1 immune responses on being exposed to Aspergillus fumigatus.

Dimorphic Fungi : Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis, Paracoccidioides brasiliensis
Histoplasmosis is common among AIDS patients because of their suppressed immunity. Recombinant antigen such as heat shock proteins hsp 60 and hsp 70 have been utilized for vaccine trials, which have shown to confer protection in mice. Subcutaneous injection of apoptotic phagocytes containing heat killed H.capsulatum results in cell mediated protection. B.dermatitidis is a dimorphic fungal pathogen which when inhaled may cause disease in immunocompetent hosts. One of the most important vaccine candidate is Blastomyces adhesin 1 (BAD 1) antigen, formerly called as WI-1, which has been found to stimulate both cell mediated and humoral response. Coccidioidomycosis can result in severe pulmonary infection and dissemination to meninges, skin, bone and joints in immunocompromised individuals. A mutant of Coccidioides posadasii having a double gene knockout, CTS2 and CTS3 (chitinase genes) was used as a live attenuated vaccine candidate. The vaccinated mice, which were challenged with intranasal organisms, showed a mixed Th1, Th2 and Th17 type immune response. Other antigens studied include spherule outer wall (SOW) antigen and recombinant protein chimera of Antigen 2/Proline-rich antigen (Ag2/PRA) cell wall antigen combined with Coccidioides-specific Antigen (CSA). There was lack of toxicity but the immunogenic potential was not significant. Therefore further clinical trials were not undertaken. Alkali-soluble water-soluble antigen (C-ASWS) showed partial protection in mice. Paracoccidioidomycosis is a fungal disease that is characterized by a pyogranulomatous tissue reaction, found principally in the tropical and subtropical areas. Vaccine containing Gp43 antigen can reduce the burden of P. brasiliensis in the lungs of mice inoculated intratracheally and reduces the severity of inflammation in lungs.

Trials in Immunocompromised individuals : Pneumocystis jiroveci
Pneumocystis pneumonia is an important disease of immunocompromised humans, particularly patients with HIV. The various vaccine candidates include a recombinant p55 antigen and glycoprotein A antigen.
Active immunization against *Pneumocystis jirovecii* with p55-v3 DNA vaccine in rats has shown encouraging results\(^{10}\). Major surface glycoprotein (MSG) or glycoprotein A is an immunodominant antigen injection into mice lead to a reduction in the burden of *P. jirovecii* compared to that in controls\(^{11}\).

**Conclusions**
There is a growing need for preventive or therapeutic vaccines to limit the rising incidence of fungal infections. There have been few clinical trials in humans till date. A major reason for the lack of human trials has been the very high costs relating to good manufacturing process and production of the antigens, toxicity studies in animals, and high costs for clinical trials in humans. Prototypic antigens have been identified, against which protective immunity can be induced. But concern regarding the safety and efficacy of fungal vaccines has been raised including safety in immunodeficient hosts. Therefore further clinical testing should be undertaken to evaluate the efficacy of fungal vaccines in humans.

**References**
Infectious Disease Challenges in Transplant Recipients
Suraiya Ansari, Sonal Saxena, VS Randhawa, Renu Dutta
Department of Microbiology, Lady Hardinge Medical College, New Delhi

Introduction
Transplantation is one of the many lifesaving procedures that occur every day throughout the world. People have attempted to transplant organ for over 1000 years but first successful human organ transplantation occurred in 1956 (kidney transplant). Successful organ transplantation became a reality only after the discovery of immunosuppressive agents. Graft failures are still very common despite the use of variety of newer immunosuppressive agents. Most common cause is Rejection followed by infections as the second most common cause of graft failure and is now the main barrier to disease free survival after organ transplant.

Impact of infections on transplantation
Infections are the leading causes of morbidity and mortality in all transplantation. More than fifty percent of all organ recipients have active infection post-transplant. Their effects can be direct or indirect and results in global depression of host defenses, opportunistic super infection allograft injury and may even contribute to some malignancies.

Challenges in transplant related infections
Immunosuppression which is an obligation for transplant survival is also accompanied by some negative effects. Inflammatory responses associated with microbial invasion are impaired leading to diminished and muted clinical and radiological findings. As a result infections are often advanced at the time of detection. This imposes diagnostic challenges. So, often aggressive and invasive investigations are required even for minor findings. Moreover there is greater diversity of organism and faster rate of progression of disease. Also there can be medication related toxicities and drug interactions which require continuous monitoring of drug levels.

Factors contributing to infection in transplant recipients
Pre-transplantation host factors: Underlying medical condition & chronic infection may persist or even worsen after transplantation. Prior latent infection may reactivate producing clinical infection. Prior colonization with organism is a risk factor and even pre transplant medication may influence post-transplant susceptibility to infection.

Transplantation related factors: Type of organ transplanted is an important determinant of type of infection. Site of transplantation itself is most common site of infection. Ischemic injury to allograft or any allograft reaction further decreases resistance to infection. Surgical factors contributing to infection include duration of surgery, surgical stress, loss of blood, tissue damage and metabolic derangements.

Immunosuppression: It is the most important factor contributing to infection. Besides the therapeutic effect of suppressing rejection, there can be acquired immunodeficiency’s leading to Infections or direct or indirect toxicity to host tissue. Among the various immunosuppressive agents, corticosteroids broadly inhibit variety of immune responses and are associated with very high risk of infection related morbidity and mortality. So nowadays, early steroid withdrawal or steroid free regime are prescribed by many transplant centers. Among the newer agents Azathioprine and Mycophenolate mofetil (Antiproliferative agents) are associated with lower rate of infections. Calcineurine inhibitors like Cyclosporine and Tacrolimus may slightly increase the risk of CMV and herpes infection. Recently available monoclonal and polyclonal antibodies like Daclizumab and Basiliximab are almost associated with no added infectious risk.

Common Microbial agents causing infection after transplantation
Bacterial agents: Gram negative bacteria including enteric bacteria, Pseudomonas, Acinetobacter, Serratia, Bacteroides are the most frequent cause of bacterial infections, mainly associated with superficial wound infections, urinary tract infections, infections of lung, thorax, abdomen etc. Legionella infection is acquired nosocomially from contaminated water supply. Gram positive bacteria include Staphylococcus aureus, CONS, Streptococcus spp, Enterococcus spp, Listeria monocytogenes, Nocardia. VRE have become the major pathogen in liver transplant recipients. Infections with MRSA and Staphylococcus epidermidis have increased in frequency. Gram negative coccobacilli include Haemophilus influenza and Moraxella spp which are particularly important in lung transplant.

Fungal agents: The most common fungal agents are Candida, Aspergillus, Cryptococcus, Histoplasma, Coccidioidomycosis, Blastomycosis, Pneumocystis jirovecii.

Viruses: Most common viruses include Herpes group (HSV, CMV, EBV, HHV-6 & 7, HHV-8), Hepatitis B & C, HIV, Adenovirus, Rota virus, Influenza & Para influenza, West Nile, Polyoma virus- BK, JC and SV. Herpes infections are most common as individuals are latently infected that gets reactivated on immunosuppression. Donor transmission is an important source of CMV, HSV, EBV, Hepatitis B & C. HIV positivity precludes organ donation but nowadays organ transplantation is being done from HIV positive donors to HIV positive recipients with well controlled infection. Influenza virus, Rota virus and adenovirus infections are associated with community outbreak. Polyoma virus nephropathy is particularly important in renal transplant recipients.

Parasites: Most common parasitic agents are Toxoplasma gondii, Strongyloides stercolalis and Trepanosoma cruci (primarily in heart and lung recipients). Others are rare and include Acanthamoeba, Cryptosporidium, Giardia, Babesia, Echinococcus, T.sollium, Leishmania donovani.
Donor derived infections
Donor-derived infections are of particular significance, as evidenced by several reports of infectious diseases transmitted through transplanted organs. They include viruses (hepatitis B and C, herpes viruses, human T-cell lymphotropic viruses (HTLV) 1 and 2, West Nile virus, rabies, LCMV, polyomavirus BK/JC, HPV, parvovirus B19, HIV), mycobacteria (tuberculous and nontuberculous mycobacteria), meningococcus, syphilis, parasites (malaria, Babesia, Toxoplasma gondii, Trypanosoma cruzi [Chagas disease], S. stercoralis), and several fungal organisms. Donor-derived drug-resistant bacteria may also be transmitted, including vancomycin-resistant enterococci, MRSA, and fluconazole-resistant Candida species.

Time scale of infection after transplantation
There are similar patterns of infection in all forms of organ transplantation and there is a consistent timetable for when different infections occur after transplantation. This timetable is most easily organized into three segments: the first month, one to six months, and more than six months after transplantation. The clinician may use this timetable as a tool for developing a differential diagnosis in transplant recipients who present with infectious diseases, a tool for detecting excessive environmental exposure to pathogens that cause deviations from the timetable, and a guide to the design of cost-effective, targeted preventive strategies.

Table 1: Major infective agents transmitted by donated organs

<table>
<thead>
<tr>
<th>Type of tissue</th>
<th>Infective Agent(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney, Heart, Liver, Lung, Bone marrow</td>
<td>CMV</td>
</tr>
<tr>
<td>Heart, Kidney</td>
<td>Toxoplasma</td>
</tr>
<tr>
<td>Heart</td>
<td>Trypanosoma cruzi</td>
</tr>
<tr>
<td>Kidney, Liver</td>
<td>HSV</td>
</tr>
<tr>
<td>Kidney</td>
<td>HHV-8</td>
</tr>
<tr>
<td>Kidney, Heart, Liver</td>
<td>HIV, HBV, HCV, West Nile virus</td>
</tr>
<tr>
<td>Kidney, Liver, Lung</td>
<td>LCMV</td>
</tr>
<tr>
<td>Kidney, Liver, Cornea</td>
<td>Rabies</td>
</tr>
<tr>
<td>Blood</td>
<td>CMV, EBV, HIV, HBV, HCV, HTLV 1</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>CMV, HIV</td>
</tr>
</tbody>
</table>

Figure 1: Changing timeline of infection after organ transplantation
Infections specific to transplant type

**Lung Transplant:** Infections are most commonly related to technical complications. Post-transplant survival is less because of high risk of infection. Besides MRSA and VRE, *Burkholderia* and *Pseudomonas* are a special threat especially in cases of Cystic Fibrosis. There are high chances of Tuberculosis reactivation. CMV and other Herpes group of viruses are most common opportunistic infections after bacterial infections. Among fungal agents, *Candida* is a common cause of blood stream infection. *Aspergillus* is the most feared pathogen. Its detection triggers increased concern for rejection.

**Liver Transplant:** Liver transplants are also associated with very high rate of infections like liver abscess, cholangitis, peritonitis, occasional BSI and Surgical site infections. Most common agents are bacterial including gram negative enteric bacilli, Enterococci etc. *Candida* is a predominant organism, overall, one third are non albicans because of fluconazole prophylaxis.

**Kidney Transplant:** Kidney transplants have the highest 5year survival rate (80-90%) because of reduction in infection related morbidity. Urinary tract infections are most commonly bacterial. Nephropathy caused by BK virus is an important cause of graft dysfunction.

**Heart Transplant:** Infections are the leading causes of mortality. Pulmonary infections, Mediastinitis, sternal wound infection and endocarditis are quite common after heart transplant. Nocardia infections . Endemic mycoses and parasitic infections like toxoplasma are more frequently reported after heart transplant.

**Intestinal Transplant:** Intestinal transplant recipients are at a higher risk of infections because it is rich in lymphoid tissue and contains a large burden of microorganism. It is technically complicated and very few centers are doing it.

**Approach to the febrile organ transplant recipient**

Diagnosis in febrile organ transplant patient is one of the most challenging situations. A strategic approach is followed by reviewing the time frame around specific infection, careful evaluation of organ specific consideration and recognition of specific associations. We can also use a framework of infection risk assessment based on community acquired pathogens, reactivation of latent infections, specific epidemiological exposure infection specific to donor, healthcare associated infection and specific travel associated pathogens.

**Preventive strategies**

Preventive strategies include vaccination, universal and targeted prophylaxis covering pre, peri or post treatment period and should be directed against bacterial, viral, fungal or other agents.

**Pre-transplantation donor and recipient screening**

Donor & recipient are screened pre transplantation by careful epidemiologic & clinical history, assessment of high risk behavior, serologic testing (HIV, HBV, HCV), microbiological culture (blood, urine, sputum), nucleic acid tests based assays and radiological investigation (chest x ray). Risk factors can be categorized into high risk with usage of corticosteroids, early graft rejection, graft dysfunction, active or latent infection and technical complications or low risk after technically successful surgery with good graft function, surgical prophylaxis, effective antiviral prophylaxis and having appropriate vaccination.

**Figure 2: Pre transplant donor and recipient screening**

Challenges with donor and recipient screening

Certain challenges are faced during donor screening by limitation in proper history taking, serology may be confounded and cost and logistical limitations to NAT testing (turnaround time). Other important question arises as to the reliability of serologic tests because it will rule out most infected individuals but false negatives are of concern and window period for detection is key. Window period for HIV is 17-22 days (NAT-5-6days). HCV is approximately 70 days (NAT-3-5days) and for HBV it is 35 – 44 days (NAT-20-22days). Guidelines for US Donor screening 2011 recommends NAT Testing in all deceased donors for HIV and HCV regardless of time relative to transplant and HBV testing only if risk factors within 12 months are present. For living donors it is recommended to test for HIV and HCV as close to the time of organ recovery. Recipients should get informed consent for high risk donors and undergo post-transplant monitoring at the time of transplant, 1 & 3 months post-transplant and 12 months post-transplant. They should be monitored for infection and immune function by standard serologic tests for HBsAg screen, HBeAb ,HCV Ab , HIV Ab , CMV Ab , EBV Ab , HSV Ab , VZ Ab and Toxoplasma Ab. Other standard assays include microbiologic cultures and susceptibility testing, Quantitative viral load assays, Histopathological examination & Immunostaining . Advanced assays include multiplex quantitative assays, genomic arrays, nongeneric
Immunoassays for degree of Immunosuppression and assays of pathogen specific immunity by Interferon release assay and Enzyme linked immunospot assay.

Vaccines
Recipients should be administered all usual vaccines and boosters prior to transplant as it is less effective during immunosuppression. Inactivated vaccines are safe to use starting 3 months after transplant. Avoid live virus vaccines after transplant (minimum 4 weeks from live vaccine to transplantation). They should also receive influenza vaccine annually and pneumococcal vaccine every 3-5 years.

Prophylaxis
Universal prophylaxis includes antimicrobial therapy to all at risk patient for a defined interval whereas preemptive therapy includes routine use of specific microbiologic assay to monitor patient at risk and antimicrobial therapy to a clinical event in asymptomatic recipients. Antimicrobial therapy with Trimethoprim sulfamethoxazole is given in UTI, PCP, Nocardia, Legionella, Toxoplasma and Listeria infections. Quinolones for bacteremia in neutropenic patients, antivirals include Ayclovir in HSV seropositive, lung & liver transplant recipients. Other antivirals include Gancyclovir, Valacyclovir, Valgancyclovir etc. Antifungals include Fluconazole, Nystatin (for Candida), Itraconazole, Voriconazole, Posaconazole (for aspergillus). Isoniazid for M. tuberculosis. Other modalities include selective decontamination of gut, oral administration of non-absorbable antibiotics like Polymyxin E, Nystatin, Gentamicin before intestine & liver Transplant. Faecal bacteriotherapy/transplant is also a promising modality.

Prevention of exposure to infections
Transplant recipients should be prevented from hospital and outdoor acquired infections. Hospital Exposures can be minimized by using standard precautions, restricting access to visitors and staff with cold or use masks and gloves and remove patients from areas of construction or erecting barriers around construction site. Outpatient Exposures can be minimized by cooking meat thoroughly and wash fresh fruit & vegetables, by avoiding drinking water from lakes, ponds, and contact with faeces, unpasteurized milk, juices & raw eggs. Avoid contact with patients of chicken pox if seronegative, avoid jobs requiring frequent animal contact, advising patient to use safe sexual practice and consult infectious disease specialist before international travel.

Conclusions
Screening of transplant donors is limited by available technology and short period during which organs from deceased donor is used. Donor and recipient screening should be improved by using more sensitive & rapid assays. Augmented screening is recommended on regional basis for endemic and epidemic infections. There should be mandatory reporting of transplant associated infection to increase awareness and also recognizing emerging infectious diseases is still challenging because of non-standardization of donor evaluation.

References
Toll like receptors
Charu Nayyar, Sonal Saxena, Renu Dutta
Department of Microbiology, Lady Hardinge Medical College, New Delhi

Introduction
Our immune system is a remarkably versatile defense system that has evolved to protect us from invading pathogenic microorganisms, chemicals and cancer. It is broadly classified into: Innate immunity and adaptive immunity. The innate immunity comprises of four defensive barriers: Anatomic, Physiologic, Phagocytic and Inflammatory. The anatomic barrier consists of skin, mucous membrane etc. The physiologic barrier consists of various factors like temperature, pH, certain soluble and cell associated molecules. The physiologic barrier, in turn, stimulates the phagocytic and inflammatory barriers. Many of the components of physiological barrier have “pattern recognition” properties, i.e. ability to recognize a given class of molecules. The concept of pattern recognition consists of two entities; Pathogen associated molecular Patterns (PAMP) and Pattern recognition receptors (PRR). PAMP are the highly conserved structural motifs that are expressed exclusively by the microbial pathogen. These molecules could be nucleic acids, proteins, carbohydrates etc. They are not expressed by the healthy mammalian tissue. PRRs are the protein molecules which recognize these PAMP. They are present in blood, tissue fluids and on surface of cells like macrophages, neutrophils, dendritic cells etc. These molecules are germ line encoded and serve as receptors for innate immunity. These PRRs are of two types; cell associated PRRs and soluble recognition molecules. Amongst the cell associated PRRs, the most important ones are Toll like receptors and Nod like receptors.

Toll like receptors
Toll in German means ‘great’. They are single, membrane spanning proteins, present on immune cells (macrophages, dendritic cells etc.) and non-immune cells (epithelial cells of digestive, lungs, female reproductive tract, pancreatic beta cells, keratinocytes etc.) Their function is to recognize pathogen associated microbial patterns (PAMPs) expressed by the microbes as well as danger associated molecular patterns (DAMPs) - endogenous molecules released from dying/necrotic cells. There stimulation results in activation of innate immune system. Toll like receptors, along with Interleukin-1- receptor form a superfamilly known as the ‘Interleukin-1 Receptor / Toll-Like Receptor Superfamily’, which shares a common cytoplasmic TIR (Toll-IL-1 receptor) domain. Toll receptor was initially discovered in Drosophila as an important receptor in dorso-ventral embryonic pattern. It was observed that flies with mutation of this receptor, referred as Toll mutants, were not able to establish a proper dorso-ventral axis. Later, Hoffman and colleagues showed that Toll-mutant flies were susceptible to fungal infections, i.e., Toll gene had antifungal properties. Mammalian homologues of this gene were discovered and were designated as Toll Like Receptors (TLR).

Structure of TLRs
The TLR consists of an exterior region, a membrane spanning domain and an interior part called the TIR domain. The exterior domain contains many Leucine rich repeats (LRRs) which serve as the ligand binding site. The TIR domain interacts with the TIR and the other members of the TLR signal transduction pathway. There are 3 conserved sequences of amino acids called Box 1, 2, 3 which are essential for this interaction and are the characteristic feature of TIR domain.

Different TLR proteins are labeled as TLR1, 2, 3 etc. Till now, eleven TLR proteins have been identified in mammals. In humans, TLR 1-10 have been studied and recently TLR 12 and 13 have been identified in murine models. TLR 1,2,4,5 and 6 are outer membrane associated and respond to bacterial surface PAMPs whereas, TLR 3,7,8 and 9 are present on surface of endosomes, Golgi bodies etc. and respond to bacterial and viral nucleic acid. TLR 10, 11, 12 and 13 are also surface associated – very little known. Different TLRs, their ligands and target molecules are shown in Table 1.

TLR signal pathway
The activation of TLR signaling pathways originates from the cytoplasmic TIR domains. The TLR signalling pathway consists of two different pathways, MyD88 dependent pathway which is common to all TLRs except TLR3 and MyD88 independent pathway which is peculiar to TLR3.
Figure 2. TLR signal pathway

Table 1: TLRs, their ligands and target microbes

<table>
<thead>
<tr>
<th>TLRs</th>
<th>Ligands</th>
<th>Target microbes</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR1</td>
<td>Di &amp; Tri-acetyl lipopeptides</td>
<td>Mycobacteria</td>
</tr>
<tr>
<td>TLR2</td>
<td>Peptidoglycans, GPI-linked proteins, Lipoproteins, Zymosan</td>
<td>Gram positive bacteria, Mycobacteria, Yeasts and fungi</td>
</tr>
<tr>
<td>TLR3</td>
<td>dsRNA</td>
<td>Viruses</td>
</tr>
<tr>
<td>TLR4</td>
<td>LPS, F-protein</td>
<td>Gram negative bacteria, RSV</td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td>Bacteria</td>
</tr>
<tr>
<td>TLR6</td>
<td>Diacyl lipoproteins, Zymosan</td>
<td>Mycobacteria, Yeasts and fungi</td>
</tr>
<tr>
<td>TLR7</td>
<td>ssRNA</td>
<td>Viruses</td>
</tr>
<tr>
<td>TLR8</td>
<td>ssRNA</td>
<td>Viruses</td>
</tr>
<tr>
<td>TLR9</td>
<td>CpG unmethylated di-nucleotides</td>
<td>Bacterial DNA</td>
</tr>
<tr>
<td>TLR10,11</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Consequences of TLR activation

**Inflammatory responses:** TLR activation contributes to host inflammatory responses. Activation of NF-κB allows for transcription of immunomodulatory genes including genes for various cytokines and chemokines. The production of cytokines and chemokines in turn triggers inflammation through recruitment of host immune cells and activation of antimicrobial defences. Although this may aid in clearing an infection, inflammation triggered through TLR may also harm the host through the damage of host tissues or development of septic shock.

**Phagocytosis:** TLR activation stimulates phagocytosis by promoting internalization of pathogens, causing maturation of host phagosomes and triggering inflammatory responses to phagosome contents. The mechanism thought to be responsible for this is the upregulation of scavenger receptors by the MyD88, IRAK protein etc. In animal models, it has been observed that the absence of TLR2/TLR4 or MyD88, phagocytosis of bacteria including E.coli, Staphylococcus aureus, Salmonella has been impaired due to impaired phagosome maturation.

**Triggering of direct antimicrobial pathways:** TLR activation promotes release of non-specific antibacterial molecules such as antimicrobial peptides.
increased β defensin 2 productions from alveolar epithelium on activation of TLR2. These peptides trigger more TLR activation which in turn activates other cells, like β defensin 2 activated dendritic cells through TLR4. TLR activation can also lead to release of certain reactive oxygen and nitrogen species which aid in killing intracellular pathogens such as Mycobacterium tuberculosis in mice.

**Development of adaptive immune response:** TLR activation facilitates and instructs the development of adaptive immune responses. Certain cytokines produced by TLR activation may instruct differentiation of T-cells into Th1 or Th2 subset which guides the pattern of adaptive response the host will launch against the pathogen. For instance, human monocyte-derived DC stimulated with lipopeptide from M. tuberculosis secrete IL-12 over IL-10, skewing the host’s adaptive immune response toward a Th1 pattern, characterized by a cellular, cytotoxic T cell response. In contrast, activation of an adaptive Th2 immune response by cytokines such as IL-10 and IL-4 is characterized by the involvement of B cells and antibody production.

**Apoptosis:** TLR activation may also lead to apoptosis. LPS has been shown to induce apoptosis through TLR4. The 19 kDa lipoprotein from M. tuberculosis has been shown to trigger apoptosis in macrophages, and activation of TLR2/6 with Mycoplasmal lipoproteins has also been shown to induce apoptotic cell. This activation of TLR2 with bacterial lipoproteins has been shown to signal apoptosis through MyD88, Fas-associated death domain protein, and caspase 8. The induction of apoptosis in infected cells through TLR ligands may help the host in eliminating the infection.

**TLRs and Immune disorders**

Several lines of evidence indicate that TLRs are implicated in inflammatory and immune disorders. In a prolonged state of infection and/or tissue damage, there are sustained high levels of inflammatory proteins which lead to autoimmune, inflammatory diseases and even cancer. In an experimental model, it was seen that defective IL-10 signalling led to constitutive activation of innate immune cells which resulted in chronic enterocolitis. On introduction of TLR4 deficiency in such mice, there was improvement of intestinal inflammation suggesting a role of TLR4 in the pathogenesis. In another study, it was seen that mice deficient in the apolipoprotein E were at a risk of development of atherosclerosis. However, the disease was averted on introduction of MyD88 deficiency. The MyD88-dependent pathway is involved in induction of autoantibodies in SLE, rheumatoid arthritis and also in allograft rejection.

**TLRs and Cancer**

There are many evidences suggesting that chronic inflammation is crucial to the onset and progression of multiplicity of cancers. The exact link between chronic inflammation and carcinogenesis is unclear; however, many studies have shown that pro-inflammatory cytokines (TNF-α, IL-6, IFN-β) are important for proliferation, survival, metastasis and escape from immune surveillance of many cancers. It is now recognized that TLRs are important in development of carcinogenesis and tumour progression. Multiple TLRs have been implicated in a variety of cancers. For eg: TLR3 is associated with Breast, Colorectal, Hepatocellular carcinomas; TLR4 with Bladder, Breast, Brain, Cervical; TLR7 with Chronic lymphocytic leukemia; TLR8 with lung cancers etc.

**Negative Regulation**

Stimulation of TLRs by microbial components triggers induction of inflammatory cytokines. When the cytokines are produced in excess, they induce serious systemic disorders. Therefore, there are mechanisms for modulating the TLR-mediated responses. Combination of these negative regulators coordinates TLR signalling pathway and thus limit exaggerated innate responses causing harmful disorders.

**Figure 3: Negative Regulation**


**Therapeutic Role**

In order to be considered as potential therapeutic targets, molecules must fulfils certain criteria which include Overexpression in disease, Knock-out mice being resistant to disease in disease models, Ligands exacerbating inflammation in disease models and Genetic differences (or their signalling proteins) correlating with the risk of disease. Extensive scientific research has already indicated that TLRs can be used effectively as therapeutic targets for a number of diseases. TLR can be targeted as TLR agonists and TLR antagonists.

**Receptor Agonist:** Imidazoquinolinamines—Immune Modulators: Imidazoquinoline compounds (ICs) have similar structure to nucleosides found in all living organisms. Imiquimod and resiquimod are two compounds that are efficient at activating the immune system. These are TLR7 agonists and act by stimulation of TLR pathways thus resulting in secretion of inflammatory cytokines. They also enhance apoptotic pathways in cancer cells. It binds to...
the high affinity adenosine receptors and suppresses its ability to regulate negative feedback. They are also known to have antiviral activity. Imiquimod has been authorized for treatment of genital warts caused by HPV. Imiquimod has also been used to activate the immune system in many diseases like actinic keratoses, BCC, SCC, and melanoma. It is also used in the patients of Herpes simplex virus where it serves as an alternative therapy for people resistant to conventional treatment. Its use in the treatment of HCV is still under investigation. Two other immunomodulators which function through TLR 7, loxoribine, and bropirimine have also been used. These are currently in clinical studies for treatment of carcinomas.

**Role in Vaccine:** Linkage of a TLR agonist to antigen increases antigen uptake by Dendritic cells, thus reducing the needed dose and also facilitate antigen processing and MHC class I and II antigen presentation. Thus TLR agonists are extensively being used as vaccine adjuvants. For eg: Multiphospholipid adjuvant, which is a TLR4 agonist has been approved by FDA to be used in Hepatitis B vaccine. Its use as adjuvant to Human papilloma virus is under preapproval stages. CpG-ODN, which are unmethylated sequences of Cytosine and Guanosine act as TLR9 agonist and their use as adjuvants to Influenza and HIV antigen is also under clinical trials.

**Cancer Therapy:** TLR agonist show significant promise for treatment of cancers. They act on cancer cells and cause enhancement of innate immunity, T cell immunity, cytotoxic antibody function and induction of Apoptosis in TLR positive tumors. They can be used as monotherapy where they provide benefit to certain types of cancer. Interest is being centred on the potential to complement existing modes of therapy with radiation, monoclonal antibodies or cytotoxic drugs.

**TLR agonists:** These are structural analogues of agonists. They block action by neutralizing ligands, block receptors or prevent signaling thus reducing the release of host derived mediators. They also block the proliferation of and autoantibody production by autoreactive B cells. There role in inflammatory and autoimmune diseases is under clinical trials. For eg; role of Lipid A derivatives such as TAK 242, Erikoran which are TLR 4 antagonist is being studied for treatment of sepsis.

**Conclusions**

TLRs represent a conserved group of receptors which help the immune system to function properly. Different TLRs are associated with several diseases. TLR agonist and antagonist have great potential for treatment of inflammatory, autoimmune diseases as well as cancers.

Further research is needed to discern the relationship between specific TLRs and the corresponding disease in order to harness their therapeutic potential. This relationship is being targeted to develop newer therapeutic strategies. TLR agonist and antagonist show significant promise for treatment of inflammatory, autoimmune diseases and cancers.

**References**

Non Tubercular Mycobacteria (NTM) with special emphasis on drug resistance mechanisms, susceptibility testing and therapy

Vrushali Patwardhan and Anita Chakravarti
Department of Microbiology, Maulana Azad Medical College, Delhi

Introduction
NTM encompass a group of mycobacteria other than Mycobacterium tuberculosis complex and M. leprae. They are also known as atypical / anonymous mycobacteria, MOTT(Mycobacteria Other Than Tuberculosis). Potentially pathogenic environmental mycobacteria, Tuberculoid mycobacteria. Currently more than 125 NTM species have been catalogued. They are ubiquitously present in the environment as saprophytes & are associated with opportunistic infection in animals and humans. No man to man transmission or between animals and humans has been yet documented. Unlike TB, NTM infections are non-contagious. Human disease is suspected to be acquired through environmental exposure to water, aerosols, soil, and dust – through inhalation, ingestion, and through breaks in the skin. High level of interest in NTM disease is the result of association with AIDS, recognition of NTM lung disease with increasing frequency from immunocompetent patients, as a cause of skin and soft tissue infections especially in postoperative cases. Being ubiquitous it’s difficult to conclude if it is a contaminant or the pathogen. More importantly, infections caused by them are difficult to treat because of their intrinsic resistance to the major classes of drugs.

Epidemiology
NTM infections account for < 5% of total Mycobacterial infections worldwide incidence being around 0.4 -1.8 cases/100,000. Out of this around 94% are pulmonary infections, 3% lymph nodal and 3% soft tissue infections. The incidence of notified cases of clinically significant pulmonary disease rose from 2.2 (1999) to 3.2 (2005) per 100,000 population worldwide. The isolation rate of NTM from India has been reported to be ranging from 0.5% to 8.6%.

Drug resistance mechanisms in NTM
Resistance in NTM is a result of natural resistance, inducible resistance or mutational resistance (acquired during suboptimal drug exposure and selection).

Natural resistance mechanism: Natural resistance to antimicrobial drugs is conferred by cell wall which is important for the uptake of the drugs inside the cell and biotransformation of the drugs inside the cell with the help of various cytoplasmic enzyme systems.

Cell wall: Three critical factors contribute towards drug resistance mechanisms are the content of cell wall (hydrophobic/ lipophilic nature), porin channels and efflux mechanisms (inducible drug resistance)

Role of cell wall content: Being lipid-rich it acts as a physical barrier to the penetration of antimicrobial compounds. Several genes and systems involved in cell wall maintenance are important which include protein kinase G, fbpA , asnB (M. smegmatis), kasB in Mycobacterium marinum, Maa2520 and pks12 of M. avium. Disruption of these genes reduces susceptibility to lipophilic antibiotics (Rifamycins, macrolides, ciprofloxacin, vancomycin, imipenem and penicillins).

Role of porin channels: In mycobacteria, porins are important in nutrient acquisition, its activity determines susceptibility to small hydrophilic antibiotic molecules (Norfloxacin, Chloramphenicol and beta-lactam antibiotics and also the hydrophobic Vancomycin, Erythromycin and Rifampicin).When these porin channels are disrupted NTM strains become resistant to above mentioned antibiotics.

Role of Biotransformation: This mechanism has been described for Penicillins: Penicillins are not used in the treatment of mycobacterial infections, owing to lactamase activity in all mycobacteria.

Quinolones: Biotransformation of this class of drug is carried out by two enzymatic processes - acetylation & nitrosation which create molecules that have 2–1000 times less antimycobacterial activity. This mechanism has been noted in rapidly growing Mycobacteria for both Norfloxacin and Ciprofloxacin.

Aminoglycoside: susceptibility to this group of drugs is influenced by three distinct classes of aminoglycoside modifying enzymes aminoglycoside O-nucleotidyltransferase, aminoglycoside O-phosphotransferases, aminoglycoside N-acetyltransferases. The latter two have been identified in the genomes of Mycobacterium species. Phosphotransferase conveys streptomycin resistance in M. fortuitum and M. abscessus. Distinct N-acetyltransferases have been identified in the genomes of the Mycobacterium tuberculosis complex and the rapid growers M. fortuitum, M. smegmatis, M. abscessus.

Inducible drug resistance: Confirmed by Target binding disruption. It has been described for Erythromycin, Rifampicin and active efflux pumps for Tetracycline, Aminoglycoside, Quinolones & Rifampicin.

Target binding disruption: The best known inducible resistance mechanism in mycobacteria is the group of erythromycin resistance methylase (erm) genes. It confers Macrolide resistance through methylation of the 23S ribosomal RNA which impairs binding of the macrolides to the ribosomes. These methylases have been characterized in M. abscessus and M. fortuitum. An inducible mechanism by which tolerance to Rifampicin is increased is the RNA polymerase binding protein A (RbpA), which has been characterized in M. tuberculosis and M. smegmatis. This protein binds to the RNA polymerase, where it hampers binding of Rifampicin. Whether this protein is also present in slowly growing nontuberculous mycobacteria that are treated with Rifampicin based regimens remains unknown. Whereas porins can restrict entry of molecules into the cell, efflux pumps are utilized of the mycobacterial cell to...
evacuate potentially harmful substances out. The P55 is an efflux pump that is likely present in all Mycobacterium species that permits efflux of Tetracycline and Aminoglycosides. The best characterized efflux pumps in NTM are: Tap for Tetracycline, tetV for Aminoglycoside, lfrA for Quinolone, efpA for Rifamycins and Isoniazid

**Acquired genomic mutations:** Mutations lead to modification in the drug binding targets. In NTM the impermeability of the cell wall is a more important driver of drug resistance than polymorphism in the drug target. Mutational resistance to Macrolides is documented in NTM. However, mutations can be prevented by the use of multidrug regimens that also include Rifampicin and Ethambutol.

Mutations in codon 2058 or 2059 of the 23S ribosomal RNA gene (rrl) have been associated with high level Macrolide resistance in MAC, M. abscessus. In a case series of patients with Macrolide resistant MAC disease, 96% of patients had isolates with mutations in these two codons. Prior Macrolide monotherapy or regimens including only Quinolones and Macrolides were risk factors for the development of macrolide resistance. The rrl is also the target of Linezolid. In experimental settings, mutations in codons have decreased susceptibility to Linezolid in M. smegmatis.

**Drug susceptibility testing (DST) of NTM**
The only available and standardized drug susceptibility testing methods for NTM are CLSI recommendations.

**Broth macrodilution:** Relatively fast method the radiometric BacTec460 system has been largely abandoned as a tool for primary culture of mycobacteria. But production of its supplies has been terminated in the year 2011. Its successor, the Mycobacterial Growth Indicator Tube (MGIT) system, has been set up for DST of M. tuberculosis, likely to become the gold standard. It has not yet been adapted for DST of NTM. More extensive studies are still needed to establish the potential of MGIT-based DST for NTM. Thus for all practical purposes its broth microdilution method is the standard recommended method for DST of NTM

**Broth microdilution:** Freeze dried microtite plates are commercially available with broth already added to it. Inoculum suspension is prepared by sweeping confluent portion of growth on a solid medium with a sterile cotton swab or directly from the broth culture. Growth on the swab is transferred to 4.5 ml sterile water until the turbidity matches to 0.5 Mc Farland units. Suspension is mixed vigorously on vortex mixer for 15-20 sec & allowed to settle and the supernatant is used as inoculum suspension. Final inoculum is prepared by transferring 50 µl of suspension to 10 ml of cation supplemented Mueller Hinton broth only for rapid growers, Calion supplemented Mueller Hinton broth + 5% OADC for slow growing mycobacteria. Final inoculum suspension is mixed well & then 100 µl is transferred to each well of microdilution tray. Inoculated tray is covered with an adhesive seal and incubated in ambient air. Inoculation temperature and timing varies with different species of NTM growth density is measured optically and compared to growth in drug-free control vials, to determine MICs.

**CLSI recommendation for MAC isolates to be tested for DST**

Initial isolates from previously untreated MAC lung disease should be tested to Clarithromycin to establish baseline values. Also, isolates from patients who had previously received Macrolide therapy to determine whether or not the isolates are still Macrolide susceptible, isolates from patients with MAC pulmonary disease on Macrolide-containing regimens who relapse or failure after 6 months of Macrolide-containing therapy and isolates from patients with AIDS who develop bacteremia on macrolide prophylaxis shall be subjected to AST for clarithromycin.

**Antimicrobial agents to be tested for MAC DST:**
Correlation between in vitro MAC susceptibility and therapeutic outcome is demonstrated only with Clarithromycin and Azithromycin. However, both these drugs share cross resistance and susceptibility so either of the drug is to be tested. Untreated MAC isolates which are susceptible usually have MICs of 4 µg/ml or less to Clarithromycin, 32 µg/ml or less for Azithromycin. Relapse strains after treatment and no longer respond to treatment with macrolides. Inevitably have Clarithromycin MIC of 32 µg/ml or greater and Azithromycin MIC 256 µg/ml or greater. Strains which are intermediate in susceptibility to Clarithromycin rarely occur and should be confirmed by another testing event. Patients with these intermediate MICs should be followed closely for possible emergence of macrolide resistance. Macrolides should be included in treatment regimens for these patients unless the isolate is found on subsequent testing to be macrolide resistant. Only transparent colony type is preffered if possible (more virulent) & resistant to antimicrobials than opaque variant. Incubation is at 33°C to 37°C in ambient air. For microdilution trays are first examined at 7 days if growth is insufficient then reincubated and re examined at days 10-14 of incubation.

**Quality control (QC) for MAC DST:** M. avium ATCC 700898 recommended for QC for Macrolides. Range of acceptable results for Clarithromycin 1-4 microg/ml( Azithromycin it is 8-32 micro grams/ml). M.marinum ATCC 927 is an acceptable alternative for QC (acceptable range for Clarithromycin 0.25 – 1 microgram/ml). QC for second line drug testing ( Moxifloxacin / Linezolid) same strains are used.

**M. Kanssii DST**
Susceptibility only to Rifampin should be routinely performed. Treatment failure is usually associated with resistance to Rifampin. In vitro susceptibility pattern with INH does not correlate clinically. ( INH should not be tested). Resistance to Isoniazid and Ethambutol is usually associated with resistance to Rifampin. Isolates that are susceptible to Rifampin are also susceptible to Rifabutin. Rifabutin is substituted for Rifampin in HIV-infected patients being treated with highly active antiretroviral therapy (HAART), including some protease inhibitors and non-nucleoside reverse transcriptase inhibitors. If the isolate is Rifampin resistant, susceptibility to secondary agents, including Amikacin, Ciprofloxacin, Clarithromycin, Ethambutol, Rifabutin, Streptomycin, Sulfonamides and Isoniazid should be tested. Susceptibility to the new 8-methoxy fluoroquinolone, Moxifloxacin should be performed separately because Ciprofloxacin is the class representative for Ciprofloxacin, Ofloxacin and Levofloxacin only.
QC strains and their acceptable ranges: M. kansasii ATCC 12478 (less than 1 microg/ml) and M. marinum ATCC 927 (0.25 – 1 micro gram/ml).

M. Marinum DST
Routine susceptibility testing of this species is not recommended. There is no appreciable variability in susceptibility patterns to clinically useful agents. Isolates of M. marinum are susceptible to Clarithromycin as well as the Sulfonamides, the Tetracyclines, Rifampin and Ethambutol. Ciprofloxacin is not recommended because some strains are resistant and monotherapy carries the risk of mutational resistance. However, some experts report that the newer 8-methoxy fluoroquinolones, Moxifloxacin, is more active in vitro and could be considered for multidrug therapy. Susceptibility testing should be considered for patients who remain culture positive after more than 3 months of therapy.

Rapid growers DST
Antimicrobial agents to be tested for all rapid growers are Amikacin, cefoxitin, ciprofloxacin, clarithromycin. Doxycycline, imipenem, linezolid Moxifloxacin, trimethoprim-sulfamethoxazole. For M. chelonae only, Tobramycin is also used for DST (should not be used in M. abscessus, M. fortuitum). Incubation at 28°C - 32°C in air for 72 hrs to 4-5 days is recommended. Day 5 is the final reading day for all drugs except Clarithromycin to be read at 7-10 days, if susceptible then again at day 14 for detection of inducible macrolide resistance. If Clarithromycin resistance MIC > 8 µg/ml is recognized earlier then the report can be finalized at that time.

Pitfalls in drug susceptibility testing of nontuberculous mycobacteria
Degradation of antimicrobials: Doxycycline is known to degrade rapidly, i.e. in 14 days, in Mueller-Hinton medium. Test materials thus need to be prepared fresh. Broth microdilution plates must be read no later than after 3 days of incubation; high imipenem MICS (>8 g/ml) merit retesting. Reduction to <50% activity was noted within 1 week for Trimethoprim and Minocycline. Within two weeks for Kanamycin, Amikacin, Trimethoprim + Sulfamethoxazole and Rifampicin.

Effects of pH: The pH of the medium is important when testing macrolide susceptibility. In vitro, the activity of clarithromycin decreases at an acidic pH. The broth microdilution method commonly applies cation-adjusted Mueller-Hinton broth with pH of 7.3–7.4. At this pH clarithromycin MICs are twofold lower and separate interpretation criteria must thus be used. Similar decreases of activity at lower pH have been recorded for Ciprofloxacin, Ciprofloxacin, and Ethambutol.

Establishment of breakpoint concentrations: The clinical utility of DST relies on consistency between in vitro susceptibility to a drug & good outcome of treatment with the respective drug, in vivo. Hence, breakpoint concentrations must reflect MIC distributions of wild-types and resistant mutants, pharmacokinetics and pharmacodynamics of the drug in human infections and must aid to predict outcome of treatment. For MAC, only macrolide susceptibility testing is advised and Clarithromycin is the preferred class representative (CLSI, 2011).

Currently available susceptibility breakpoint does not take into account that MAC disease is mostly treated with a combination of a Rifamycin (Rifampicin or Rifabutin), Ethambutol and a Macrolide. Simultaneous use of rifamycins significantly decreases the serum concentrations of Clarithromycin. As a result, the breakpoint concentration based on efficacy of Clarithromycin monotherapy may not correctly predict the outcome if Rifamycins and Clarithromycin are used in combination. For M. kansasii, only the Rifampicin breakpoint has been validated clinically and hence only Rifampicin testing is currently recommended (CLSI, 2011). Treatment failure has been associated with high MICs for rifampicin. In cases of Rifampicin resistance, CLSI recommends, with caveats, testing of clarithromycin, ciprofloxacin, moxifloxacin, amikacin, ethambutol, linezolid, rifabutin, streptomycin and trimethoprim-sulfamethoxazole, with most breakpoints extrapolated from MAC (Clarithromycin) or M. tuberculosis (CLSI, 2011).

Therapy of diseases caused by NTM
**Recommended drug treatment for MAC lung disease:** involves multiple drugs; therefore, risk of adverse drug reactions and/or toxicities is relatively high. Necessary to include companion drugs with the macrolide (albeit drugs with less activity against MAC) to prevent the emergence of macrolide-resistant MAC isolates. The macrolides should never be used as monotherapy for treatment of MAC lung disease.

**Treatment recommendations for macrolide susceptible MAC isolates:** Intermittent drug therapy is not recommended for patients who have caviarty disease, patients who have been previously treated, or patients who have severe disease. The primary microbiologic goal of therapy is 12 months of negative sputum cultures while on therapy; therefore, sputum must be collected from patients for AFB examination throughout treatment. Patients respond best to MAC treatment regimens the first time they are administered; therefore, it is very important that patients receive recommended multidrug therapy the first time they are treated for MAC lung disease.

**Treatment recommendations for macrolide-resistant MAC lung disease:** Macrolide-resistance is due to macrolide monotherapy or treatment with macrolide and inadequate companion medications. A parenteral Aminoglycoside (Streptomycin or Amikacin) may be used in such cases and surgical resection (“debulking”) of disease may be contemplated.

The four-drug regimen of Isoniazid (300 mg/d), Rifampin (600 mg/d and Ethambutol (25 mg/kg/d for the first 2 months, then 15 mg/kg/d) with Streptomycin for the initial 3 to 6 months of therapy should be followed. Amikacin could be substituted for streptomycin, and isoniazid should be considered optional for these patients. Including a Macrolide in treatment regimens for macrolide-resistant MAC isolates is not recommended.

**Treatment Recommendations for disseminated MAC disease:** Clarithromycin 500 mg orally twice daily alternatively Azithromycin 500 mg daily + Ethambutol 15 mg/kg orally daily + Rifabutin 300 mg orally daily.
Treatment recommendations for Disseminated M. kansasii disease: The treatment regimen for disseminated disease should be the same as for pulmonary disease. For HIV-infected patients on antiretroviral regimen Rifampicin is substituted with a Macrolide or Moxifloxacin for the Rifamycin. There is no recommended prophylaxis or suppressive regimen for disseminated M. kansasii disease.

Duration of treatment
Diseases caused by rapid growers should be treated for 4-6 months. M marinum infections should be treated 3 months; 12 months if deeper structures are involved. Pulmonary infection (MAC) is recommended to be treated for duration of 18 months (at least 12 months after last positive culture). Disseminated MAC in HIV/AIDS are lifelong if no immune reconstitution. If successful antiretroviral therapy is ensued, stop MAC treatment after 12 months’ therapy if CD4 count restored to >100 cells/mm3 for six months or more.

References
4. V.M. Katoch Infections due to non-tuberculous mycobacteria (NTM); Indian J Med Res. 2004;120:290-304.
Crossword Puzzle 0314
Dr Reetika Dawar
Indraprastha Apollo Hospitals, New Delhi

ACROSS
2 Robinson's medium is used for cultivation of this microbe
7 Virus causing foot & mouth disease
9 Disease caused by Chlamydia psittaci
10 Corynebacterium minutissimum causes this skin infection
12 Disease for which commercial serological tests should not be used as recommended by WHO
16 The other name of Rat Bite fever caused by ingestion of contaminated food or water.
18 Metapneumovirus (MPV) belongs to this family
20 Griseofulvin is an antifungal produced by this microbe
21 Required for growth of Abiotrophia
22 Metallo-beta-lactamase producing Pseudomonas require this metal for their activity
23 Japanese encephalitis virus belongs to this genus
24 Microbe of the this genus produce violet coloured colonies

DOWN
1 Cefsulodin-Irgasan-novobiocin agar is selective for this genus
3 Species of anaerobic cocci which is mostly sensitive to sodium-polyanethol-sulphonate
4 Subacute sclerosing panencephalitis is a late complication of this disease
5 Bartonella species causing Cat Scratch disease
6 Genus of anaerobic saccharolytic bile sensitive species which produce black/brown colonies
8 Another name for Fonseca's disease
11 New species of Candida phylogenetically related to C. haemulonii
13 Oxidase positive H2S producing gram negative bacilli with polar flagella.
14 Elongation in the presence of penicillin is a useful criterion for differentiation
15 Causative agent for Whipple's disease
17 A Gram positive coccus intrinsically resistant to vancomycin
19 For Leptospira the media should contain serum of this animal
Solutions: Crossword Puzzle 0314

Dr Reetika Dawar, Indraprastha Apollo Hospitals, New Delhi