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JULY - SEPT. 2004
VOL. 17 NO. 3

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PRESIDENT WRITES

Dear Fellows and Members,

Let me express my sincere appreciation and gratitude to all fellows, members especially the Mayo foundation, the Mayo Clinic, Dr. Harvinder Luthra and his colleagues in making the IMSACON-2004 with the theme "Genomic Proteomic - a new revolution in medicine" a grand success. It was indeed an intellecutual revolution for the delegates.

The use and abuse of antimicrobial agent is a global problem. Countries with stringent criteria, the problem still exist. Medical fraternity has to be periodically updated on the antimicrobial strategies to primary physicians through CME and other media.

Our efforts should be to educate and reeducate at primary physicians level, the various do's and don'ts to ensure the drug policy. The infrastructure for the proper testing must be made available at the primary level. The WHO guidelines booklet on microbial diagnostics will readily help the physicians. The real test of the system is at times of epidemics, like the one we had, ie Pasteurella pestis, SARS, BSE, AIDS and Ebolla. In all these situations good policies help human being.

As we conquer more microbes, newer ones throw further challenges to be solved.

Dr. K. Jagadeesan,
President, IMSA

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JIMSA

JOURNAL INTERNATIONAL MEDICAL SCIENCES ACADEMY

JULY - SEPT. 2004
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FROM EDITOR'S DESK

Dear Colleagues,

From time to time, it is important to ponder about the past, look at the present critically and also make an attempt to get a glimpse of the future; it is with this objective every issue of JIMSA focuses on the topics of current interest belonging to different disciplines of medicine, through symposia or special issues published alternately, since the year 1996. JIMSA is planning to introduce some of the incentives like the *"best published article award"* for the young scientists so as to attract quality articles for its forthcoming issues.

There is a growing and justified concern about the public health and economic impact of *infections* associated with modern health care. During the past quarter of a century; HIV infection has emerged as a major hazard and there is no continent on this earth from where devastating effects of AIDS have not been reported. The expanding problem of antimicrobial resistance have become increasingly complex to monitor and quite difficult to contain; emergence and dissemination of nosocomial and also the drug resistant staphylococcus aureus and extended spectrum β-lactamase-producing *E.coli* in many parts of the world, especially the Indian subcontinent, illustrate this trend. Other ancient diseases like malaria, tuberculosis, intestinal parasitosis, are still with us and have over the years learned to survive against the growing number of antimicrobials; all these problems have raised certain questions. Are the current infection prevention and control programmes geared to address these challenges adequately? Do we have enough adequately trained professionals to look after the infection-control strategies effectively?

The present issue: ***"Advances in clinical microbiology and infection disease practice in India"*** ably compiled and edited by **Dr. Chand Wattal**, sheds light on most of currently debated infectious disease-related topics in great depth. I am extremely grateful to Dr. Wattal and other contributors for bringing out this excellent monogram; I am confident readers will find the articles informative and stimulating. Dr. Wattal has put in lot of hard work not only in its compilation but through his innovative and brilliant ideas has also given JIMSA' an improved look. I wish to congratulate him for this fruitful effort.

I also take this opportunity to thank all members of editorial / advisory board for their help and also several pharmaceutical firms for financial assistance; without which publication of this issue would not have been possible. Editorial advice and help rendered by Byword Viva Publishers Pvt. Ltd. needs to be duly acknowledged.

P.D. Gulati

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JIMSA 2004: 17(3)

Our Guest Editor

Dr. Chand Wattal - born in the year 1955 is presently working as Hony. Senior Consultant & Head of Clinical Microbiology Department, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi, INDIA. He did his MD (Medical Microbiology) from P.G.I., Chandigarh, in 1982; he has a total professional experience of more than 20 years. Prior to his present assignment he worked as associate professor of clinical microbiology from 1989 to 1990 (incharge Unit of Parasitology), Institute of Medical Sciences, Kashmir, India; later on he was consultant to Sunder Lal Jain and Tirath Ram Shah Hospital, Delhi

Dr. Wattal has been a post graduate teacher and a guide/co-guide for MD since 1985 and for DNB Microbiology since 2003. He is an astute academician; he has attained international recognition; his bibliography has been included in : Marquis directory (Founded in 1899) of Who's Who in USA 1997-98, Who's Who in World for the year 1999. International Biographical Centre of Cambridge - Outstanding people of the 20th Century and International Man of the Year for 1999/2000: he is a member of advisory board of Indian J. Pediatrics and also an expert to the technical advisory committee, Ministry of Health & Family Welfare, Government of NCT of Delhi. The department of Clinical Microbiology Sri Ganga Ram Hospital (SGRH) which Dr. Wattal is heading; brings out SGRH *Microbiology Newsletter* twice every year giving the details of the antibiogrammes and other articles of academic interest to the clinicians and clinical microbiologists ever, since 1995. He is the Editor for the SGRH Newsletter, a quarterly publication of the hospital. Dr. Wattal has 28 research publications to his credit out of which, 12 are in international journals; he has made contribution in Book Series/ Lung Biology in Health and Disease on a topic - "Pulmonary Hydatid Diseases in India, Diagnosis and Management" *Publisher:* Marcel Decker. Inc., 270 Madison Avenue 1991, *New York,* USA. In recognition of his academic achievements; he has been assigned the task of Chief Investigator WHO (Geneva) Project on Rational Antibiotic Usage Phase 1. He has participated as guest lecturer and made scientific presentations in 30 National and International conferences.

Dr. Wattal has been the recipient of several awards and brought laurels to his institution through such awards. Sir Ganga Ram Hospital Management has been awarded Hospital Management Asia Award 2002 for best hospital infection control programmes; practised, this was out of 72 hospital nominations received from 21 countries. Credit for this prestigious award goes to Dr. Wattal who was responsible for designing the polices; these programmes were intelligently implemented by him as a secretary of the Hospital Infection Control Committee. He has been awarded by the Lt. Governor of Delhi the 'Dharma Vira Award' for his professional excellence for the year 2003; this award is given every year to the best consultant of the hospital.

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EDITORIAL

Medical microbiology and the infectious diseases burden: Is India prepared?

The need for having infectious diseases (ID) as a specialty in India is overwhelming. This can be appreciated by the data quoted below in this over view.

It may not be wrong to say that in India, the specialty of ID does not exist. It is an irony of fate that the country which requires it the most, does not have it. The internist or medical graduate, including specialists in any field with whatever primitive knowledge, tackle most of the burden of ID in India. An attempt was made to address this problem, when the specialty of Medical Microbiology was started as a part of the MD course in the late 1960s. The intention good but the means for its implementation for the benefit of the community appears to be lacking. The enormous talent of medical graduates especially post graduates in medical microbiology is wasted because of its non-application in the field of ID.

No microbe causes disease in cent percent of its hosts, making it difficult to justify a definition of infection synonymous with disease.[1] A distinction between colonization and disease has to be made. For example, the recovery of *Neisseria meningitidis* from the blood or cerebrospinal fluid of an individual could appropriately be a case of meningococcal meningitis. On the other hand, the recovery of *N. meningitidis* from a non-sterile site, e.g. the nasal passage in a symptom-free person, should be considered colonization rather than infection, despite the fact that the organism was isolated in the host. In each situation, the patient has infection since he/she harbours *N. meningitidis*, but in the former, the outcome of the infection is disease, whereas in the latter, it is colonization or carriage. Hence, isolation alone does not reflect the clinical status of patient nor does it suggest a need for any type of intervention and/or therapy. Presently, we lack adequate quantitative and qualitative measures of damage to establish objectively whether the states of commensalism and colonization are harmless to the host, protect the host, or will eventually produce damage resulting in disease. Fortunately, the clinical signs, symptoms and syndromes that characterize the diseases caused by most microbes are relatively well defined and agreed upon. The recognition of these clinical entities has an important bearing in the therapy of infections and thereby call for the need for ID as a specialty.

Poverty is a medium for diseases to grow exponentially. The economic system that we live in today is focused on high profits. Investment in public health, more so in ID, has a low priority. It has to be realized by the world that prevention of illnesses has always resulted in the economic growth of a country.

The statistics of the global burden of water-related infectious diseases alone are startling: each year, approximately 4 billion cases of diarrhoeal diseases (including cholera) lead to 2.2 million deaths, mostly among children under the age of 5 years. This is equivalent to 20 jumbo jets crashing every day. These deaths represent approximately 15% of all child deaths under the age of 5 in developing countries.[2] Research has shown that improving the quality of water reduces diarrhoeal diseases by 16%, whereas improving sanitation brings about a reduction of 32%, and handwashing with soap results in a further reduction of up to 45%.[2]

Opportunistic fungal infections have emerged as an important cause of morbidity and mortality in immunocompromised patients and remain a major challenge for clinicians. Invasive fungal infections, besides their increased frequency, are still associated with unacceptably high mortality-up to 40% in bloodstream infections caused by *Candida albicans* and more than 50% in invasive aspergillosis.[3]

The epidemic of human immunodeficiency virus (HIV) infection claiming young lives in Africa is a disaster for that country where infants, even before achieving their productive years of life, are consumed by this disease. As it appears, India is sitting on a volcano, if WHO figures are to be believed. The prevalence of hepatitis B virus (HBV) is already between 4% and 7% and that of hapatitis C virus (HCV) is fast growing (0.8%-1%).

Malaria is the world's most important parasitic infection and ranks foremost among the major health and developmental challenges in the developing countries of the world.[4] Despite global economic development, more people die from malaria nowadays than 40 years ago.[5] The estimate for annual mortality due to malaria ranges from 0.5 to 3.0 million people.[6] In a well-documented study, it was concluded that chloroquine resistance has resulted in a 4-8 fold increase in mortality.[7] Every year we are visited by the dengue virus, another mosquito-borne scourge of mankind directly related to public hygiene.

The incidence of sepsis has increased by 329% over a period of 20 years, between 1979 and 1999, according to new research presented at the American Thoracic Society's 98th International Conference (May 17-22, 2002, Atlanta, USA).[6] Although better supportive care for patients with sepsis has lowered the mortality rate, many more patients are now actually dying from sepsis' as commented by Dr Greg Martin, Emory University School of Medicine, Atlanta, GA, USA.[8] There are now more people with altered immunity because of organ and bone marrow transplantation, chemotherapy and HIV infection, which lowers the immunity in patients. Add to this the geriatric population pool. Apart from the loss of life and morbidity caused by sepsis, it is also a drain on resources.

Despite significant advances in the fight against tuberculosis over the past 10 years, formidable challenges still remain as the incidence of tuberculosis is rising. Developments in tuberculosis research include progress in the understanding of multidrug resistance, development of rapid DNA-typing

techniques and the genetic sequencing of *Mycobacterium tuberculosis*. Experts addressing tuberculosis control noted that 23. 'high-burden countries' account for 80% of the world's tuberculosis cases and India is one among them. It is estimated that worldwide, one in three people is infected with tuberculosis and that there are 8 million active cases each year, resulting in 2 million deaths.[9]

Hepatitis B virus infection is prevalent in Asia, Southern Europe and Latin America, where the rate of hepatitis B surface antigen (HBsAg) carriage in the general population ranges from 2% to 20%.[10] About 2 billion people, one-third of the world's population, have serological evidence of infection and develop liver disease. Worldwide, 350 million people with chronic HBV infection have a 15%-25% risk of dying from HBV-related liver disease, including end-stage cirrhosis and hepatocellular carcinoma.[11] Each year, acute and chronic HBV infection causes roughly 1 million deaths.[12]

Unfortunately vaccines for many pathogens, including HIV, hepatitis (HCV, HEV), malaria (*Plasmodium falciparum*) and *M. tuberculosis* are either ineffective or unavailable. The traditional immunization arsenal includes vaccines that use live-attenuated or inactivated organisms. From an immunological standpoint, live-attenuated vaccines are the vaccines of choice.

In this era of concern about the number of babies who are born infected with HIV, the increasing number of babies with congenital syphilis unfortunately receives little attention. This discrepancy is surprising, more so when congenital syphilis can be prevented by well-established screening and treatment approaches, whereas the prevention of mother-to-child transmission of HIV is less effective and more expensive. Congenital syphilis is now a rare disease in affluent countries, but it remains a severe, adverse pregnancy outcome in many less-developed countries. The infection is transmitted vertically, and in women with infectious syphilis, at least two-thirds of foetuses are affected.[13] In the early part of the twentieth century, tertiary syphilis was the cause of illness in about a fifth of patients in institutions for the mentally ill in the USA. In many developing countries, this situation applies at present. WHO estimates that each year, a million pregnancies are adversely affected by syphilis due to maternal infection (about 270 000 babies are born with congenital syphilis, 460 000 pregnancies end in abortion or perinatal death, and 270 000 babies are born prematurely or with low birth weight.[14] There is limited information on the prevalence of syphilis among pregnant women in Asian countries, although studies from China and India in the 1990s found rates of between less than 1% and 5%.[15-16]

Keeping in view the above data, India needs a robust ID set-up which can be achieved by the rejuvenation or transformation of Medical Microbiology into Clinical Microbiology. Here, the specialty of Clinical Microbiology or ID needs to be introduced immediately and nurtured with conviction. It should not be difficult with the infrastructure of medical colleges that exists in this country, where well-drafted programmes can be initiated.

The teaching of ID at the undergraduate or postgraduate level in almost all specialties is dismally poor. It is the need of the hour that *pundits* who frame or have a say in drafting the medical curriculum keep in mind the needs of the society, so that the tremendous efforts and the most fruitful years of the life of medical graduates and Medical Microbiology postgraduates are not wasted. Sensitization to this crucial, non-existent specialty has to begin during undergraduate training itself, which can be further groomed at the postgraduate level. Clinical teaching should be ensured in the Medical Microbiology curriculum if we want to reap the fruits of training a medical graduate in ID. No conscientious medical practitioner in India can survive without acquiring a reasonable knowledge of ID, which is abundantly, clear from the statistics quoted above. There have been some exceptions such as the Sanjay Gandhi Post-Graduate Institute, Lucknow, which has started a postdoctoral course in ID but this may not be enough.

An attempt has been made in this issue to highlight some of the important ID related issues most relevant to our country. The readers will appreciate the endeavour of every author by the quality of their articles, which are thought-provoking. I am hopeful that topics such as typhoid reviewed by Dr A. Aayagiri, endocarditis by Dr A. Arora, emerging infections by Dr A. Sharma, significance of *Candida* (non-*albicans*) by Dr A. Chakrabarti and application of molecular assays by Dr K. J. Prasad should make interesting reading. The TORCH panel investigation is a prominent grey zone in the serodiagnosis of this group of infections. This investigation, reviewed by Dr J. K. Oberoi, appears to be very simple, but in fact, is far from being so. While the gap is narrowing between infections above and below the navel, the antenatal and perinatal evaluation of TORCH assays continue to be challenging and baffling to medical practitioners and gynaecologists alike.

The concept of HIV-1 and its strains in India has been of interest to researchers in India and no less than the authority in the field of Virology, Professor P. Seth, has made an earnest attempt to explain and review the article for us.

As organ transplant has gained momentum in our country, it is important for us to understand its outfalls (collateral damage) by way of immunosuppressive therapy. Opportunistic pathogens play a dominant role in immediate as well as delayed infections affecting the morbidity and mortality of patients who have undergone a transplant. Distinguished authors with a long-standing reputation in this field Dr V. Kher and Dr R. Sardana have done full justice to the topic.

We always are on the lookout for new antibiotics, which unfortunately are not forthcoming and Dr A. Rattan, who is passionately pursuing newer molecules, has aptly brought home the fact. The significance of coccidian intestinal parasites cannot be overemphasized and the same has been reviewed by the eminent Medical Parasitologist Dr N. Malla.

Genitourinary tuberculosis has been a difficult area in clinical

practice. Dr S. Khanna and Dr S. Joshi have done full justice to the article by highlighting and sharing their concerns regarding the pitfalls in its diagnosis. Rare presentations can be appreciated in the images section provided by Dr Kamlender Singh.

It has been a pleasure as well as a learning experience to be associated with this special issue of JIMSA as its Guest Editor. I wish to thank Dr P. D. Gulati for giving me this chance. My sincere thanks are due to all the contributors who very readily agreed to my request and have put in a lot of efforts in helping me produce this issue in time. I wish to dedicate this issue to the policy-makers of this country who frame the medical curriculum, besides all those patients who battle infections, especially in the intensive care units of our hospitals day in and day out.

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Diagnosis of septicaemia with special reference to enteric fever

ARCHANA AYYAGARI, INDRANIL ROY

Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow

Abstract: Sepsis is the most common cause of death in the non-coronary intensive care unit. The mortality ranges from 16% in patients with sepsis to 40% in patients with septic shock. In some cases, the focus is known, e.g. pneumococcal pneumonia, while in some cases, it is unknown. The organisms most often come from the patient's own flora. Sepsis is becoming more common in immunocompromised and critically ill patients than ever before. Drug resistance is rampant in the isolates making therapy difficult and a good number of cases show no growth in conventional culture systems, obviating the need for special techniques to look for fastidious organisms that may be involved in such cases.

Introduction

While dealing with cases of blood stream infection, the terms bacteraemia, septicaemia, sepsis, etc. are often used synonymously and indiscriminately. This causes much confusion in understanding the disease process and can hamper proper management. The recommended definitions are:[1]

Bacteraemia: The presence of viable bacteria in the blood.

Sepsis: The systemic response to infection that is manifested by two or more of the following conditions as a result of infection: temperature >38 °C or <36 °C; heart rate >90/min; respiratory rate >20/min or $PaCO_2$ <32 torr; WBC >12 000 cells/cmm, <4000 cells/cmm or 10% immature (band) forms.

Septic shock: Sepsis with hypotension, despite adequate fluid resuscitation, along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration of mental status.

In a study it was found that of the total cases with septicaemia, 50% were due to community-acquired infections; the commonest isolate was *Salmonella* species out of which one-third were *S. typhi*.[2] Multidrug-resistant *S. typhi* has emerged in various parts of the world since 1989. Such strains have been isolated from Asian countries such as India and Bangladesh as well as from the western world. In India, several epidemics of typhoid have broken out in the past two decades. These isolates are often resistant to chloramphenicol, ampicillin and co-trimoxazole. In 1983, an outbreak of *S. typhi* infection in Chandigarh was caused by chloramphenicol-resistant strains.[3] An increase in the number of cases caused by *S. paratyphi* A has been noticed in Delhi since 1996 and an outbreak caused by this bacteria was detected in 1999. About 32% of the 16 strains isolated were resistant to more than two antibiotics.[4] Plasmid-mediated chloramphenicol resistance was detected in the isolates of *S. typhi* since the early 1970s, both in the northern and southern parts of India.[3,5] Armed with drug-resistant genes, typhoid fever continues to be a rogue disease affecting over 20 million people each year resulting in 700 000 deaths worldwide. In a SENTRY antimicrobial surveillance programme in Europe during 1997–98, *Escherichia coli*, *Staphylococcus aureus* and coagulase-negative *Staphylococcus* topped the list of pathogens isolated from the blood.[6] In children and those admitted in the neonatal intensive care unit (ICU), Gram-negative bacteria are the most frequent invaders of the blood stream with *Klebsiella pneumoniae* being the most frequent isolate. There have been several reports of outbreaks of Gram-negative septicaemia among neonates caused by *K. pneumoniae*, *Pseudomonas aeruginosa, Acinetobacter* species and *Enterobacter*. Invasive procedures such as exchange transfusions via the umbilical vein potentially expose the neonate to nosocomial infection. Blood stream infections are the most common mode of nosocomial infection.

From January 2001 to July 2002, a clear predominance of Gram-negative bacteria (67.8% of isolates) over Gram-positive cocci (32.2%) isolated from the blood was seen in the Sanjay Gandhi Post Graduate Institute of Medical Sciences, a tertiary-care hospital. *E. coli* was the most common Gram-negative bacteria in the samples from the ward and ICU, and *S. aureus* was the commonest Gram-positive organism from these places. Kapoor *et al.* reported a predominance of Gram-negative isolates (67.9%) in the ICU and nurseries.[7] In the burns unit, there was a fall of *Pseudomonas* and rise of *K. pneumoniae* isolates. In other reports, bacteraemia caused by *P. aeruginosa* was the major cause of death in burn patients.[8]

Another Gram-negative bacteria that is emerging as an important nosocomial pathogen is the *Enterobacter* species. As far as hospital-acquired blood stream infections are concerned, coagulase-negative staphylococci have made a comeback and their presence in the blood can no longer be regarded as being mere contaminants, especially among patients in critical care units. Arterial lines, central lines and other artificial devices are a major source of this infection.

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Pathophysiology and clinical features

The complications of septicaemia can range from hypotension, bleeding, leucopenia, thrombocytopenia, haemolytic uraemic syndrome to organ failure. The whole range of manifestations is the result of an interaction between bacterial products and host factors. Bacterial endotoxin is one of the best studied products, though others such as formyl peptides, exotoxins and proteases from Gram-negative bacteria, and exotoxins, enterotoxins, haemolysins, peptidoglycans and lipoteichoic acid from Gram-positive bacteria may also be involved. These toxins stimulate the release of cytokines from macrophages that trigger off a systemic inflammatory cascade. Tumour necrosis factor (TNF)-α and interleukin (IL)-1 play a major role in this process.

The most important mediator of septic shock seems to be nitric oxide, which is a potent vasodilator. The expression of adhesion molecules may be upregulated on endothelial cells and neutrophils during septic shock. These include CD11/CD18 on neutrophils and intercellular adhesion molecules (ICAM)-1 and ICAM-2 on endothelial cells. Adhesion and transmigration of neutrophils to the extravascular space lead to microvascular and tissue injury. There may be activation of the extrinsic pathways leading to disseminated intravascular coagulation. The host factors which can increase the risk of septicaemia include invasive procedures, extremes of age and immune suppression. Acquired immunodeficiency syndrome (AIDS) and/or severe immunosuppression can be associated with an increased risk of Gram-negative septicaemia, especially among young children.

Bacteria causing bacteraemia can come from a variety of sources. They can be from an infective focus in the body such as pericarditis, peritonitis, pneumonia, osteomyelitis, skin and soft tissue infections, or they may come from medical devices and the hospital environment. Microbes such as *Staph. epidermidis* are frequent colonizers of catheters and can easily invade the blood stream. In neonates, low birth weight, presence of a central venous pressure (CVP) line, paediatric risk of mortality (PRISM) score and length of stay in the hospital have been identified as notable risk factors.

Septicaemia in enteric fever deserves a detailed discussion. Enteric fever caused by *S. typhi* and *S. paratyphi* (A, B and C) has been defined as a generalized infection of the reticuloendothelial system and intestinal lymphoid tissue accompanied by sustained fever and bacteraemia. Typhoid fever is more common than paratyphoid fever and rarely other serovars of *S. enterica* may cause a similar clinical condition. Bacteraemia occurs twice during the course of infection—once, when the infectious dose reaches the intestine, the bacteria pass through the epithelium without damaging it to enter the lymphatic channels and then into the blood stream producing a transient and usually symptomless primary bacteraemia. The second episode of bacteraemia results when the bacteria multiply at the end of the incubation period in the reticuloendothelial system and spill over into the blood stream producing obvious signs and symptoms. It does not produce a fulminant clinical picture resembling septic shock; rather, it begins as a subacute illness and progresses gradually to the acute stage. The patients are bacteraemic during the first and second weeks of the illness but after that, when the disease worsens, the patients are mostly free of bacteraemia. Also, in typhoid fever, compared with other Gram-negative bacteraemic illnesses, the bacterial load is low ($<10^3$ bacteria/ml), whereas a count of $\geq 10^5$/ml is usual for other Gram-negative bacteraemia. In spite of the low level of circulating lipopolysaccharides (LPS) in typhoid fever, the toxin may still act locally in the liver, spleen and elsewhere to release endogenous pyrogens into the blood.

A key factor in the pathogenesis of enteric fever is the survival of the organisms within macrophages. After reaching the small bowel, the bacteria are rapidly internalized by the host cells and transported to the submucosal lymphatic tissue.[9] Internalization occurs by what is called bacteria-mediated endocytosis. In this process, the bacteria are internalized into the membrane-bound vacuoles through which they are transcytosed from the apical to the basolateral surface. In the lymphoid tissue, they interact with the macrophages. They gain entry inside the macrophages by the induction of macropinocytosis rather than receptor-mediated endocytosis. The macropinosomes may fuse to give rise to spacious phagosomes containing *Salmonella. Salmonella* can induce death of the macrophages after phagocytosis and can also induce phagocytosis by macrophages, thus, surviving engulfment by neutrophils, which are very effective killers of *Salmonella*. Survival within macrophages is mediated by regulatory proteins PhoP/PhoQ that regulate the gene complex, which is also responsible for resistance to cationic antimicrobial proteins, acid pH and invasion of epithelial cells. Regulatory genes implicated in the pathogenesis include *crp/cya*, *ompR/ envZ* and *katF*.[9]

Diagnosis of septicaemia

Blood culture: The role of blood culture in typhoid fever cannot be overemphasized. In 1907, a review of the literature reported that 89% of blood cultures were positive in the first week of typhoid fever; 73% were positive in the second; 60% were positive in the third; and only 26% were positive in the fourth week and thereafter.[10]

Collection: Blood samples should be collected prior to the administration of antibiotics. Blood meant for culture must be collected under aseptic conditions. Though collection by venepuncture is preferred over collection from indwelling intravenous or intra-arterial catheters, a comparison of these two practices showed 96% correlation in positive cultures and 98% correlation with negative cultures. It has been recommended that less than 15 colony forming units (CFU) of bacteria obtained by catheter culture is less likely to reflect true septicaemia.

Culture methods: It has been observed that in about 10%–30% cases, the aetiological agents of septicaemia cannot be grown in culture medium. There can be several reasons for this: (i) prior antimicrobial therapy; (ii) presence of fastidious bacteria in the blood that need a special growth environment; (iii) slow-growing bacteria, e.g. *Brucella,* which needs longer incubation; and, (iv) inappropriate anticoagulant that may be inhibitory to some bacteria, e.g. sodium amylosulfate (SAS) is inhibitory to *K. pneumoniae,* and sodium citrate (0.5%–1.0%) may be inhibitory to some Gram-positive cocci. The lysis centrifugation system is particularly good for fungaemia.

Of the automated blood culture systems, both BacT/Alert and the BACTEC 9440/9120 detect changes in the CO_2 concentration in the blood–broth mixture. While BacT/Alert uses spectral light to detect the change, BACTEC uses fluorescent light. The extra sensing power (ESP) blood culture system monitors multiple factors such as CO_2 pressure, changes in the concentrations of H_2 and O_2 in addition to CO_2. The Vital blood culture system differs from BACTEC in the incorporation of a soluble fluorescent molecule directly in the broth. The OASIS blood culture system measures headspace gas pressure by a scanning laser sensor.

Apart from these automated systems, there are also some manual systems designed for the rapid detection of growth of bacteria in blood. These include the Oxoid signal system and the Septi-Check system. Some comparative studies indicate that BacT/Alert is superior or equivalent to BACTEC.[11] The FAN bottles of BacT/Alert have a higher rate of positive results while the patient is on antibiotics. The BACTEC Aerobic Plus/F culture system is more rapid in detection than Septi-Check, though the latter shows a greater rate of recovery[12] and the ESP system detects growth much earlier than Septi-Check.[13]

The non-culture techniques include latex agglutination tests for group B streptococci, *Haemophilus influenzae* type B, *S. pneumoniae*, *Neisseria meningitidis* and staphylococcal teichoic acids. The limulus amoebocyte lysate assay is a highly sensitive test for endotoxin detection. Gas liquid chromatography and lysis filtration techniques have also been tried.

The problem with blood culture in typhoid fever is that the bacterial load in the extracellular compartment of the blood is low, as two-thirds of the bacterial population remain confined within the phagocytic cells. Also, the proportion of patients with a positive culture decreases with increasing duration of illness and volume of blood. Isolation techniques are also important factors that determine the yield from blood cultures.

Clot culture: Several methods have been tried as alternatives to whole blood culture. One of these is clot culture. In this method, blood is allowed to clot, loosened with streptokinase and incubated in broth for subsequent subcultures. This gets rid of the bactericidal substances present in the serum. Also, the serum can be used for biochemical or serological testing.

Buffy coat culture: Wain *et al.*[10] used direct plating of the buffy coat as an alternative to whole blood culture. They found that the method was as sensitive as whole blood culture and allowed earlier identification of the organism and antimicrobial sensitivity testing. Other advantages included less contamination, higher yield from a small volume of blood, especially in children having higher bacterial load and availability of plasma from the same sample, which can be used for biochemical and serological tests.

Lysis direct plating lysis centrifugation (LDP–LC): This method was tried by Saha *et al.* in 1992 on the blood samples of Bangladeshi children and evaluated for its effectiveness in the rapid identification of *S. typhi*.[14] They found the time for generating a report was less by this method than the conventional techniques.

Bone marrow culture: Enteric fever is the only bacterial infection in humans for which bone marrow examination is routinely recommended while investigating pyrexia of unknown origin. Bone marrow culture is superior to blood culture because it increases the diagnostic yield by about one-third compared with those from blood.[15] Quantitative studies have revealed that in typhoid fever blood bacterial counts do not correlate either with the outcome or the clinical and laboratory measures of severity.[10]

The marrow samples remain positive for up to 5 or more days after starting fluoroquinolone therapy. Cell-culture experiments have shown that serovar typhi can replicate in human macrophages to reach an average of 14 organisms per cell. In contrast, peripheral blood monocytes from patients infected with serovar typhi contain an average of only 1.3 CFU/cell. In one study, it was found that there were over 10 times more bacteria in the bone marrow than in the blood, which means that compared with 10 ml of blood, only 1 ml of bone marrow will be needed to yield a positive result.

Molecular techniques for detection of S. typhi in blood: Song *et al.*[16] developed a polymerase chain reaction (PCR)-based test for this purpose using a 343 bp fragment of *flagellin* gene of *S. typhi* as the target. They concluded that PCR was a rapid, simple and specific method for the early diagnosis of typhoid fever, particularly useful in culture negative-antibiotic treated cases. A nested PCR has also been developed based on the *Via B* sequence that codes for the Vi antigen, which has been found to be highly sensitive.[17]

Tests for the diagnosis of enteric fever

Stool cultures are positive in less than half the patients and urine culture in even less. Stool cultures may be useful when the patient is on antibiotics and the bacteria have been cleared from the blood. Culture of biopsy specimens from rose spots can be positive in about two-thirds of the cases. The Widal test has been in use for more than 100 years but its use in the diagnosis of typhoid fever is limited.[18] The minimal titres defined as positive for O and H antigens should be established for individual geographical areas to obtain credible results. The test is more reliable in areas from which data on the titres in control groups without enteric fever are available. Other limitations are cross-reactions and false-positive results in the acute settings.

Though several assays to detect antibodies against

Salmonella have been developed, the value of enzyme immunoassay (EIA) for routine diagnostic purposes is limited because there are numerous *Salmonella* serotypes and antigens. Counter-immunoelectrophoresis to detect *Salmonella* antigens in the blood or urine lack sensitivity and specificity. Dot enzyme immunosorbent assay for rapid serodiagnosis has been described by various workers and is available commercially as easy-to-do kits.[19]

Management

The choice of antimicrobials in sepsis depends on a multitude of factors such as whether the infection is community- or hospital-acquired, the organism involved, immune status of the patient, tolerance of a drug in a particular individual and the existing pattern of antimicrobial resistance in a particular ward. Multiple antibiotics are often administered and the value of such a regimen cannot be denied. Combination therapy covers a large range of organisms while the culture reports are awaited; it prevents the emergence of resistance and may act additively or synergistically. For community-acquired infections, first- and second-generation cephalosporins are reasonable choices. For nosocomial infection, aminoglycosides with β-lactam drugs is a better regimen. For the neutropenic patient, the β-lactam should be active against *Pseudomonas*. Indiscriminate use of third-generation cephalosporins may give rise to extended spectrum β-lactamase producing strains. Use of these drugs as well as imipenems in places such as ICUs should be made judiciously to prevent the emergence of resistance. The final choice obviously depends on the results of the culture and antibiogram. It should be remembered that it may take 4–5 days before the fever peaks abate, therefore, this should not be viewed as resistance or a need for combination therapy.

The use of antiserum to treat bacterial infections is not a new concept. Several such agents have been tried for sepsis, e.g. E5 monoclonal antibody to endotoxin, human monoclonal antibody HA-1A, monoclonal antibody to human TNF-α, etc. Other agents that have been tried include recombinant human IL-1 receptor antagonist, platelet activating factor (PAF) receptor antagonist[20] and activated protein C.

For enteric fever, chloramphenicol was the drug of choice until resistant strains became widespread. The most frequently used group of drugs now is the quinolones. Ciprofloxacin has good activity inside phagocytes. A short course of ofloxacin has been evaluated for multidrug-resistant *S. typhi*. Quinolones are cheap, well tolerated and have good efficacy. There has been some concern regarding their use in children but other reports say that no arthropathy or cartilage damage has been observed with their use in humans and they can be used safely even in neonatal septicaemia with good results.[21] The other drug with high efficacy against *Salmonella* is ceftriaxone. The shortening of the duration of fever has been observed with the use of this drug.[22] However, a resurgence of chloramphenicol-sensitive *S. typhi* has been noted recently, which is probably due to the restricted use of the drug for the treatment of typhoid fever.[23]

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Coccidian intestinal parasites: Diagnosis and treatment

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Abstract: Intestinal coccidian parasites such as Cryptosporidium, Isospora, Cyclospora and Sarcocystis have gained importance as emerging pathogens in the era of AIDS. Several protozoan infections that had previously been considered rare are now much more commonly recognized in the immunocompromised group and, more importantly many new species of parasitic protozoa have been recognized as human parasites. All coccidian parasites have a complex life-cycle involving asexual, sexual and sporogenous cycles, and details are discussed here.

Cryptospordium

The coccidian parasite *Cryptosporidium* causes a disease called cryptosporidiosis. It infects the epithelium of gastrointestinal tract to produce self-limiting in immunocompetent but potentially life-threatening diarrhoea in immunocompromised patients, especially in those with AIDS. It is responsible for 2.2% (range 0.26%-22%) of cases of diarrhoea in immunocompetent persons in developed countries and 6.1% (range, 1.4%-41%) in developing countries. It has been reported in up to 7% of children with diarrhoea in developed countries and up to 12% in developing countries.[1] Cryptosporidial infection in immunocompromised patients, especially those with AIDS, leads to persistent watery diarrhoea, malabsorption and weight loss, and tends to involve the biliary tract, pancreas, stomach and the middle ear. In developed countries, it occurs in 14% (range 6%-70%) of patients with AIDS and diarrhoea; in developing countries, it occurs in 24% (range 8.7%-48%) of such patients. The proportion of general population excreting oocysts is 1%-3% in developed countries and 10% in developing countries.[1] In India, HIV serosurveillance 1996-1997 showed an HIV positivity rate of 21.07 per 1000 population and C. *parvum* infection in 27.5% of these patients.[2] A study conducted at the Post-Graduate Institute of Medical Education and Research, Chandigarh has documented this infection in 10.8% of HIV/AIDS patients.[3] In Manipur, 94.4% of HIV-positive drug abusers were infected with C. *parvum*.[4]

There are 10 species of this parasite but *C. parvum* is the only species responsible for human infections. Further, it has been categorized into two genotypes the human type 1 and bovine type 2. Most human infections occur directly by the faecal-oral route or indirectly by fomites. Zoonotic transmission from cattle and sheep to humans is known and the latter are considered as important reservoirs. *Cryptosporidium* oocysts may be found in all types of water including untreated surface water, filtered swimming pool water and even chlorine-treated or filtered drinking water. This is a growing concern since waterborne outbreaks have been reported worldwide.[5] The 1993 outbreak in Milwaukee, U.S.A. resulted in the death of several immunocompromised patients and caused illness in many previously healthy persons.[6]

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Diagnosis

The simplest tool available is microscopic examination of the stool, sputum or bile. These specimens should be submitted as fresh material or in 10% formalin or sodium acetate-acetic acid-formalin (SAF) preservative. Fixed specimens are recommended because the cysts are highly infectious and resistant to disinfectants. Potassium dichromate solution is routinely used as a storage medium to preserve oocyst viability.[7]

Stool examination

A minimum of three samples collected on alternate days need to be examined. This is especially true for formed stool specimens, which contain fewer cysts than diarrhoeal stools. The stool samples are examined after concentration by either Sheather sugar flotation technique, zinc sulphate (specific gravity 1.18), or saturated salt solution (specific gravity 1.27), or sedimentation techniques such as formol-ether or formalin-ethyl acetate. To detect *Cryptosporidium* cysts, it is advisable to centrifuge the stool specimen at more than 500 *g* for at least 10 minutes.[7]

Unstained wet mount preparation: Faeces may be examined using phase-contrast or bright-field microscopy. However, the oocysts are considered infectious and resistant to routinely used disinfectants. Hence, stained smears are better then wet preparations.

Staining techniques: Most recommended stains for *Cryptosporidium* oocysts cannot be performed on stools commonly preserved in polyvinyl alcohol fixative. The widely used techniques include modified acid-fast staining and negative staining.[8,9] Less common staining methods include auramine-rhodamine and acridine orange.[10] All stained preparations should be examined using a high dry (40x) or oil immersion objective.

Immunofluorescent (IF) *antibody:* Procedures employing

Cryptosporidium-specific polyclonal or monoclonal antibodies have been developed and may provide the most sensitive method for the diagnosis of cryptosporidiosis.[11] The sensitivity of acid-fast (AF) immunofluorescence for detecting *C. parvum* oocysts in human stool has been reported to be 10 000 oocysts per g of watery stool while in formed stools 50 000 or 500 000 oocysts are required for a positive IF or AF test, respectively.[12]

Autofluorescence: The green autofluorescence of the oocysts under violet or ultraviolet (UV) light illumination can be used to detect *Cryptosporidium* in cell cultures. However, due to similarly fluorescing spores and yeast cells of identical size,[13] identification of oocysts in faecal samples using only autofluorescence is not recommended.

Serodiagnosis

Specific IgG or IgM antibodies have been detected by enzyme-linked immunosorbent assay (ELISA) using crude oocyst preparations as antigens. However, detection of specific antibodies only indicates prior exposure. Antigen detection: Immunoassays using uncentrifuged, fresh, frozen or fixed faecal material can be used for antigen detection. Currently, enzyme immune assay kits are available. The sensitivity and specificity of such tests are in the range of 90%-100%. Some of these, particularly the combination of direct fluorescence product used to identify both *Giardia lamblia* and *Cryptosporidium* cysts, are being widely used in water testing and outbreak situations.[7]

Flow cytometry

Flow cytometric assays for the detection of *Cryptosporidium* oocysts in stool have been shown to be ten times more sensitive than conventional IF assays.[14]

Molecular techniques

Application of molecular techniques has contributed greatly to the understanding of the genetic diversity among *Cryptosporidium* isolates. Polymerase chain reaction (PCR), with its various modifications, has been used by many groups of workers. A sensitivity of 97% in nested PCR assays to detect *Cryptosporidium* DNA in fixed, paraffin-embedded tissues and water samples has been reported.[15] An important advantage of PCR is its ability to directly differentiate between genotypes of *Cryptosporidium.*

Treatment

The clinical course of cryptosporidiosis depends largely on the immune status of the host and, therefore, the treatment options vary. Generally, asymptomatic and immunocompetent persons need no specific therapy. Supportive therapy with oral or intravenous fluids and electrolyte replacement helps to correct the dehydration that accompanies acute diarrhoea and the patient recovers spontaneously. In children, spiramycin (100 mg/kg/day) may shorten the duration of oocyst excretion and diarrhoea, although the data on this aspect are conflicting. In patients with AIDS, the best treatment is improvement of immune function with highly active antiretroviral therapy (HAART), which also helps to resolve *Cryptosporidium* infection. If HAART is not possible, combination therapy with an antimicrobial and an antidiarrhoeal agent helps. More than 100 antimicrobial agents have been tested so far, but none has been found to be consistently curative. Some clinical improvement and decrease in oocyst shedding has been seen with the non-absorbable aminoglycoside paramomycin (1 g twice daily) and azithromycin (600 mg daily) for 4 weeks followed by paramomycin monotherapy for an additional 8 weeks.[16] Nitazoxanide, a nitrothiazole benzamide compound with a wide spectrum of activity against protozoa, helminths and bacterial pathogens, appears to have some efficacy against human *Cryptosporidium*. Severely immunocompromised AIDS patients with refractory cryptosporidiosis may show variable response to letrazuril. Ongoing trials with immunological intervention hold promise. The best treatment for biliary cryptosporidiosis in patients with AIDS is still HAART. It can help to resolve infection but may not eradicate the organism from the biliary tract. For patients with pain or cholangitis associated with papillary stenosis, endoscopic sphincterotomy may provide striking symptomatic relief.[5]

Control and prevention

Most conventional water treatment methods do not effectively remove or kill all oocysts. Thus, preventive measures include health education about proper handwashing and boiling or filtration of water (filter pores < 1µm diameter).

Cyclospora

Both immunocompromised as well as immunocompetent persons can be infected. The mode of transmission of infection is water or food. Waterborne outbreaks of diarrhoeal disease have been reported from different regions throughout the world. A number of food items, especially fresh fruits, vegetables and dishes containing items (raspberries, baby lettuce and basil) have been implicated. Two outbreaks had been reported in the USA and Canada in 1996 and 1997 and were related to the import and consumption of Guatemalan raspberries.[17] Prevalence rates of *C. cayetanensis* in HIV patients in India have been reported to range from 3% to 5.2%.[3,18,19] The infection is seasonal, and occurs particularly during the warm and rainy seasons. The signs and symptoms of the disease include explosive diarrhoea, abdominal cramps, vomiting, anorexia, fatigue and weight loss. In immunocompetent patients, the diarrhoea is prolonged but self-limiting and lasts for 1-6 weeks. In immunocompromised patients, the diarrhoea is even more protracted. *Cyclospora* resides in the upper small bowel and can cause villus atrophy, crypt hyperplasia and inflammatory changes.[20]

Diagnosis

Diagnosis of cylosporiasis is mainly dependent on the detection of oocysts or its antigens/DNA in faecal samples or, less commonly, in the jejunal aspirates of infected persons.

Unstained wet mount preparation

C. cayetanensis can be detected by examining an unstained normal saline preparation under a microscope at a magnification of 440X. The oocysts appear as non-refractile, round, hyaline structures containing an arrangement of refractile membrane-bound globules. Measurement of the oocyst is essential to differentiate it from other coccidia, especially *Cryptosporidium.* The oocysts of *Cyclospora* measure 8-10 µm in diameter, while *Cryptosporidium* oocysts measure 4-6 µm in diameter and Isospora oocysts are 22-33x10-19µm in size.[20]

Staining methods

With acid-fast stains, oocysts show variable staining (compared to *Cryptosporidium* oocysts where the majority of oocysts take up the stain), with the colour varying from deep red to pink to unstained; some may contain granules or have a bubbly appearance. It has been observed that in fresh faecal samples, most of the oocysts show good staining, while in older samples, the majority may be decolorized. A strong decolorizer should not be used. Sulphuric acid solution (1%) is recommended for the staining of all coccidian parasites. A more consistent and rapid staining method based on a modified safranin technique stains oocysts of *Cyclospora* a brilliant reddish-orange colour. Other stains that have been used are auramine-O, methylene blue, trichrome, iron-haematoxylin and methanamine silver. These staining procedures are usually more cumbersome and less sensitive.

Auto fluorescence

Primary fluorescence of oocysts is a feature that appears to be unique to *Cyclospora* and *Isospora* oocysts. This method allows the detection of oocysts even if they are covered with faecal debris. The colour of the oocysts appears as neon blue at the excitation wavelength of 330-380 nm and green at 450-490 nm.

The oocysts *of Cyclospora,* excreted in the faeces, sporulate outside the host in about 2 weeks resulting in two sporocysts, each containing four sporozoites. The oocysts can also be induced to sporulate in the presence of 5% potassium dichromate.[14]

Molecular methods

There are only a few studies which have tried to use PCR for the diagnosis of cyclosporiasis. Though this may not find application in the field at present, it may help in the epidemiological investigation of oocysts in foods such as raspberries, etc.

Treatment

The drug of choice is trimethoprim/sulphamethoxazole (TMP-SMX) at a dose of 160/800 mg twice daily for 7 days. This eradicates the organisms and decreases symptoms**.** In patients with AIDS, the treatment recommended is similar. However, maintenance therapy with TMP-SMX 3 times a week or sulphadoxine 500 mg + pyrimethamine 25 mg once a week is given to prevent relapse. Ciprofloxacin 500 mg twice a day for 10 days has been reported to be a reasonable alternative in patients unable to tolerate TMP-SMX.[21]

Control and prevention

It is extremely difficult to control and prevent cyclosporiasis because of the limited ability to detect low infective doses of oocysts that may contaminate products such as raspberries. The oocysts are resistant to conventional water treatment procedures such as chlorination and therefore, boiling of drinking water is recommended. Fresh fruits and vegetables should be thoroughly washed and/or peeled before consumption. The role of handwashing cannot be overemphasized.

Isospora

All species of *Isospora* are obligate intracellular parasites, mainly in vertebrates. In humans, two species of *Isospora* have currently been identified, *I. belli* and *I. natalensis*. The latter was reported from South Africa in the early 1950s and apparently there are no further reports in human beings. *I. belli* has a worldwide distribution, with most cases occurring in the tropics. Enteric infections with *I. belli*, once considered rare, are increasingly being recognized in patients with AIDS. It produces chronic, intermittent secretory-like diarrhoea that leads to dehydration. In patients with AIDS, recurrence of symptoms is a common manifestation despite treatment. Prevalence rates in India have been reported to range from 2.5% to 31%.[3,18,19] Animal sources of human infection have not been identified and infection is usually thought to occur by ingestion of oocyst-contaminated food or water. Histologically, *I. belli* infection can induce villus atrophy and crypt hyperplasia.

Diagnosis

Specific identification of the organism requires examination of the faeces, which may contain the infective oocysts. However, examination of fresh material, either as a direct smear or as concentrated material by wet preparation, is recommended rather than permanent stained smears. The oocysts are either pale and transparent and can be easily missed. A biopsy may be positive while no organisms may be seen in the stool because of the small numbers. Oocysts of *I. belli* are elongate, ellipsoidal and are 20-33 x 10-19 µm in size. The oocysts are either unsporulated or partially sporulated and can sporulate in less than 24 hours.[20]

Unlike the sporulated oocysts of *Cyclospora*, both sporocysts and oocysts of *Isospora* autofluoresce a neon-blue colour when illuminated by UV light of 330-380 nm wavelength and viewed under an epifluorescence microscope. The organisms may also be demonstrated by acid-fast staining.

Treatment

The treatment of choice is TMP-SMX (160-800 mg) 4 times a day for 10 days followed by twice a day for 3 weeks. In patients with AIDS, maintenance therapy with the same drug is given. In patients allergic to sulphonamides, pyrimethamine alone (50-75 mg daily) has cured infections. Ciprofloxacin has been found to be a reasonable alternative for patients unable to tolerate TMP/SMX.[21] Nitazoxanide has also been found to be effective in eliminating *I. belli.*

Sarcocystis spp.

Humans serve as a definitive host for *Sarcocystis hominis* and *Sarcocystis suihominis* and also as accidental intermediate hosts for several unidentified species of *Sarcocystis*. Sarocystis spp. cause a disease called sarcoystosis, the symptoms of which vary with the species causing the infection. It may present as intestinal or muscular sarcocystosis. Infection is acquired by ingesting uncooked beef (*S. hominis*) containing sarcocysts. *S. hominis* is only mildly pathogenic compared to the more pathogenic *S. suihominis* (found in pork). *The* oocysts and sporocysts of *Sarcocystis* are discharged over a period of several months; they are resistant to freezing and drying, and spread by invertebrate transport hosts. There is little or no immunity to reshedding of sporocysts, which are passed in the infective form.[5]

Diagnosis

Diagnosis of intestinal sarcocystosis is easily made by faecal examination. Oocysts are colourless, thin-walled and contain two elongated sporocyst, each of which contains four elongated sporozoites and a granular sporocyst residuum. The thin oocyst wall often ruptures, releasing the sporocysts in the intestinal lumen from where they are passed in the faeces. The sporocysts that are recovered in stool are oval, measuring 9-16 µm and contain four mature sporozoites and the residual body. Sporocysts or oocysts of *Sacrocystis* are shed fully sporulated in the faeces whereas those of *I. belli* are often shed unsporulated. Till date, it has not been possible to distinguish one species of *Sarcocystis* from another.

Treatment

No specific therapy is known to be effective for *Sarcocystis* infections.

Control and Prevention

Prevention includes adequate cooking of pork and beef.

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Genomic diversity of human immunodeficiency viruses: The Indian scenario

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Abstract: The human immunodeficiency virus type 1 (HIV-1) strain exhibits an extraordinary degree of genetic diversity. It has been divided into 3 groups M for main, O for outlier and N for non-M non-O. The M group of viruses have been further divided into ten distinct genetic subtypes A-H and J, K and L. The diversity of HIV-1 is achieved by a high rate of mutation and replication, immune selection of resistant strains and recombination. In an infected individual, the virus exists as a population of genetically distinct, yet related genotypes called quasispecies. The distribution of HIV-1 subtypes shows a geographical predilection. Globally, subtypes A and C account for most current infections. The identification of subtypes and circulating recombinant forms provides a means of tracking the dissemination of the pandemic worldwide. The molecular epidemiological data regarding HIV infection in India is still limited. Both HIV-1 and HIV-2 are found in India. Studies revealed the presence of multiple subtypes of HIV-1, namely A, B and C, subtype C being predominant (the majority belonged to subtype C3). Even within a genetic subtype of HIV-1, the extent of genetic and antigenic diversity is enormous. However, it is observed that some features of the envelope glycoprotein structure of the virus are conserved. It would be desirable to express such conserved structures in vaccine antigens aimed at inducing a broadly reactive humoral immune response. The analysis of genetic subtypes and inter-subtype recombinant genomes is necessary to elucidate the geographical distribution and historical evolution of the strains; to detect the introduction of divergent strains; to develop subtype-specific serological techniques; to study the pattern of transmission and degree of transmissibility among variant strains; to delineate the host range, relation to disease progression and drug resistance patterns; and to design vaccines.

Introduction

More than 40 million people are infected with human immuno deficiency virus (HIV) globally. A majority of these patients experience similar symptoms regardless of the region they come from. These patients show similar responses to the same regimen of antiretroviral therapy, meaning thereby that all HIV-positive individuals are infected with an identical version of the virus. On the contrary, these are the different variants of HIV, which are members of one large family. This phenomenon is called HIV diversity. Globally circulating strains of HIV-1 exhibit an extraordinary degree of genetic diversity, which may influence various aspects of their biology such as infectivity, transmissibility and immunogenicity.

HIV-1 strains have been divided into three groups on the basis of phylogenetic analyses of complete genome sequences. The groups were originally named M for main, O for outlier, and N for non-M non-O.[1] Group M includes viruses that dominate the global epidemic,[2] and are responsible for more than 99% of infections.

HIV-1 genetic subtypes

The M group viruses have been divided into ten distinct genetic subtypes or clades (A through H and J, K and L).[1] Prototype viruses representing the genetic subtypes E and I have not yet been found. The viruses originally identified as subtype E (the predominant group of viruses involved in heterosexual transmission in Thailand) and I (a small group of viruses from the Mediterranean region) are now considered inter-subtype recombinants.

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Mechanisms for HIV-1 diversity

A study of isolates from the Democratic Republic of Congo indicates central Africa as the epicentre of HIV-1 diversity, with a large number of different genetic subtypes and subtype recombinants circulating. The prevalence of inter-subtype recombinant strains is increasing and creates even more HIV-1 antigenic diversity. Several recombinant viruses have now spread epidemically to establish distinct lineages.

The diversity of HIV-1 strains is achieved by: (a) the high rate of mutation, (b) high rate of virus production, (c) immune selection of resistant strains, and (d) recombination. The viral enzyme reverse transcriptase (RT), which transcribes the viral RNA into proviral DNA, is error prone. which is approximately 1 in 4000 nucleotides. In addition, HIV-1 maintains a very high replication rate producing 10 billion virus particles per day in an infected individual. Assuming that RT creates 2-point mutations during each transcription and 1 million reverse transcriptions are taking place each day, then only every single point mutation is produced daily in an infected individual. In an

infected individual, the virus exists as a population of genetically distinct, yet related genotypes called quasispecies. The diversity also greatly increases through the recombination of divergent viral strains within a population. Further, immune-mediated selection within a host also increases the HIV-1 diversity.

Recombination events between segments from two different viral strains in the same individual have resulted in the emergence of circulating recombinant forms (CRFs). Overall, CRFs constitute 10%–20% of newly characterized strains and recombination between CRFs has also been reported.[3] Phylogenetic analyses in areas of high HIV-1 transmission have shown the presence of multiple mosaic genomes.[4]

Geographical distribution of HIV-1 subtypes

The distribution of HIV-1 subtypes shows a geographical predilection.[5] Globally, subtypes A and C account for most of the current infections followed by subtype B and the inter-subtype recombinants. Subtype B is dominant in Europe, the Americas and Australia. Subtype C probably currently infects more people worldwide than any other, accounting for more than 50% of infections; it is common in southern Africa and India. Subtypes A and D infect a large number of people in central and eastern Africa. The other subtypes infect a relatively small number of people in central Africa and South America. For example, subtype F includes isolates from Brazil and Romania, and subtypes G and H include viruses from Africa, Russia and Taiwan. Subtype K, whose *env* C2-V5 sequence branched within group M but remained distinct from all known HIV-1 subtypes, was reported from the Cameroon.[6]

In recent years, the prevalence of non-B infection has markedly increased in several European countries.[7] Although non-B infections are infrequent in North America, a study in New York identified non-B infections in a few US citizens.[8]

Even though it is the behaviour and not the occupation of an individual that determines the risk of HIV-1 infection, some groups of individuals, particularly travellers, contribute to increasing the diversity of HIV-1 worldwide. They include, in particular, immigrants, intravenous drug abusers, tourists, truck drivers, military troops and seamen. In large countries such as Russia, China, India, Brazil and South Africa, internal migrants contribute largely to the spread of HIV-1 diversity. As HIV-1 continues to spread globally, the geographical restrictions are increasingly breaking down.

Tracking the dissemination of the pandemic

As mentioned above, the identification of subtypes and CRFs provides a means of tracking dissemination of the pandemic. The world map illustrates that most of the subtypes and CRFs are present in central Africa. This distribution indirectly suggests that a few individuals initiated the earliest HIV-1 spread outside Africa.[5] It has been documented that the initial spread of the pandemic in a previously unaffected area is generally characterized by a founder effect, which translates into the rapid spread of a single subtype or CRF in a defined risk group (that is, subtype B in men having sex with men in North America, western Europe, and Australia, and subtype A in intravenous drugs users [IDUs] in Russia).[9] By contrast, in Thailand, two separate epidemics occurred almost simultaneously with subtype B and CRF01 AE in two different risk groups.[10-12] IDUs were infected with subtype B, whereas CRF01 AE predominantly infected patients through heterosexual contact. This finding suggests that the source case for HIV-1 infection in IDUs was from Europe, the USA, or Australia, whereas the source case in the heterosexual risk group was probably derived from central Africa. Similarly, Maitra *et al.*[13] noted in their series that infection due to subtype A in a patient with idiopathic thrombocytopenic purpura (ITP) and a husband-wife pair from Haryana was probably derived from central Africa. Little is known about the factors driving changes in the prevalence of subtypes in the same geographical areas, such as quasi absence of subtype B in Africa with the exception of homosexuals in South Africa.[14] Similarly, replacement of subtype B in the northeastern region of India by subtype C is quite intriguing (Seth P, unpublished).

The Indian scenario

The scenario of HIV infection in India is extremely grim; the molecular epidemiological information is still limited. Till date, a few preliminary studies that have been conducted on these lines have all suffered from drawbacks such as small sample size, lack of proper representation from various geographical regions and inclusion of different risk groups and modes of transmission. Sahni *et al.*[15] studied 125 HIV-1 seropositive individuals from different regions of India. In their study population, most of the infections were heterosexually acquired (84.8%). The other modes of transmission were blood and blood products (8.8%), IDU (3.2%), vertical transmission (1.6%) and transmission through homosexual and artificial insemination (0.8%).

Initial studies have indicated that both HIV-1 and HIV-2 are present in India.[16] Subsequent studies have indicated the presence of subtype C. The subtype C isolates were found to cluster with South African isolates of NOF and ZAM 18.[13,17] Maitra *et al.*[13] studied the genomic diversity of HIV-1 in India by partial sequencing of the *gp120* gene (C2-V3-C3 region). The study revealed the presence of multiple subtypes of HIV-1, namely subtypes A, B and C. However, the predominant subtype was C (82% of sequences analysed). Most of the subtype C sequences obtained in India were related to the African subtype C sequences in the C2-V3-C3 region. Most of the subtype B sequences were obtained from IDUs from the northeastern state of Manipur and were related to subtype B sequences circulating in Thailand. The subtype A sequences were related to central and east African subtype sequences.

In another study,[15] heteroduplex migration analysis (HMA) was used as a tool for epidemiological typing of HIV-1 isolates. A majority of the strains present in India belonged to subtype C (78.4%). Subtypes A, B and E were found in samples from the north and northeast. Further analysis of HMA data suggested that subtype C3 was the predominant strain of HIV-1 circulating in India (68%), whereas subtype C2 and C4 were present in fewer samples (8% and 2.4%, respectively). Subtype A was found in a patient with ITP who had received a blood transfusion in 1987 in Delhi and also in a husband-wife pair from Haryana where the husband had acquired infection through a female sex worker (FSW). Of the 11 patients with subtype B (Thai type subtype B), 6 acquired infection through FSW in the northeastern region of the country and 3 acquired infection through intravenous drug use in Manipur in the northeast. One husband-wife pair from Delhi was infected with subtype B' where the wife was inadvertently infected through artificial insemination in South Africa and she later infected her husband. Subtype E was found in a husband-wife pair from Uttar Pradesh in north India where the husband got infected in Manipur through a commercial sex worker (CSW) and then apparently infected his wife through unprotected intercourse. In 11 samples, HMA could not clearly identify the subtype of the envelope sequence. It is possible that these samples were recombinant HIV (homotypic or 'intra-clade' recombinants and heterotypic or 'inter-clade' recombinants). In a recent study on HIV-1 subtypes circulating in the eastern and northeastern regions of India,[18] subtype C was again found to be predominant, with subtype C3 accounting for 50% and subtype C2 13%. The same study documented subtype C (68%) and B (20%) among the strains from IDUs in Manipur.

Implications of molecular heterogeneity of HIV-1

It is important to emphasize that the genetic subtypes or recombinant lineages of HIV-1 are not analogous to classic viral serotypes. The HIV-1 genetic diversity currently present in the human population dwarfs anything that has been described for other human viral infections. To put the situation into perspective: a few amino acid changes in one of the envelope glycoproteins of the influenza virus may be sufficient to trigger a new epidemic and reassortants of influenza virus envelope genes may lead to devastating pandemics.[19] Yet, in HIV-1, replicating viruses can differ as much as 10% in the amino acid sequence even within a single individual. Therefore, within a genetic subtype, the extent of HIV-1 genetic and antigenic diversity is enormous when compared to the diversity found for viruses for which effective vaccines have been developed. The degree of genetic, and hence antigenic, diversity is daunting from the perspective of HIV-1 vaccine development. However, the description of a small number of human monoclonal antibodies that do neutralize many different HIV-1 isolates, including ones from different genetic subtypes, suggests that some features of the envelope glycoprotein structure are conserved.[20] It would, therefore, be desirable to express such conserved structures in vaccine antigens aimed at inducing a broadly reactive humoral immune response. Knowing that genetic variants can escape immunosurveillance, information on genetic subtypes becomes important. Nonetheless, if phylogenetically defined groups broadly correspond to antigenic groups, development of vaccine using the most appropriate (antigenically related) strain will require information regarding the genetic subtypes of the regionally found strains that are most likely to pose a challenge. In addition, distribution of subtypes helps us understand the global HIV epidemic.

In Thailand, the prevalent subtypes have been shown to vary according to the risk group within the same city.[10] The predominance of phylogenetically clustered strains in some geographical areas may reflect introduction by single individuals into high-risk groups.[10] The prevalence of subtype and degree of inter-isolate variation in any region or group also provides us with clues about the origin and spread of HIV. Thirdly, in the face of this global variation of HIV-1, little is known about the correlation of genetic diversity to the biological properties of the virus. For example, HIV-1 subtypes B and E have caused two parallel epidemics in Thailand.[11] The subtype B infection, predominating in the IDU population, is different from the North American and European subtype B strains, and is generally referred to as subtype B. The subtype E strains, which are actually recombinants of subtype A and E with the *gag* gene of subtype A and the *env* gene of subtype E, are associated mostly with heterosexual transmission.[12]

The reasons for analysing genetic subtypes and inter-subtype recombinant genomes are compelling. These include: molecular epidemiological studies to elucidate the geographical distribution of strains co-circulating in a particular region; to study the historical evolution of strains in a population group; detect divergent strains being introduced; develop subtype-specific serological techniques such as synthetic peptide enzyme immunoassays for accurate diagnosis and improved sensitivity and specificity; to study the patterns of transmission and degree of transmissibility among variant strains; to delineate the host range and specificity of viral strains such as NSI and SI and their relation to disease progression; to study the drug resistance patterns among subtypes and quasispecies in an individual over time; and to design vaccines based on individual sequence subtypes. Moreover, information on the presence of multiple HIV infections caused by distinct viral subtypes circulating within a population, and the long-term observation of patients dually infected with distinct HIV strains may contribute to a better understanding of the pathogenesis of HIV infections. To study the magnitude of the HIV-1 epidemic in India, more extensive molecular epidemiological analysis of the high-risk population from different geographical regions of the country will be required to substantiate the extent of subtype representation in individual regions. Sequencing of full-length genes rather than sub-genomic fragments of the viral genome are the order of the day.

Acknowledgement

This study was supported by a research grant from the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi.

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Antibiotic policy: Why and for whom?

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Abstract: There is no place for the indiscriminate use of antibiotics. If we continue with the practice, the fact that we have antibiotics may not matter in due course of time. The basis of an antibiotic policy rests in generating microbiological data and prescription auditing at any one geographical place. The concept of a class of antibiotics that is time-and concentration dependent, with an understanding of their pharmacokinetics and pharmocodynamics are important factors for successful antibiotic therapy. It is important to recognize that antibiotics have a high (ampicillin, ciprofloxacin, imipenem, ceftazidime, tetracycline, etc.) or low (piperacillin, quinolones other than ciprofloxacin, third-generation cephalosporins other than ceftazidime, meropenem, etc.) resistance potential. Various issues involved in formulating an antibiotic policy are discussed.

Introduction

Appropriate antibiotic use is one of the main goals of the medical community.[1] Overuse of antimicrobial agents has been described worldwide in both community[2,3] and hospital[4,5] settings. In addition to the effect on patients,[6] antibiotic misuse can provoke the emergence of bacterial resistance[4] and increase healthcare costs.[7] It is evident that optimizing antibiotic use is a challenge that deserves to be undertaken.

It has been observed that the infectious diseases physician plays a crucial role in controlling antibiotic use in the hospital,[8] as does a multidisciplinary team approach with the active involvement of a clinical microbiologist and a pharmacist.[7,9] Bantar *et al.*[10] published an alarming rate of bacterial resistance in a surveillance study involving 27 Argentinian healthcare centres and noted a high rate of nosocomial infection, surgical prophylaxis errors leading to unnecessary cost increases in the hospital,[11] and confirmation of misuse of antibiotics in the same hospital.[5] These findings provide compelling evidence of the need for more rational use of antimicrobial agents. To our knowledge, a systematic strategy for the control of antibiotic use in India has not been undertaken or published.

Inappropriate and empirical antibiotic therapy is widespread and associated with increased mortality in critically ill patients. The initial selection of antibiotic must account for a variety of host, microbiological and pharmacological factors. Institution-specific data, such as susceptibility patterns and local antibiotic use, must be considered. Tailoring antimicrobial therapy according to the culture and sensitivity results will help reduce cost, decrease the incidence of superinfection, and minimize the emergence of resistance.

A few years back, I wrote an article for the souvenir of the Annual Conference of the Hospital Infection Society of India held in New Delhi titled 'Have we reached the dead end?' and I summed up by saying 'If we are not discrete and act now, after another 20 years, it may not matter if we have antibiotics.' But now, I revise this deadline further to only 10 years. We, the human species have to remember that whatever we may do, bacteria shall remain ahead of us by the sheer number of years of their existence on earth, which far outnumber ours. Bacteria are genetically better evolved than us.

'Antibiotic policy: for whom and why'. The answer to both these questions is similar the medical fraternity, at large, feels safe at present with the usage of antibiotics at the slightest pretext.

The concept of antibiotic policy is not new and a great deal of effort goes into this exercise wherever it is undertaken. It is its implementation in letter and spirit that requires serious thinking. When we say that a dead end has been reached, it signifies that we are at the end of a road from where we have nowhere to go. This further means that while treating a case of overwhelming infection, it is unlikely that the patient can be salvaged. With the current anti-infective therapies, multidrug resistant (MDR) organisms have come to stay unless we change our practices. The matter is rendered more complicated due to the presence of extended spectrum β-lactamase (ESBL) producing organisms as a result of the unbridled use of cephalosporins, particularly ceftazidime, a strong inducer of ESBL organism.[12] Though this is the scenario the world over, in India we are more vulnerable due to the overwhelmingly indiscrete use and over-the-counter availability of antibiotics.

Rational antibiotic usage

We have to start from somewhere to begin an era of rational antibiotic usage, which includes the type of antibiotic chosen, its dosage and duration of therapy. The concept of pharmacokinetics and pharmacodynamics (PK/PD) while treating a patient of sepsis with an antibiotic, in the background of the knowledge of the minimum inhibitory concentration (MIC) of the organism in question is, unfortunately, yet to evolve in this part of the world. All this is not attempted in the

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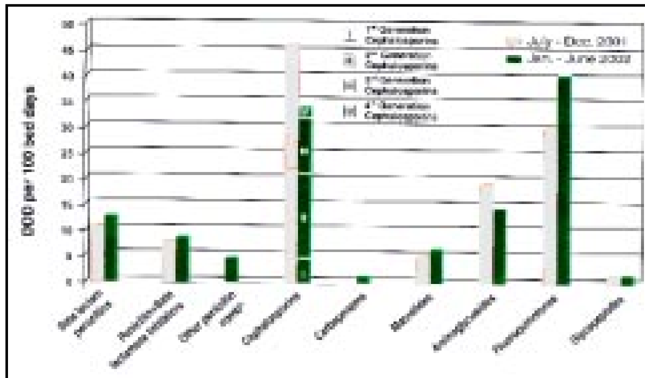
name of increasing the burden of the cost of therapy! The cost-benefit ratio loses its significance when faced with the outcome of irrational treatment with expensive antibiotic usage. Moreover, the concept of a concentration-dependent and-independent class of antibiotics has not emerged and a scientific way of treating infections guided by various scientific parameters remains to be developed.

Prescription Auditing

In light of this, the onus falls on the medical fraternity to evolve an empirical antibiotic policy for every clinical setting and at a given place, based on the existing knowledge of antibiogrammes and antibiotics, their bioavailability and pharmacokinetics. To formulate an antibiotic policy in any hospital or geographical area it is necessary to know the prevailing antibiogramme of that area besides knowledge of the most commonly isolated pathogens both in the outpatient and inpatient departments. It is necessary that such data be generated and antibiotic preferences studied using prescription auditing as a tool (Fig. 1) for the same period of the respective pharmacy that dispenses antibiotics in that area or institution.



I, II, III, IV : first-second-third-fourth generation cephalosporins respectively; July-December 2001; January-June 2002; DDD: daily defined dose

At Sir Ganga Ram Hospital, we are lucky to have the infrastructure whereby this kind of study is being undertaken on a six-monthly basis since 1995. It was interesting to see the prescription auditing figures getting reflected in our antibiogrammes. Keeping this in mind, an antibiotic policy was framed at our hospital. It is necessary to have the following specialties come together with a consensus opinion, i.e. the Department of Internal Medicine and its allied specialties, Department of Clinical Microbiology, and Department of Surgery and its allied specialties. Every institution has a peculiar situation or a set of problems to tackle. Similarly, the intensive care expectations of an institution also vary from one place to another.

Antibiotic class and resistance

Various parameters are central to this theme such as the class of antibiotics being used in their order of preference. Though there is a feeling that class resistance or shift of resistance is limited to a few classes of antibiotics, the key concept of antibiotic resistance is that it is agent specific and not related to an antibiotic class or duration of use. If antibiotic resistance has to emerge in a particular agent, it occurs very early in the course of therapy rather than later.[12] Amikacin, which has been in use for over a decade, is an example, and the level of resistance is still low. Antibiotics with a high resistance potential should be restricted for use; for example, ampicillin, carbenicillin, gentamicin, tetracycline, ciprofloxacin, imipenem and ceftazidime. Antibiotics with little or no resistance potential should not be restricted; for example, piperacillin, piperacillin + tazobactum, amikacin, doxycycline, quinolones (other than ciprofloxacin), third-generation cephalosporins (except ceftazidime), cefipime and meropenem.[12]

To formulate antibiotic policy at any place, besides the need for the availability of local data, it is also important to understand the genesis of antibiotic resistance. Antibiotic resistance with some organisms has become a worldwide problem such as methicillin-resistant *Staphylococcu s aureus* (MRSA), pencillinase producing Neisseria gonorrhoeae (PNG), aminoglycoside, ceftazidime and ciprofloxacin resistant *Pseudomonas.* Resistance among pathogens such as *Moraxella, Streptococcus pneumoniae and Haemophilus influenzae* has also started causing concern in the medical world. The emergence of MDR *Enterobacter* and *Escherichia coli* is worrying. Resistance in anaerobes is by and large not clinically significant. A distinction has to be made between increase in prevalence versus emergence of resistant organisms, which is seen with enterococci. Vancomycin-resistant enterococci (VRE) are not an example of increasing antibiotic resistance but a change in the selective pressure of the faecal flora favouring VRE as a colonizer. However, in the Indian scenario we are lucky to have almost no vancomycin resistance in Gram-positive organisms vancomycin-resistant Staphylococcus aureus [VRSA/E] epidermidis even though VRE have started to emerge.

In vitro susceptibility testing and its pitfalls

While we are familiar with natural and acquired resistance it is equally important to understand the methodology of susceptibility. Moreover, we know that *in vitro* susceptibility testing does not necessarily reflect *in vivo* efficacy, because of the variability of both host and pathogen, culture media used, conditions of incubation, method of identifying the organism and its sensitivity. There is no universal agreement on how to conduct a susceptibility test; for example, in anaerobes, undue emphasis on *in vitro* susceptibility can be misleading at times. Similarly, it is necessary to know the synergy test results of beta-lactams and aminoglycoside groups of antibiotics for the treatment of enterococci or group D streptococci isolated from samples other than the urine. Non-synergistic Kirby-Bauer (disc diffusion) antibiotic sensitivity results can be misleading for these organisms. Thus, knowledge of the variabilities can help clinicians to use the data to their advantage in a given clinical setting.

Gross resistance to a group of antibiotics requires the use of another class of antibiotics to eliminate the resistant organism. However, with some organisms, gross resistance to one antibiotic within the class does not necessarily mean that others in the same class are also resistant. For example, ciprofloxacin resistant *Strept pneumoniae* are sensitive to levofloxacin and strains of gentamicin-resistant *P. aeruginosa* are sensitive to amikacin. It should be noted that third-generation cephalosporin-induced ESBL organisms are resistant to all third-generation cephalosporins. In such cases, treatment should be commenced with an effective agent from a different antibiotic class; for example, carbapenems. When ESBL organisms are prevalent in any clinical or institutional setting, it is worthwhile considering the use of antibiotics combined with β-lactamase inhibitors; for example, piperacillin + tazobactum, cephalosporins with β-lactamase inhibitors (cefoperazone, sulbactum), or ticarcillin clavulanic acid empirically as the first-line antibiotic in severe infections. 'Susceptibility drift' is a term that has also been used. It refers to a temporary decrease in susceptibility to one antibiotic caused by another in the same group; for example, the use of ceftazidime may result in decreased susceptibility to cefpirome in *P. aeruginosa* infection. It is suggested that replacement of ceftazidime with cefpirome may restore sensitivity in *P. aeruginosa* to cefpirome after some months. This implies that the simultaneous use of ceftazidime and cefpirome by a particular medical centre should be discouraged. Nor should a step-up therapy from ceftazidime to cefpirome be considered in the same patient in case of treatment failure. Resistance of *P. aeruginosa* to imipenem implies that it is also meropenem resistant.

Predictor of antimicrobial efficacy

A good predictor of antimicrobial efficacy has emerged from the concept of the area under curve (AUC)/MIC ratio. However, it has not been found to be superior to traditional kill ratios, i.e. antibiotic serum levels to MIC ratios. However, AUC/MIC ratios also cannot predict the development or emergence of resistance. The best means of controlling antibiotic resistance is to recognize the resistance potential of a particular antibiotic class. The control of resistance in the community is dependent on the selective use of antibiotics by physicians, particularly oral antibiotics. A physician, out of habit or compulsion, prescribing an antibiotic for outpatients, that too in an inadequate dose for an imprecise duration, can have far-reaching consequences in terms of resistance. For example, oral ampicillin or ciprofloxacin prescribed for a short period of time results in the selection of resistant mutants in the gut. This resistant organism can re-infect the same patient even after cessation of therapy, or others within the family or community, and this should be a matter for concern.

Antibiotic resistance in hospitals

We are usually faced with one of the two forms of antibiotic resistance in hospital settings.

(i) Generalized resistance to multiple antibiotics primarily due to non-selective formulary usage of high-resistance potential antibiotics, e.g. ciprofloxacin, ceftazidime, ampicillin.

(ii) Outbreaks of infection due to highly resistant organisms in selected areas of the hospital, e.g. CCU, ICU, Urology Department, etc. Barrier nursing (handwashing) is an effective measures controlling such outbreaks in hospital settings.

In a non-outbreak situation, hospital formularies can play a major role. The magnitude of antibiotic resistance as a result of the unbridled dispensing of antibiotics by formularies in Delhi has resulted in a scenario such as that seen in Table 1. It is interesting to see the prescription auditing get reflected in the development of resistance (Fig. 1). I believe the situation in the rest of the country is not very different. The use of antibiotics at the slightest pretext gives a false sense of security while an infectious disease might still be raging within the victim.

Table I – Percentage Antibimicrobial Resistance

Organisms	1995	1996	1997	1998	1999	2000	2001
Staphylococcus aureus:							
Penicillin	55	75	70	79	83	91	92
Oxacillin	24	33	30	29	39	46	43
Clindamycin	20	18	22	18	26	33	26
Ciprofloxacin	19	29	45	43	48	51	47
Gentamicin	21	23	15	37	41	38	42
E. coli:							
Gentamicin	38	62	59	62	65	57	63
Cefotaxime	37	44	55	60	63	64	67
Ofloxacin	40	58	69	77	73	53	62
Coamoxyclav	55	61	77	80	82	88	82
Pseudomonas aeruginosa:							
Ceftazidime	50	42	57	76	42	50	52
Ciprofloxacin	42	51	50	62	49	54	58
Amikacin	20	24	30	51	30	45	49

We were all aware that VRE would be a problem at some stage in India and our first case (VRE VanB phenotype) was isolated in July 2001. By September 2003, we had 15 such cases. Any institution, in which the prescribing rate of third-generation cephalosporins is high, has been found to be associated with a higher incidence of MRSA. Once the rate of MRSA is high, the likelihood of having to use glycopeptides is very high. This is a classical setting for VRE or VRSA/E to emerge.

The increasing prevalence of resistance to penicillin and other drugs among pneumococci (PRP) has considerably complicated the empirical treatment of community-acquired pneumonia. Penicillin resistance is a worldwide occurrence. We do not have the exact figures for PRP from India. From our centre approximately 33% PRP was documented in the year 2003. Resistance to other classes of antibiotics traditionally used as alternatives in the treatment of pneumococcal infections has also been reported. In some areas of the USA, Europe and East Asia, a prevalence of macrolide resistance as high as 35% or more has been reported recently.[13] Resistance to fluoroquinolones remains low but several treatment failures have been reported from different parts of the world. Resistance or increased MIC to quinolones in *Salmonella* infections has

already been reported.[14] We have isolated 72 strains that are moderately sensitive to quinolones (ciprofloxacin) having an MIC of ≥4 µg (Vitek System, BioMerieux) and all these strains were nalidixic acid resistant (in print). Penicillin G remains the mainstay of therapy for the treatment of penicillin-susceptible pneumococcal pneumonia. Penicillin-resistant pneumococcal pneumonia (MIC <4 µg/ml) can be safely treated with high-dose beta-lactams. The newer fluoroquinolones are very active and effective in pneumococcal pneumonia.[13]

Formulation of an antibiotic policy

Several strategies for regulating antimicrobial prescribing practices have been proposed, such as formulary replacement or restriction,[15] introduction of order forms,[16] healthcare provider education, feedback activities[17] and required approval from an infectious diseases physician for drug prescription.[18] Although most of these interventions have been assessed separately, data from prospective studies evaluating the impact of these different strategies applied systematically over time in the same hospital setting remain scarce. In addition, the results of a coordinated approach by a multidisciplinary team composed of infectious disease physicians, clinical microbiologists and pharmacists have rarely been reported.[9]

We have shown a significant reduction in antibiotic use when the Hospital Infection Control Committee (HICC) performed feedback activities. This is not surprising, because we noted a number of unjustified prescriptions during the baseline phase. A further decrease in antibiotic consumption was reached during the subsequent intervention periods (framing of an antibiotic policy, education and active control phase with feedback activities). Linezolid remains a reserve drug in our hospital only for use in glycopeptide resistance or overwhelming MRSA infections.

Because of the alarming prevalence of bacterial resistance found during the baseline period, our education strategy should emphasize 3 major issues. An effort should be made to document the infection microbiologically before starting antimicrobial therapy and the use should be avoided of certain antibiotics known to be associated with the emergence of bacterial resistance.[12,18,19] A proactive approach by the HICC is the intervention of choice.

Recommendations

- Perform focused diagnostic tests and procedures according to the Fever Assessment Guidelines before instituting antibiotic therapy.
- Start haemodynamically unstable patients with a suspected infectious aetiology on broad-spectrum antibiotics preferably guided by the local antibiotic policy for empirical therapy.
- Convert direct empirical antimicrobial selection into definitive therapy as soon as possible and justify continuation of antibiotics in any given patient (antibiotic audits).
- Base the use of antibiotics on the culture and susceptibility results where ever available.
- Monitor the usage of antibiotics in any institution.

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Infections in organ transplant recipients

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Abstract: Infections still remain a bugbear for the recipients of organ transplantation due to the need for lifelong immunosuppression. Improvements with newer immunosuppressive agents, and more specific diagnostic and therapeutic agents to prevent and treat infections have led to considerable reduction in fatal infections and have improved survivals. Infections can be community-acquired, nosocomial and often opportunistic. Often the clinical manifestations are atypical and altered or masked by the immunocompromised state. Infections are often disseminated and with mixed and multiple organisms, e.g. tuberculosis and Nocardia in the same patient and at the same time. In the absence of typical clinical features, a high degree of clinical suspicion and an aggressive diagnostic approach is required for the early diagnosis and appropriate, specific management of infections.

Introduction

Transplants have become widely accepted as a successful modality for treating end-stage organ diseases. However, the success of organ transplantation depends on a compromise between achieving sufficient immunosuppression to avoid rejection of the graft and maintaining a sufficient level of immune competence to protect the recipient from infections. Improved methods of immunosuppression, development of prophylactic strategies for bacterial, fungal, viral and protozoan infections, and progress in the diagnosis and treatment have led to a consistent decline in the incidence of fatal infections.

Factors determining the state of immunosuppression

A Pre-operative condition of the patient
- Presence of end-stage organ disease (cirrhosis, renal failure, etc.)
- Nutritional status
- Splenic function
- Autoimmune diseases
- Neutropenia
- Lymphopenia

B Nature of immunosuppressive therapy
- Type of drug used
- Dosage
- Duration
- Sequencing of immunosuppressive agents

C Infection profile
- Presence or absence of infection in the recipient
- Type of infection (e.g. immunomodulatory viruses implicated, i.e. Cytomegalo Virus [CMV], Epstein-Barr Virus [EBV], Hepatitis B Virus [HBV], Hepatitis C Virus [HCV] and Human Immunodeficiency Virus [HIV])

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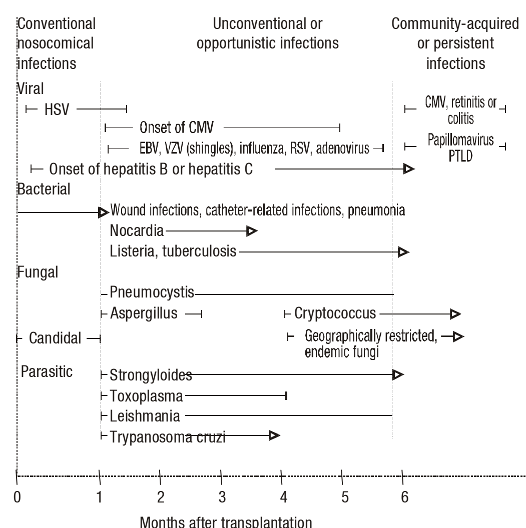
D Surgical techniques used
- Presence of devitalized tissues
- Tissue complicated injuries
- Undrained fluid collection
- Presence of indwelling devices

Other risk factors include age,[1] diabetes, neutropenia,[2] hepatitis, poor graft function and splenectomy.[3]

The time graph of infection after transplantation

Due to the unique nature of solid organ transplant programmes, the similarity of anti-rejection regimes is depicted in the consistency with which a time graph can be drawn of infections occurring after transplantation (Table 1).[4,5] However, it should be remembered that there are geographical variations beyond the scope of the graph.

Table 1. Temporal sequence of infections after organ transplantation



BACTERIAL INFECTIONS

These infections, occurring especially in the first month post transplantation, can be urinary tract infections (UTIs) and upper and lower respiratory tract infections including pneumonias. Among the late-occurring infections, tuberculosis has an incidence of 11%–17% in tropical countries.

Urinary tract Infection (UTI)

Urinary tract infection occurs in 50% or more patients in the first 3 months following transplantation. However, with prophylaxis using low dose-trimethoprm sulphamethoxazole (TMP -SMZ), the incidence has reduced.[6] UTI's in renal transplant is commonly associated with pyelonephritis, bacteraemia and frequent relapses after standard antibiotic therapy for 10-14 days, even in the absence of urological abnormalities.[7] Asymptomatic bacteriuria requires treatment for at least 10 days and acute pyelonephritis and/or positive bacteraemia for 4-6 weeks. Since Gram-negative organisms are most commonly implicated, the initial treatment may be with cephalosporins in case of extended spectrum beta-lactamase prevalence carbapenems (meropenem) or with aminoglycosides in severe cases. Candidial infections may require fluconazole. However, careful pre-transplantation screening of recipients and donors can prevent UTI.

Wound infections

Wound infections are now rare in most centres. This may be attributed to improved surgical techniques and newer antibiotics. Important predisposing factors are wound haematomas, urine leaks and lymphocele. Treatment is mainly surgical drainage, antibiotic therapy and aseptic daily dressing.

PULMONARY INFECTIONS

Pulmonary infections remain the most serious in renal allograft recipients (Table 2). Since there is diverse aetiology and a lack of specificity of clinical and radiological findings (Table 3), an aggressive diagnostic approach is often indicated.

Diagnosing pulmonary infections

Along with sputum examination, Gram-stained smear examination, specific cultures and radiological tests including computed tomography (CT) scan, fibreoptic bronchoscopy with tomography, biopsy and bronchoalveolar lavage (BAL) are the most frequently used techniques for the diagnosis of specific aetiological agents. BAL cytology has a diagnostic yield of 75%. Lung biopsy is appropriate when a patient with pneumonitis is worsening. Open lung biopsy is traumatic and may require assisted ventilation. Thoracoscopic biopsy, if available, is less invasive and is appropriate for diffuse or peripheral lung lesions. An aggressive approach for early diagnosis is more rewarding and leads to specific therapy.

Table 2. Main atiological agents of pulmonary infections

Bacteria	*Pneumococcus, Haemophilus influenzae, Staphylococcus aureus, Legionella* spp., *Mycobacterium* spp. *Nocardia* spp.; *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Chlamydia* spp.
Viruses	*Cytomegalovirus, Varicella* zoster
Fungi	*Aspergillus* spp., *Cryptococcus* spp., *Candida* spp., mucormycosis
Parasites	*Pneumocystis carinii, Strongyloides stercoralis,* etc.

Table 3. Radiological and clinical characteristics of pulmonary infections

Radiographical abnormality	Acute development	Chronic development
Nodular infiltrate	Bacteria	Fungi, *nocardia asteroides*, tuberculosis, *Pneumocystis carinii*
Cavitations	Bacteria (*Legionella*), fungi	Tuberculosis
Consolidation	Bacteria (*Legionella*)	Fungi, *Nocardia asteroides*, tuberculosis, viruses, *Pneumocystis carinii*
Diffuse interstitial infiltrations		CMV, *Pneumocystis carinii*, fungi (rare)
Peribronchovascular abnormality	Bacteria, viruses (influenza)	CMV, *Pneumocystis carinii*, fungi, tuberculosis, *nocardia asteroides*

Bacterial pneumonia

Normally occurring in first month after transplantation are pneumonias caused by Gram-negative bacilli (*Escherichia coli, Pseudomonas aeruginosa, K lebsiella pneumoniae*) and *Staphyflococcus aureus*. While awaiting the culture results, a third-generation cephalosporin with or without an aminoglycoside can be administered. In resistant cases, meropenem, ciprofloxacin or vancomycin may be administered.

Legionella pneumonia

This can cause nosocomial pneumonia. In this case, a chest X-ray shows irregular, nodular shadows that progress to a lobar or diffuse consolidation, cavitation can also occur. Identification may be done by direct immunofluorescent staining of sputum or biopsy samples, or by urine antigen assay. Macrolides are the drugs of choice, however, they interfere with cyclosporin and tacrolimus pharmacokinetics. Rifampicin has also been found to be effective, besides doxycycline and the fluoroquinolones.

Nocardiosis

The diagnosis must be suspected in any case of chronic pneumonitis not responding to antibiotics. The associated presence of cerebral focal abscess and cutaneous manifestations is highly suggestive of the diagnosis. The diagnosis must be confirmed with aspiration, microscopy and culture. Treatment with TMP-SMZ is usually successful.

Alternative drugs are ciprofloxacin, cephalosporins, imipenem and aminoglycosides.

Pneumocystis carinii pneumonia (PCP)

This is a complication in transplant patients, treated either with cyclosporin or with rapamycin. However, the incidence of PCP has become rare with the prophylactic use of TMP-SMZ for the first 3 months post-transplantation. Patients with PCP usually present with fever and dyspnoea, while physical signs are absent. Radiographic abnormalities are variable and non-specific; interstitial pneumonia is frequent. Severe hypoxaemia is usually present. High-dose TMP-SMZ is the treatment of choice. In patients allergic to sulphonamides, slow intravenous infusion of pentamidine (3-4 mg/kg/day) may be indicated. Alternative treatment includes the use clindamycin.

TUBERCULOSIS

The incidence of tuberculosis is more common in transplant recipients than in the general population and may be as high as 11%-17% in India.[8,9] INH prophylaxis has been advised in western countries to prevent reactivation but this poses the problem of creating resistance in countries such as India where the incidence of the disease is very high. The mean time interval for the development of tuberculosis post-transplant is around 18.1+17.4 months.[10] Treatment consists of four-drug therapy for a duration, 12-18 months.[10] Rifampicin, being an enzyme inducer; is avoided; as it reduces the blood levels of cyclosporin and tacrolimus.

VIRAL INFECTIONS

Viral infections affect transplant recipients mostly in a period ranging from 3 months to 1 year after transplantation. They account for significant mortality, morbidity and allograft dysfunction. Important viral infections are discussed below.

Cytomegalovirus infection (human herpesvirus 5)

In the western world, approximately 50% of patients awaiting transplantation have been infected with CMV in the past and have tested positive for antibodies. In the Indian subcontinent, the IgG CMV positivity is nearly 100%.

There are three forms of CMV infection in transplant patients:

1. **Primary infection:** This is the most severe variety of infection and is associated with approximately 60% morbidity. The virus can reside in the allograft and this can infect a previously seronegative recipient. These patients have more than 50% risk of symptomatic disease.[11]
2. **Secondary infection:** This occurs in previously seropositive patients because of reactivation of the patient's own latent virus or reinfection. Systemic inflammation can reactivate CMV from latency. Use of antilymphocyte globulin, OKT3 and mycophenolate have been shown to increase the incidence of CMV.
3. **Superinfection:** This occurs when the donor and recipient are both seropositive and the virus of donor origin reactivates in the recipient.

Clinical features

Typical CMV disease manifests itself with spiking, constant fever associated with weakness and malaise 4-10 weeks post transplantation. It may also be associated with anaemia, leucopenia, thrombocytopenia, mild lymphocytosis and mild hepatitis (elevated aspartate aminotransferase). Less common features are arthralgias, overt hepatitis, splenomegaly, gastrointestinal ulceration, encephalitis, myocarditis and pneumonitis. Deterioration in the renal function may also occur in some cases. CMV infection may render the patient susceptible to opportunistic infections such as *Pneumocytis carinii* or invasive aspergillosis. It may also expose the patient to increased risk of EBV infection or to lymphoproliferative disorders. Chorioretinitis with a permanent reduction in vision may occur in at least half of the patients. CMV infection may precipitate acute rejection due to upregulation of major histocompatibility complex (MHC) antigens.

Diagnosis

- Serology is helpful for detecting previous CMV infections; however, it cannot be used for early diagnosis and does not provide relevant clinical information.[12,13]
- Viral cultures of peripheral blood leucocytes (PBL) provide a direct demonstration of the presence of CMV. However, this takes 5-28 days to produce a cytopathic effect and the delay is inevitable.
- Shell vial modification of the buffy coat culture technique shortens the time of viral culture, but is still not useful for an early diagnosis[14,15] and does not correlate with the clinical course of the infection.[13]
- CMV antigen test is an immunocytochemical method that, by using a monoclonal antibody specific for the pp 65 CMV matrix protein, allows detection and quantification of positive PBL.
- Polymerose chain reaction (PCR) can also be used to detect the DNA of CMV in the plasma or leucocytes of the patient.[16] This test has a good sensitivity and specificity,[17] and can detect the presence of viraemia even before the onset of symptoms.
- In the diagnosis of CMV, it is important to distinguish between latency, active infection and disease. Nucleic acid amplification-based assay (NASBA) is the sure-shot method of diagnosing active disease and, therefore is the most popular method of detecting CMV.

In fatal infections, CMV has been cultured from many sites, including the bone marrow, kidney, liver, lung, pancreas, large bowel and brain.

Management

CMV-negative recipients, CMV-negative donors-recipients have the lowest incidence of CMV infection.[18,19] When infection does occur, it is most probably due to transfused blood. Leuco-reduction of transfused blood lowers this incidence.[18]

70%-90% of recipients CMV-negative recipients, CMV-positive donors-develop' primary CMV infection and 50%–80% have CMV disease. None of the available therapeutic regimens reliably prevent this incidence; however, prophylactic and reserve therapies with ganciclovir have, to a large extent, reduced the incidence of CMV disease. CMV hyperimmune globulin has been used as rescue therapy.

CMV-positive recipients, CMV-negative donors the transplant recipients may have reactivation of a latent CMV infection. Antiviral therapy is recommended for patients who receive antilymphocyte globulin as induction therapy.

CMV-positive recipients, CMV-positive donors the recipients, are at a higher risk for both reactivation and superinfection. USRDS and UNOS reveal the worst graft and patient survival at years post transplantation in this group. Antiviral therapy is recommended for those who receive antilymphocyte globulin.

Treatment

Clinical CMV disease requires intravenous administration of ganciclovir, a guanine analogue, at 5 mg/kg every 12 hours, with dose adjustment in case of renal dysfunction. This is continued for 2 weeks followed by oral ganciclovir for up to 3-6 months. To prevent relapse or resistance, treatment should be continued until clearance of viraemia. In case of overt CMV infection, a decrease in immunosuppression and administration of TMP-SMZ for prevention of *P. carinii* infection has been recommended. For patients who develop ganciclovir resistance, foscarnet given intravenously at 60 mg/kg every 8 hours initially followed by 120 mg/kg/day maintenance dose can be useful. However, it is nephrotoxic, causing a reversible nephropathy. Recently a new anti-CMV molecule, cidofovir, has been licensed, which is also nephrotoxic.

Prevention of infection and disease

Primary CMV infection in seronegative recipients can be avoided if the transfused blood and especially the transplanted kidney come from seronegative donors. Live-attenuated CMV vaccine has been administered in the pre-transplant period to immunize seronegative recipients.

Passive immunization with immunoglobulin over the first 4 months after transplantation reduces CMV-related illness in CMV-seronegative recipients from CMV-seropositive donor organs. Oral acyclovir (800-3200 mg depending on the renal function) has been advocated for use as prophylaxis for the first 12 weeks following renal transplantation. However, its efficacy has not been established.

Other antiviral agents

Valganciclovir: This is a valyl-ester prodrug of oral ganciclovir. It has a bioavailability of nearly 70% at doses of 450-900 mg. It produces ganciclovir levels that are similar to intravenous administration of 2.5–5.0 mg/kg of ganciclovir.[20]

Leflunomide: This is a pyrimidine synthesis inhibitor and immunosuppressive agent that has been utilized to prevent CMV and herpes simplex virus (HSV-I) replication by interfering with virion assembly.[21] Experimental trials are still continuing regarding its role in the control of CMV infection.

Pre-emptive antiviral treatment has to be based on laboratory tests that indicate CMV activity. In CMV seronegative recipients of a seropositive organ, a programme of weekly testing for the first 2-3 months has a positive predictive value of 80%.

Epstein-Barr virus (EBV)

This virus is endemic in all human populations and after infection is carried lifelong, as a latent infection of the lymphoid cells with only occasional viral replication. The effect of immunosuppression of the cytotoxic T cell population results in its failure to check the proliferation of EBV-infected B lymphocytes. Chemoprophylaxis with acyclovir in EBV-negative transplant recipients has been carried out to prevent lymphoproliferative disorders, but the results are not convincing. The role of a very early and prolonged intravenous administration of ganciclovir is under investigation.[22] Rituximab (anti-CD20 monoclonal antibody) has been shown to remit EBV-induced post-transplant lymphoproliferative disorder.

Varicella zoster virus (VZV)

Chickenpox is rare in graft recipients but zoster occurs annually in approximately 3% of renal transplant patients (10 times more than in non-immunocompromised patients). One of the clinical features is rash, which may become confluent, bullous, haemorrhagic or gangrenous. Pneumonitis, encephalitis or meningitis may be fatal. Oral acyclovir (800 mg, four times a day for 7 days) or intravenous acyclovir in the most severe cases (250–500 mg/m^2 every 8 hours for 7 days) may halt the progression of herpes zoster but it may precipitate renal dysfunction. Valacyclovir at dosage of 1 g three times a day for 7-14 days can also be used. If a patient without antibodies to VZV is exposed to chickenpox, then immunoglobulin is a dose of 125 µl/10kg of body weight should be given within 72 hours of exposure.

Herpes simplex virus (HSV)

Primary infection with HSV is rare in transplant patients. Reactivation, 40% of which is asymptomatic, is more common. Infection usually involves the orolabial region and less commonly the anogenital area, conjunctiva or cornea. Most

patients respond well to oral acyclovir (200-400 mg five times per day for 7-14 days). In the case of encephalitis or other visceral localization, high dose intravenous acyclovir (100 mg/ kg every day for 10-14 days) is the treatment of choice.

Human herpesvirus 6 (HHV-6)

Such infections occur in 31%-55% of organ transplant recipients, usually 2-4 weeks after transplant as a result of reactivation caused by intense immunosuppression. In many cases there is a co-infection of HHV-6 and CMV or a reactivation together.[23] Two strains have been identified. HHV–6A and HHV-6B. Renal transplant recipients are exclusively infected with HHV-6B. Clinical sequelae may range from a self-limiting febrile illness to disseminated disease. Bone marrow suppression, meningoencephalitis, interstitial pneumonitis and a mononucleosis like syndrome are the most commonly reported types of clinical disease. HHV-6 responds to ganciclovir and foscarnet, but is resistant to acyclovir.

Human herpesvirus 8 (Kaposi sarcoma)

It may develop in transplant recipients either as a consequence of reactivation or as a primary infection which may be transmitted through the graft.[24] A response to treatment with cidofovir has been reported in post-transplant Kaposi sarcoma. Cancer therapy may be required.

Polyomavirus

Serological studies have shown that up to 90% of the population have been exposed to the polyomavirus by adulthood. Three species-BK virus (BKV), JC virus (JCV) and Simian virus (SV 40)-infect humans. In fact, BKV was first discovered in 1971 from renal transplant patients who developed ureteric stenosis.[25] Initial infections are usually occult and occur via the respiratory tract or through blood transfusion. Serological activation and shedding of the virus in the urine has been reported in 60% of kidney transplant recipients with graft dysfunction.[25] BK viraemia has been seen in 11%, and viruses in 27% of paediatric renal transplant patients.[25] JC viraemia was also discovered in about 13.5% of cases. Polyomavirus nephropathy (PVAN) is characterized by mononuclear cell interstitial infiltrates and tubulitis, which can be confused histopathologically with acute cellular rejection. Recognition is critical as the therapeutic choices are diametrically opposite, requiring reduction of immunosuppression. Early cessation of corticosteroid therapy from immunosuppressive protocols has been associated with a decline in the incidence of PVAN.[25] Male sex and donor seropositivity coupled with recipient seronegativity are further risk factors for the development of PVAN.

Hepatitis B and hepatitis C viruses

These viruses remain a cause for concern in patients who have been on *long-term* haemodialysis prior to transplantation. They also carry a significant mortality and morbidity. However, a discussion about them is beyond the scope of this article.

FUNGAL INFECTIONS

The major fungal infections include those caused by *Aspergillus* species, *Histoplasma capsulatum, Coccidioides immitis* and *Crytococcus neoformans.*

Aspergillosis species

This can be found as saprophytes but the repeated demonstration of hyphae on direct microscopy and growth in culture in a patient with unexplained pneumonia is highly diagnostic. It can cause a patchy infiltration followed by consolidation and abscess formation, usually around 1-5 months post transplantation. Mortality is extremely high, especially when dissemination in the form of rhinosinusitis, cerebritis or abscess takes place. Successful treatment depends on three factors: early diagnosis, aggressive antifungal therapy and ability to reduce immunosuppression.[26] Amphotericin B is the drug of choice; its lipid formulation is less nephrotoxic and can be used at higher dosages. Itraconazole (200 mg three times a day for 3 days) followed by 200-400 mg/day may be used. New antifungal agents are voriconazole and echinocandins.

Histoplasmosis

This is relatively frequent in endemic areas (America, Africa). It usually presents with fever, malaise, myalgias, non-productive cough, arthralgias and erythematous skin lesions. Hilar adenopathy and small, irregular disseminated infiltration may be seen on the chest X-ray. Recovery of the fungus from the lung biopsy is highly diagnostic. Treatment with amphotericin B is the first-line of therapy. Alternative agents are itraconazole or ketoconazole for at least 6-8 months.

Cryptococcosis

It presents with cough, chest pain, mucopurulent expectoration, haemoptysis and dyspnoea. Fever may be absent. A single nodule or focal or disseminated infiltrates may be the only findings on the chest X-ray. Central nervous system disease may present as subacute meningitis. Amphotericin B with flucytosine is the treatment of choice. India ink staining and latex agglutination tests of the cerebrospinal fluid are highly diagnostic and require early initiation of therapy.

Central nervous system infections

These usually occur within 1-12 months post transplantation. Presentation may be different than in normal patients. The onset may be subacute, and systemic signs may be lacking.

The most reliable symptoms are headache and unexplained fever. Patients should be subjected to CT scan examination and a lumbar puncture to clinch the diagnosis (Table 4).

Table 4. Central nervous system infections in transplant receipient

Syndrome	Most frequent aetiological agent	Clinical characteristic
Acute meningitis	L. monocytogenes	• Fever, altered sensorium and headache • 40 % may have no signs • CSF: increased TLC and proteins may be lacking • 33% focal radiological findings, 25% - seizures • Ampicillin 14-21 days + aminoglycosides 7-10 days
Subacute/chronic meningitis	C. neoformans H. capsulatum N. asteroides S. stercoralis M. tuberculosis	• Fever or headache over several days or weeks • Non-specific presentation • Concomitant lung or skin infection in some cases
Focal brain abscess	A. fumigatus	Seizures, focal neurological abnormalities. Not found in CSF Concomitant skin, lung and kidney involvement in some cases
Multifocal leucoencephalopathy	Papovavirus HSV CMV EBV	Progressive dementia

HSV : herpes simplex virus; CMV: cytomegalovirus; EBV: epstein-Barr virus; TLC: total leucocyte count

PREVENTION OF INFECTION IN ORGAN TRANSPLANT RECIPIENTS

This is one of the primary goals in the management of organ transplant recipients. Theoretically, the best chance of prevention is represented by the use of low immunosuppression. Availability of more specific immunosuppressive agents have reduced the incidence of severe and fatal infections.

Before transplantation

Patients accepted into a transplant programme should be screened for the presence of active infection. Thorough screening should be done by taking nasal swabs for *Staphylococcus,* as well as dental examination and assessment of the chest and urinary tract.[27]

During hospitalization

The risk of postoperative infection can be reduced by minimizing the presence and duration of drainage catheters, stents, vein catheters and other foreign bodies.[27]

Since infections in transplant patients may often have devastating effects, their early diagnosis and treatment forms the crux of the management. Because of the non-specific symptomatology as a consequence of immunosuppression, it is imperative to have a high index of suspicion.

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Newer antibiotics: Current concepts

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Abstract: Antibiotic resistance is a global problem. In the community, resistance can result from nosocomial acquisition of resistant bacteria, emergence of resistance consequent upon its use in the community or acquisition of resistant pathogens as a result of travel. Resistance can also occur as a result of using antimicrobial agents as 'growth promoters' in animals. Since resistance is often a result of the selective pressure exerted by the use or misuse of antibiotics, prudent and appropriate employment of these valuable agents is likely to reduce the emergence of resistance and prolong their usefulness. Besides prevention of selective pressure, physicians can also contribute to the control of spread of these infections by instituting appropriate isolation protocols. New agents can be developed to deal with resistance. In this review, various attributes of four new agents: linezolid for hospital-acquired infections, telithromycin for community-acquired respiratory tract infections, and moxifloxacin and gatifloxacin are described with the hope that a better understanding of their attributes would translate into appropriate deployment of these agents.

Oxazolidinones (Linezolid)

One way forward would be to discover and develop a new class of antimicrobials that are completely synthetic in nature. This would ensure that the target pathogens have no prior exposure and, therefore, no pre-existing resistance to the drug. The (S)-3-aryl-5-acetamidomethyl-2-oxazolidinones, first discovered and reported in 1987, are a novel class of synthetic antibacterial agents. Oxazolidinones have a number of intriguing attributes: (i) a unique mechanism of action that involves the inhibition of protein synthesis at a very early stage, providing a lack of cross-resistance with existing antimicrobials; (ii) a spectrum of activity that includes a number of important bacterial species; (iii) activity in animal models of human infection when administered by either the oral or parenteral routes; and (iv) sufficient structural latitude to allow for activity and/or toxicity modifications.

Linezolid is the first antibacterial drug of a new class, now available for the treatment of infections associated with vancomycin-resistant (VR) Gram-positive infections, e.g. *VR Enterococcus faecium* (VREF). It is effluxed out of Gram-negative bacteria and consequently its activity is restricted to Gram-positive pathogens only (that lack these specific efflux pumps).

Spontaneous mutation occurs in 10^9 to 10^{11} generations for a few strains of staphylococci on exposure to sub-inhibitory concentrations of linezolid. In clinical settings, however, resistance develops and has been reported in enterococci and staphylococci.

In vitro activity[1]

Minimum inhibitory concentrations (MIC) attained against various Gram-positive pathogen indicate that linezolid has MIC^{90} against *Staphylococcus aureus, Staph. epidermidis* and *Staph. haemolyticus* of <4 μg/ml. The presence of β-lactam, glycopeptide or quinolone resistance does not effect the MIC. Similarly, the degree of penicillin, erythromycin or clindamycin resistance of *Streptococcus pneumoniae* isolates does not affect linezolid's activity. Linezolid also demonstrates excellent activity against enterococci (Table 1). MIC do not exceed 4 μg/ml even against vancomycin A and vancomycin B phenotypes of VR enterococci (VRE).

Table 1 Laboratory testing-interpretative breakpoints for linezolid

	Organisms	Susceptible	Intermediate	Resistant
MIC (μg/ml)	Staphylococci	<4	—	—
	Enterococci	<2	4	>8
	Pneumococci	<2	—	—
Zone size (mm)	Staphylococci	>21	—	—
	Enterococci	>23	21–22	<20
	Pneumococci	>21	—	—

Clinical utility

Preclinical investigation using animal models has indicated that linezolid is effective in systemic infection caused by penicillin-resistant S. pneumoniae (PRSP), endocarditis caused by methicillir-risistant (MRSA) or VRE, meningitis or otitis media caused by *S. pneumoniae* and soft tissue infection caused by MRSA or VRE.[2] The clinical and bacteriological success rate for all infections is equivalent to that of vancomycin, though patients treated with linezolid had a slightly shorter hospital stay. Linezolid has been approved for infections caused by VRE, pneumonia caused by Gram-positive bacteria, and skin and soft tissue infections caused by Gram-positive bacteria. Linezolid has also been approved for paediatric use and diabetic foot infections.[3]

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Mechanism of action[4]

A number of marketed antibiotics control bacterial growth through the inhibition of prokaryotic RNA transcription and protein translation. Linezolid acts by inhibiting one of the first steps in the synthesis of bacterial proteins. By binding to the 50S subunit, It inhibits the formation of tertiary initiation complex and prevents translation (Fig. 1). This mechanism of action is unique to this class of agents and cross resistance, as seen with other inhibitors of protein synthesis, does not occur. Rifampicin is a potent inhibitor of bacterial RNA polymerase; macrolides, lincosamides, aminoglycosides, tetracyclines and oxazolidinones have protein translation as their site of action.

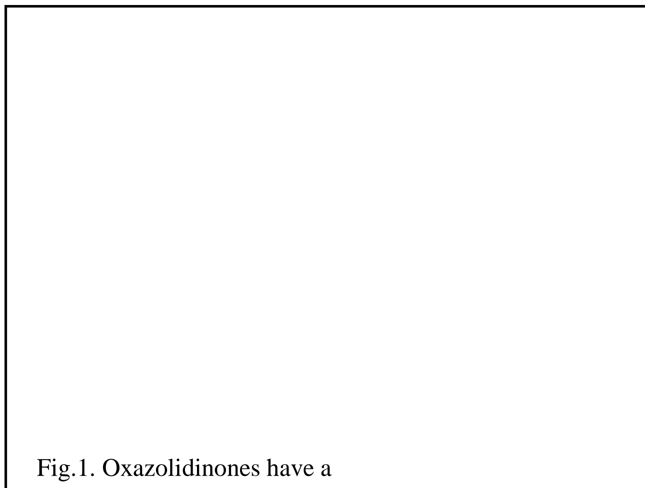

Fig.1. Oxazolidinones have a Unique Site of Action

Dose

The recommended dose of linezolid intravenous (i.v.) or per oram (PO) is 600 mg every 12 hours. Linezolid should be administered separately because incompatibility has been observed with a few drugs: amphotericin B, ceftriaxone, co-trimoxazole, erythromycin, chloramphenicol, diazepam, pentamidine and phenytoin.

Adverse effects

In general, linezolid is well tolerated. Adverse side-effects include nausea, diarrhoea, headache, vomiting, oral candidiasis and pain at the infection site. Other serious side-effects are infrequent but may include elevated liver enzymes, atrial fibrillation, increased incidence of renal failure and thrombocytopenia. The last situation is frequent when linezolid is administered for more than 2 weeks. It is recommended that platelet counts be monitored weekly.[5] Patients treated with linezolid should avoid consuming large quantities of food and beverages with a high tyramine content (e.g. aged cheese, fermented or air-dried meat, soy sauce, red wine).

Following oral administration, linezolid is rapidly and extensively absorbed. It may be administered irrespective of the timing of meals. Its absolute bioavailability is 100% and dosage adjustment is not necessary when switching from i.v. to oral administration. Linezolid is not a substrate for any of the cytochrome P450 isoenzymes.

Place in therapy

Linezolid, being an oral agent active against all clinically important Gram-positive cocci, is likely to become the drug of choice for skin and soft tissue infections, and community acquired or nosocomial pneumonia. It is envisaged that linezolid therapy would be initiated (with the i.v. formulation) in the hospital, but oral administration would allow for early discharge. It appears appropriate to consider the use of linezolid in units and patients where multidrug-resistant enterococci or staphylococci are either documented or likely to be present, but not in units or patients where most staphylococci remain oxacillin-susceptible and most enterococci remain ampicillin-susceptible.[4,6]

Ketolides (Telithromycin)

Antimicrobial resistance in community-acquired respiratory infections is an emerging problem. *S. pneumoniae*, major respiratory pathogen, is increasingly becoming resistant to penicillin, macrolides, trimethoprim-sulfamethaxazole (TMP-SMZ), tetracycline, quinolones and chloramphenicol. In the past, macrolides have been very effective in the empirical treatment of community-acquired respiratory infection as they not only provide coverage of the three key bacterial pathogens *S. pneumoniae, Moraxella catarrhalis* and *Haemophilus influenzae* but also extended coverage atypical pathogens such as *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Legionella* spp.

Of late, the efficacy of macrolides has been compromised with the emergence of resistance in *S. pneumoniae*. Macrolide resistance has two main mechanisms: modification of the target (the ribosome) and modification of antibiotic transport across the cell membrane. High-level resistance of *S. pneumoniae* and other Gram-positive organisms to 14 members of macrolides has been described as MLS_B (macrolide, lincosamide and streptogramin B) resistance. This resistance phenotype is further subdivided into two types: inducible and constitutive. The constitutive resistance (MLS_C) phenotype is highly resistant to all the three classes of antibiotics while the inducible phenotype (MLS_I) exhibits a high level of resistance to erythromycin and similar antibiotics but not to lincosamide and streptogramin. Resistance is mediated by methylation of the adenine residue at position 2058 in the ribosomal RNA (rRNA) resulting in markedly reduced binding of the antibiotic to its target. A family of genes called *erm* is the genetic determinant. The genes *erm A* and *erm C* are most common in *Staph. aureus* whereas *erm B* is the commonest in streptococci.[7]

The second mechanism by which bacteria become resistant to macrolides is efflux. The *mef* gene codes for the efflux pump.

These strains are clindamycin-susceptible, but erythromycin-resistant. They are also known as M phenotype strains.

Mechanism of action

Ketolides work by the inhibition of protein synthesis. They bind to the 23S rRNA subunit of the 50S rRNA of the bacterial ribosomes. Ketolides bind to A 2058 and A 2059 in domain V of 23S rRNA, the same site as erythromycin. In addition, ketolides bind to an additional site in domain II, A752. Ketolides also bind to L4 and L22 proteins in the ribosomes. This results in conformational change in the tunnel through which the nascent peptide passes.

Overall, the enhanced activity of ketolides compared to traditional macrolides is most likely derived from tighter binding of ketolides to the ribosome, additional ribosome binding sites, slow dissociation from the ribosome and rapid drug accumulation.

Spectrum of activity[8]

Similar to the macrolides, the spectrum of activity of telithromycin includes community-acquired respiratory tract pathogens. Owing to structural differences, telithromycin has demonstrated enhanced activity against erythromycin-resistant Gram-positive cocci (Table 2).

Table 2. NCCLS break-points of telithromycin (in μg/ml)

Against streptococci, staphylococci and enterococci		
S	I	R
<1	2	>4
Against *Hacmophilus influenzae*		
<2	4	>8

S: sensitive; I: intermediate; R: resistant; NCCLS: National Committee for clinical laboratory standards

Gram-negative bacteria : Telithromycin is active against *H. influenzae*, *M. catarrhalis*, *Neisseria gonorrhoeae* and *N. meningitidis*. It has no significant activity against *Enterobacteriaceae, Acinetobacter* and *Pseudomonas aeruginosa*.

Anaerobic bacteria : While telithromycin has some activity against the respiratory anaerobes: *Peptostreptococcus, Prevotella* and *Actinomyces,* it has modest, if any, activity against, *Clostridium, Fusobacterium* or *Bacteroides* spp.

Clinical utility[9,10]

Ketolides have the potential to address the needs for an antibiotic with potent activity against multidrug resistant *S. pneumoniae* strains as well as to provide coverage against the major pathogens of the respiratory tract encountered in the outpatient setting. The ketolides are thus expected to be useful the treatment of community-acquired respiratory infection when resistant *S. pneumoniae* strains are considered.

Post-antibiotic effect

Similar to other macrolides, telithromycin has demonstrated a post-antibiotic effect (PAE) against most community-acquired pneumonia (CAP) pathogens. The duration of PAE appears to be dependent on the concentration with a rise in PAE seen with high concentrations.

Pharmacokinetic activity

A single oral dose of 800 mg in a healthy volunteer achieved a maximum concentration (Cmax) of 1.9-2.3 μg/ml at 1 hour (Tmax [maximum time under the influence of antibiotic]). The $t_{1/2}$ was 7.2-10.6 hour and area under curve (AUC) was 7.3-9.3 μg/hour/ml. A steady-state concentration is usually achieved in 2-3 days. Telithromycin can be taken without regard to food. Telithromycin experiences 33% first-pass metabolism and has a 57% absolute bioavailability. It shows 60%-80% protein binding ability, which is mild and should have minimal clinical significance. Telithromycin achieves high tissue concentration in respiratory fluids, saliva, alveolar macrophages, epithelial lining fluid and bronchial mucosa.

Elimination

Faecal elimination accounts for the majority (75.6%) of the dose of telithromycin, whereas urinary excretion accounts for the remainder (17.4%); only a small fraction is excreted uncharged in the urine and faeces.

Renal impairment

Plasma concentration and (AUC) were 1.4-1.5 times higher in patients with mild (creatini.... clearance 41-80 ml/min) or severe (<40 ml/min) renal impairment. No dosage adjustment is necessary.

Drug interaction

In vitro, telithromycin is a competitive inhibitor of CYP 3A4 and CYP 2D6.

CYP 3A4 inhibitors*:* Co-administration of ketoconazole (a potent inhibitor of 3A4), results in a 100% and 50% increase in AUC and Cmax of telithromycin, respectively.

CYP 3A4 substrate

Cisapride: Co-administration of telithromycin and cisapride caused a 2.4–2-fold increase in AUC and Cmax of cisapride. Since cisapride causes increase in the QT interval on ECG, co-administration of these 2 drugs contraindicated.

Simvastatin: An 8-9-fold increase in AUC and 5.3-fold increase in Cmax of simvastatin seen.

Midazolam: A 2-6-fold increase in AUC of midazolam was observed. Dosage adjustment is required to avoid prolonged sedation.

Dosage

The dosage of telithromycin is 800 mg orally once a day for 7 to 10 days for CAP and for 5 days for other respiratory tract infections. No intravenous formulation is available.

Adverse effects

Gastrointestinal adverse effects are the most commonly observed. The rate varies from 7.5% to 19.9% for diarrhoea and 2.4% to 11.7% for nausea. No *Clostridium difficile*-associated diarrhoea has been reported. Blurred vision (inability to focus) has been reported in young women (<40 year of age). The effect is mild and reversible with a peak incidence occurring within 2 hours of drug intake. The sole laboratory abnormality reported is elevated liver function tests. As with other available macrolides, telithromycin has the potential to lengthen the QT interval.

Place in therapy

When compared with the available macrolides, the main advantage of telithromycin is its activity against macrolide-resistant pneumococci. Its activity is comparable to that of clarithromycin and azithromycin for most other community-acquired pathogens. The primary use of telithromycin should be for those instances in which a resistant pathogen is isolated or highly suspected. An increasing awareness of local resistance pathogens will be necessary to optimize the use of telithromycin.

Gatifloxacin and moxifloxacin

MOXIFLOXACIN

Gatifloxacin (Tequin–Bristol Myers Squibb) and moxifloxacin (Avelox–Bayer) are the newest fluoroquinolone antibacterial agents, increasing the number of approved agents in this class to 10. They exhibit bactericidal action by inhibiting the enzymes DNA gyrase (topoisomerase II) and topoisomerase IV, which are required for bacterial DNA replication, transcription, repair and recombination. Both the new drugs contain a methoxy-substituent at position 8 of the molecule (Fig. 2), and this is thought to provide enhanced activity and lower selection of resistant mutants of Gram-positive bacteria.

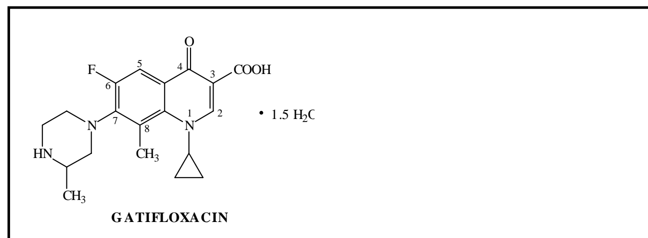

Fig. 2. Structures of gatifloxacin and moxifloxacin

Gatifloxacin Ras 1.5 molecules of H2O entrapped indicating racemic mixture of R and S enantiomers which are equally active unlike levofloxin. Gatifloxacin and moxifloxacin are active against a wide range of Gram-positive and Gram-negative bacteria and also against *C. pneumoniae* and *M. pneumoniae*. They are more active than the early fluoroquinolones (e.g. ciprofloxacin and norfloxacin) and against Gram-positive bacteria such as *S. pneumoniae.* Gram-negative bacteria that are resistant to other fluoroquinolones may be susceptible to these new agents. Both agents have been shown to possess *in vitro* activity against PRSP. However, clinical data with infections caused by PRSP strains is limited and the US Food and Drug administration (FDA) has not approved these agents for this indication (only levofloxacin [Levaquin] has been approved by the FDA for CAP caused by PRSP strains). Gatifloxacin and moxifloxacin are less active against *P. aeruginosa* than agents such as ciprofloxacin, and thus are not indicated for the treatment of infections caused by *P. aeruginosa*.[11]

Respiratory tract infections are is the primary indication for gatifloxacin and moxifloxacin, although gatifloxacin is also indicated for urinary tract infections (UTI), pylelonephritis and gonorrhoea.

An important concern with the use of both gatifloxacin and moxifloxacin is the possibility of their prolonging the QT interval and an associated increased risk of ventricular arrhythmia including torsades de pointes. This has also been a concern with grepafloxacin (Raxar, GSK), which has been withdrawn from the market because of infrequent reports of adverse events of the cardiovascular system (CVS) adverse events.

Like other fluoroquinolones, gatifloxacin and moxifloxacin may cause dizziness, nervousness and central nervous system (CNS) stimulation. It should be used with caution in patients with known or suspected CNS disorders such as epilepsy.

When used in the recommended doses, Phototoxic reactions have not been observed with gatifloxacin and moxifloxacin, but patients should be advised to avoid excessive exposure to sunlight or artificial ultraviolet light on treatment.

Fluoroquinolones, including gatifloxacin or moxifloxacin, have shown erosion of the cartilage in weight-bearing joints and other signs of arthropathy in immature animals of several species.

As with other fluoroquinolones, the absorption and activity of gatifloxacin or moxifloxacin may be markedly reduced by metal-containing products. An appropriate interval of time must separate the administration of the two.

The cytochrome P450 system is not involved in the metabolism of gatifloxacin or moxifloxacin, nor is this system induced or inhibited by these new agents.

Moxifloxacin

The mean Cmax and AUC values at steady state with a daily dosage regimen of 400 mg are:

Cmax: 4.5 + 0.53 µg/ml

AUC: 48 + 2.7 µg.hr/ml

Time: 1 to 3 hours

t1/2: 12 + 1.3 hours

Steady state is achieved after 3 days with a 400 mg once daily regimen.

Microbiology

Moxifloxacin has *in vitro* activity against a wide range of Gram-positive and Gram-negative bacteria.

Though moxifloxacin is active (MIC_{90} <2 µg/ml) against many Gram-negative facultative and true anaerobes, the significance of this has not been established in adequate and well-controlled clinical trials. The various breakpoints for testing *Enterobacteriaceae*, staphylococci, *H. influenzae, H. parainfluenzae, S. pneumoniae* and *N. gonorrhoeae* with 5 µg discs, are shown in Tables 3-6.

Table 3. Breakpoints for testing *Enterobacteriaceae* and staphylococci

	Moxifloxacin		Gatifloxacin	
	MIC (µg/ml)	Zone diameter (mm)	MIC (µg/ml)	Zone diameter (mm)
Susceptible	<2	>19	<2	>18
Intermediate	4	16–18	4	15–17
Resistant	>8	<15	>8	<14

Table 4. Breakpoints for *testing H. influenzae* and *H. parainfluenzae.*

	Moxifloxacin		Gatifloxacin	
	MIC (µg/ml)	Zone diameter (mm)	MIC (µg/ml)	Zone diameter (mm)
Susceptible	<1	>18	<0.5	>18

Table 5. Breakpoints for testing *Streptococcus pneumoniae*

	Moxifloxacin		Gatifloxacin	
	MIC (µg/ml)	Zone diameter (mm)	MIC (µg/ml)	Zone diameter (mm)
Susceptible	<1	>18	<1	>18
Intermediate	2	15–17	2	15–17
Resistant	>4	<14	>4	<14

Table 6. Breakpoints for *testing Neisseria gonorrhoeae* with gatifloxacin only

	MIC (µg/ml)	Zone diameter
Susceptible	<0.125	>38
Intermediate	0.25	34–37
Resistant	>0.5	<33

Tequin (Gatifloxacin)

Following oral administration, gatifloxacin is well absorbed and its absorption is not affected by food. Its absolute bioavailability is 96% and it is not necessary to adjust this dose when switching from i.v. to oral route. It is excreted unchanged via the kidneys. In patients with creatinine clearance <40 mL/ minute, an initial dose of 400 mg is given on the first day and the dosage is reduced to 200 mg once a day on subsequent days. Gatifloxacin is available as 200 mg and 400 mg tablets. The i.v. formulation includes a single use vials containing a concentrated solution of 200 mg (10 mg/ml, 20 ml) and 400 mg (10 mg/ml, 40 ml) of gatifloxacin in 5% dextrose injection over a period of 60 minutes. It should not be administered by rapid or bolus i.v. infusion. The single use vials must be further diluted to a concentration of 2 mg/ml with a compatible solution (e.g. 5% dextrose, 0.9% sodium chloride) prior to administration. Additives or other medication should not be added to the gatifloxacin solution or infused simultaneously through the same i.v. line. The dosing of gatifloxacin for various indications are shown in Table 7.

Table 7. Food and Drug Administration (USA)-approved dosage regimens for gatifloxacin

Indications of gatifloxacin	Daily dose (mg)	Duration
ABECB	400	7–10 days
Acute sinusitis	400	10 days
community-acquired pneumoine (CAP)	400	7–14 days
Uncomplicated urinary tract infection (UTI) Single dose		400
	or 200	3
Complicated UTI	400	7–10 days
Acute pyelonephritis	400	7–10 days
Gonorrhoea	400	Single dose

The pharmacokinetics of Gatifloxacin are linear and depend on doses ranging from 200 to 800 mg administered over a period of up to 14 days. Steady-state concentrations are achieved by the 3rd day. A comparison of *in vitro* activity[12] of the four quinolones is shown in Table 8.

Table 8. Comparison of pharmacokinetic/Pharmacodynamic parameters for four fluoroquinolones and selected targets.

				Streptococcus pneumoniae			*Pseudomonas aeruginosa*		
Fluoroquinocone	Dose (mg)	Cmax	AUC	MIC µg/ml	Cmax/MIC	AUC/ MIC	MIC µg/ml	Cmax/MIC	AUC/ MIC
Cipro	500	3	28	2	1.5	14	4	0.75	7
	750	3.6	32	2	1.8	16	4	0.9	8
Levo	500	5.7	48	1	5.7	48	16	0.36	3
Moxi	400	4.5	48	0.25	18	192	8	0.56	6
Gati	400	4.2	34	0.5	8.4	68	8	0.52	4.25

Place in therapy

In the guidelines of CAP and acute maxillary sinusitis, moxifloxacin, gatifloxacin and levofloxacin are either first-line or alternative treatment options. Gatifloxacin and moxifloxacin should not be used to treat *Pseudomonas* infection.

Mutant prevention concentration

Mutant prevention concentration (MPC)[12,13] is a novel concept

that has been employed in the evaluation of antibiotics' ability to minimize or limit the development of resistant organisms. MPC has been defined as the MIC of the least susceptible single-step mutant. By definition, cell growth in the presence of antibiotic concentrations greater than MPC requires an organism to have developed two or more resistant causing spontaneous chromosomal point mutation.

Table 9 : Mutant prevention concentration (MPC).

Fluoroquinolone	Daily dose	Cmax	*Pseudomonas* MPC	*Streptococcus aeruginosa* MPC
Ciprofloxacin	500 bid	3	2	NR
	750 bid	3.6	2	NR
Levofloxacin	500 bid	5.7	8	8
Moxifloxacin	400 bid	4.5	NR	2
Gatifloxacin	400 bid	4.2	NR	4

A strategy for restricting the development of resistance is to find compounds that have narrow mutant selective windows (MPC/MIC=1) (Table 9).

Although the AUC/MIC and Cmax/MIC ratios are useful for predicting the potential for developing drug resistance, it is suggested that the AUC/MIC should exceed 100 for Gram-positive and gram-negative species to prevent selection of resistant strains. Alternatively, Zhao *et al.* have suggested that rate at which resistance develops to a fluoroquinolone is related to its MIC and MPC.[13]

Moxifloxacin exceeds the MPC for *S. pneumoniae* and ciprofloxacin exceeds the MPC for *P. aeruginosa* (both 2 µg/ml) by achieving a Cmax of 4.5 µg/ml and 3 µg/ml, respectively.

Evidence is emerging that suggests a link between appropriate fluoroquinolone use, development of resistance against the entire fluoroquinolone class and clinical failure. The major factors associated with increasing resistance to fluoroquinolone include:

- Under-dosing, i.e. use of a marginally potent agent whose-MIC is largely reached in the serum or infected tissue
- Overuse of agents known to encourage resistant mutants
- Inability to readily detect and respond to changes in antimicrobial susceptibility.

Ciprofloxacin, levofloxacin and gatifloxacin achieve a high concentration in urine, thus, they are appropriate choices for treating UTI in the community.

For infections in which *S. pneumoniae* is anticipated to be the most likely pathogen (CAP), moxifloxacin, which currently has the best anti-pneumococcal pharmacodynamic activity and the lowest MPC against this agent, would represent a prudent therapeutic choice.

Expecting a single fluoroquinolone to be suitable for all infections is unreasonable and excessive use of any single fluoroquinolone for all indications will lead to resistance that adversely affect the entire class.[14]

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Literature Review

Compiled by Dr. Pradeep Chattree

Antimicrobial treatment in Diabetic women with Asymptomatic Bacteriuria. Godfrey K, Harding MD, Nicoble MD et al (*N. Eng J. Med. 2002;347:1576-83)*

Asymptomatic bacteriuria is common among women with diabetes, and the treatment of such infections has been recommended to prevent complications related to symptomatic urinary tract injection. Thus the study in women (>16 years of age) with diabetes, bacteriuria (>10 colony-forming units of an organism per milliliter in cultures of two consecutive urine specimens) were enrolled with no urinary symptoms. 50 were randomly assigned to receive placebo and 55 to receive antimicrobial therapy. For the first six weeks which included the initial course of treatment, the study was placebo-controlled and double blind. Subsequently, the women were screened for bacteriuria every three months for up to three years, antimicrobial therapy was provided to women in the antimcrobial therapy group who had asymptomatic bacteriuria.

Four weeks after the end of the initial course of therapy 78% of placebo recipients had bacteriuria, as compared with 20% of women who received antimicrobial agents (p<0.001). During a mean follow up of 27 months, 20 of 50 women in the placebo group (40%) and 23 of 55 women in the antimcrobial therapy group (42%) had at least one episode of symptomatic urinary tract infection. The time to a first symptomatic episode was similar in the placebo group and the antimicrobial therapy group. P=0.67 by the long rank test), as were the (ISD) rates of any symptomatic urinary tract infection (1.10+0.17) and 0.93+0.14 per 1000 days of follow up respectively; relative risk, 1.19; 95% confidence interval 0.28 to 1.81). Pyelonephritis (0.28+0.08) and 0.13±0.05 per 1000 days of follow up; relative risk, 1.93; 95% confidence interval 0.47 to 7.89). The women in the antimicrobial therapy group had almost five times as many days of antibotic use for urinary tract infection as did the women in placebo group. (158.2+1.7 ys 33.7+0.91/1000 days of follow up.

Treatment of asymptomatic bacteriuria in women with diabetes does not appear to reduce complications. Diabetes itself should not be an indication for screening for or treatment of asympstomatic bacteriuria.

Emergence of non-*albicans Candida* species

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Abstract: A steady rise in incidence of systemic candidiasis over the past two decades resulting in higher morbidity, mortality and length of stay of hospitalized patients has been well documented worldwide. The gravity of the situation is further compounded by the emergence of non. *C.albicans Candida* (NAC) species as both colonizer and pathogen. Several sentinel and population-based surveillance programmes have noted an increase in the proportion of bloodstream infections (BSI) by NAC species, especially *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* in tertiary care centres. A few outbreaks due to unusual NAC species are also reported. Certain risk factors such as neutropenia, acute leukaemia, antineoplastic chemotherapy, surgery, intravascular catheter and antifungal prophylaxis have been specifically associated with NAC species causing catheter-related fungaemia. Accurate and rapid species level identification of NAC species is necessary because of differences in epidemiology and antifungal susceptibilities among various Candida species. The inherent resistance of a few NAC species to commonly used systemic antifungals such as fluconazole and amphotericin B can pose a therapeutic challenge in the management of NAC candidaemia. New drugs including broad-spectrum triazoles and echinocandins may be used as therapeutic alternatives in such a situation. Overall, higher mortality (35%-100%) due to NAC candidaemia demands strict implementation of preventive strategies and search for new broad-spectrum antifungal drugs.

Introduction

In recent years, a rise in the incidence of invasive candidiasis has been observed in the nosocomial setting, possibly contributed by the advances in medical technology, chemotherapeutics, cancer chemotherapy and bone marrow and organ transplantation. The infection is associated with high morbidity, mortality and an increase in the length of hospital stay.[1,2] In a study from the US hospitals, it was found that *Candida* species accounted for 8%-15% of nosocomial bloodstream infections (BSI), 38% attributable mortality and a 30-day median increase in hospitalization.[2] The excess cost attributable to candidaemia in the US is estimated to be $ 1 billion/year.[3] According to the National Nosocomial Infection Surveillance (NNIS) system , USA, *Candida* species is the sixth most common pathogen (7.1%) causing infection and fourth most common isolate (10.1%) in patients in the intensive care unit (ICU). The Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) study estimated that *Candida* species is the fourth most common bloodstream pathogen in US hospitals. The annual incidence of candidaemia in the USA ranges from 6-8 per 100 000 population.[4] However, the picture in developing countries is not clear. According to the limited data available in India, *Candida* species accounted for 16.4%-34.7% of neonatal sepsis[5] and a burden of 2 per 1000 discharges in a tertiary care hospital.[6] In the Postgraduate Institute of Medical Education and Research, Chandigarh, India, the second half of the 1980s witnessed an 11-fold increase in candidaemia cases followed by a further 18-fold rise in 1995 compared with 1991, and the rate was doubled again in 1996 and 1997. However, a 2-3 fold decrease was observed in 1998-2000 after administration of antifungal prophylaxis to high-risk patients.[7]

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The overall increase in candidaemia is further complicated by the emergence of non. *C. albicans Candida* (NAC) species as both colonizers and pathogens causing nosocomial fungal BSI. A distinct increase in the proportion of cases due to NAC species has been observed.[7]

Incidence of candidaemia due to NAC species

Wingard, in a comprehensive review of all published reports during 1952 through 1992, found that 12 reports showed higher (>50%) proportion of isolation of NAC species.[8] The common NAC species isolated were *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. parapsilosis*. Other species such as *C. guilliermondii*, *C. lusitaniae, C. dubliniensis, C. kefyr, C. lipolytica* and *C. pelliculosa* were occasionally isolated. A rank order of species distribution has been obtained from population-based and sentinel surveillance data on candidaemia (Table 1).[4] The earliest population-based surveillance study conducted in 1992-1993 by the Centers for Disease Control and prevention (CDC), USA reported *C. albicans* as the commonest species, followed in order by *C. parapsilosis*, *C. tropicalis* and *C. glabrata*. Subsequent surveillance programmes have noted an increase in the proportion of *Candida* BSI by NAC species and especially an increase in the frequency of BSI due to *C. glabrata* (Table 1). In contrast, surveillance data from other countries continue to reflect the importance of *C. parapsilosis* over *C. glabrata*.[9]

Table 1. *Candida* species distribution as reported by sentinel and population-based surveillance programmes (modified from Pfaller and Diekema[4])

Surveillance programme[a]	Years	Percentage of total (%)					
		C. albicans	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*	*C. krusei*	Other species
CDC	1992-1993	52	12	21	10	4	1
NEMIS	1993-1995	56	15	15	10	0	4
SCOPE	1995-1998	53	20	10	12	3	2
CDC	1998-2000	45	24	13	12	2	4
EIEIO	1998-2001	58	20	7	11	2	2
SENTRY	1997-2000	54	16	15	10	2	3

a: CDC: Centers for Disease Control; NEMIS: National Epidemiology of Mycoses Study; SCOPE: Surveillance and Control of Pathogen of Epidemiologic Importance; EIEIO: Emerging Infections and the Epidemiology of Iowa Organisms; NNIS: National Nosocomial Infection Surveillance System.

Table 2. *Candida* species distribution in adults and neonates as reported by different surveillance programs in USA (Modified from Pfaller and Diekema[4])

Study population	Surveillance program[a]	Percentage of total (%)				
		C. glabrata	*C. parapsilosis*	*C. tropicalis*	*C. krusei*	Total NAC (%)
Adults	NEMIS	24	5	19	0	52
	NNIS	12	10	11	NA[d]	41
	CDC[b]	25	12	14	NA	52
	SENTRY	23	12	10	2	50
Neonates	NEMIS	6	29	0	0	37
	NNIS	2	38	4	0	46
	CDC[c]	0	45	0	0	47
	SENTRY	3	24	7	0	40

[a]See footnote of Table 1 for abbreviations
[b] CDC study of 1998-2000
[c] CDC study of 1992-1993
[d] NA : data not available

The importance of the age of the patient in determining the rank order of *Candida* species causing BSI has also been noted (Table 2).[4] The predominance of *C. albicans* and *C. parapsilosis* and the lack of *C. glabrata* and other NAC species have been observed in neonatal age groups. In contrast, *C. glabrata* becomes an increasingly important pathogen with increase in age. Knowledge of the above trends can have important implications in nosocomial infection control strategies as well as for prophylaxis and dosages of systemic antifungals.[10] Similar to the western world, the rise in frequency of isolation of NAC species has been observed in tertiary care centres in India as well, with isolation rates ranging from 52.4% to 96%.[5,6] The dramatic increase in NAC candidaemia (20% in 1991 to 99% in 1997) has been documented in one centre.[7] In India, *C. tropicalis* is the commonest NAC species isolated followed by *C. guilliermondii, C. krusei, C. glabrata and C. parapsilosis*. An outbreak due to *C. pelliculosa* (*Pichia anomala*) involving 379 neonates and children over a period of 23 months was reported.[11]

Risk factors for NAC species

Epidemiological studies have successfully identified several significant risk factors for nosocomial candidaemia: acute leukaemia, leucopenia, burns, gastrointestinal surgery, prematurity, prior antibiotic use (vancomycin, imipenem), haemodialysis, intravascular catheters and previous colonization with *Candida* species.[4,8] However, certain specific factors and patient populations have been observed to be significantly associated with NAC species (Table 3). Preponderance of NAC species, especially *C. glabrata,* has been reported in surgical operation theatres and the ICUs.[12] Wingard reported that NAC species were more frequently isolated in leukaemia and bone marrow transplant patients ($p<0.001$).[8] In another study, haematological malignancies ($p<0.03$), neutropenia ($p<0.01$) and antifungal prophylaxis were documented to be significantly associated with NAC fungaemia.[4] Breakthrough (BT) candidaemia (candidaemia after 5 days of systemic antifungal therapy) is more often caused by NAC species, with isolation rates of 65%-80% against 45%-55% in non-BT episodes.[4] Previous colonization with NAC species ($p<0.001$) has also been reported to be predictive for NAC candidaemia. The role of antifungal prophylaxis in increased isolation of NAC species has been intensively investigated and a highly significant association of prior azole therapy with rise in incidence of NAC candidaemia (especially *C. krusei*) has been identified. *C. parapsilosis* has been increasingly associated (17%-45%) with candidaemia in neonates with significant risk factors including gestational age (<32 weeks); 5-minute Apgar score <5; shock; disseminated intravascular coagulation; prior use of intralipid; total parenteral nutrition; central venous catheter; intubation and use of H^2 blockers.[4]

Table 3. Risk factors for non albicans *Candida* infections

Candida species	Risk factors
C. tropicalis	Neutropenia, acute leukaemia, antibiotics, antineoplastic chemotherapy
C. glabrata	Solid tumours, surgery, azole therapy, long hospital stay, antibiotics, old age
C. parapsilosis	Intravascular catheters, neonates, prosthesis
C. krusei	Neutropenia, azole therapy, old age
C. lusitaniae	Neutropenia, polyene use

Pathogenicity of NAC species

The virulence of NAC species has been suggested to differ from those of *C. albicans*. Though most NAC species have been reported to be less virulent compared with *C. albicans in-vitro* and also in animal models, severe infections in humans with fatal outcomes have been attributed to NAC candidaemia. Hence, the pathogenicity of NAC species *in-vitro* or in animal models cannot be extrapolated to humans. In neutropenic patients, higher isolation of *C. tropicalis* and *C. krusei* with a grave prognosis is noted. The reason is not clear. However,

biofilm production has been implicated as a potential virulence factor for NAC species responsible for catheter-related fungaemia. *C. tropicalis* and *C. parapsilosis* tend to frequently colonize indwelling catheters frequently in individuals receiving intravenous hyperalimentation[13]. Comparison of clinical bloodstream isolates, representing different *Candida* species, with each other and with those from other anatomical sites, revealed that biofilm production was most frequent with *C. tropicalis* (80%), followed by *C. parapsilosis* (73%), *C. glabrata* (28%) and *C. albicans* (8%).[14]

Diagnosis

The emergence of NAC species as important human pathogens demands rapid species-level identification for prompt institution of appropriate antifungal therapy. Accurate diagnosis is of paramount importance because of differences in epidemiology and in antifungal susceptibilities of various species. Diagnosis of systemic candidiasis can be established either directly after isolation of the yeast or indirectly by detection of antibodies, antigens, metabolites and nucleic acids. Indirect tests such as antibody, antigen (mannan, enolase, HSP 90, proteinase) and metabolite (arabinitol) detection tests can be successfully used in diagnosis of systemic candidiasis but species-specific identification is difficult. Conventional methods of yeast identification after isolation in culture rely heavily on Wickerham or Delft assimilation/fermentation characters, which are cumbersome, and often beyond the range of expertise available in non-specialized clinical microbiology laboratories. Various commercially available rapid identification phenotypic tests, which can be used after primary isolation of the yeast on culture, have been developed. These include: (1) rapid methods (requiring less than 5 hours): enzymatic tests after primary isolation, immunological tests with polyclonal and monoclonal antibodies, biochemical and enzymatic panels, both manual and automated; (2) methods requiring 15 hours or more: biochemical and enzymatic panels, both manual and automated; and (3) physico-chemical methods: cellular fatty acid analysis by gas-liquid chromatography and spectroscopic techniques (Fourier transformed-infrared spectroscopy, pyrolysis mass spectrometry).

The incorporation of fluorogenic or chromogenic substrates directly into growth media agar to reveal species-specific enzyme activity allows easier discrimination of some *Candida* species and high sensitivity (85%-100%) and specificity (90%-100%) are reported for species identification.[15] The Iatron serological *Candida* check kit (Iatron Laboratories Inc., Tokyo, Japan) can also rapidly identify *C. albicans, C. tropicalis, C. guilliermondii, C. krusei, C. kefyr, C. glabrata and C. parapsilosis* but its use is limited because of low specificity.

Identification systems based on direct enzyme detection by providing substrates to determine the yeast's enzymatic profile are available in both manual and automated versions. The API 20 AUX (88.5% accurate) is considered a reference method and new systems such as Auxacolor (61%-63% accurate) and Fungichrom (81%-91% accurate) have been evaluated in some laboratories. Among the automated systems, ID 32C strip, Vitek yeast biochemical card and Vitek 2 ID-YST system can correctly identify >93% of yeasts and the highest accuracy is observed with the ID-YST system.[15] Many probes have been designed for the various *Candida* species: *C. tropicalis* (Ct3, Ct14), *C. glabrata* (Cg6, Cg12), *C. dubliniensis* (Cd1, Cd24, Cd25), *C. parapsilosis* (Cp3) and *C. krusei* (CkF1, CkF2). These probes have been evaluated for rapid identification but these are available only in select centres. Specific amplification of certain target genes: *L1A1* (encoding lanosterol 1-4α demethylase), *hsp 90*, actin gene, mitochondrial DNA and ribosomal DNA can also be used for identification of *Candida* species.[15]

Management

The inherent resistance of some NAC species to commonly used systemic antifungals can pose a therapeutic problem in the management of NAC candidaemia. A proportion of *C. krusei* and *C. glabrata* isolates are either primarily or secondarily resistant to fluconazole. Resistance to amphotericin B is observed in *C. lusitaniae, C. krusei, C. rugosa* and *C. guilliermondii* strains.[16] Decreased susceptibility of *Candida* species causing BSI in older patients to both azoles and amphotericin B has been observed due to decrease in *C. albicans* and increase in *C. glabrata* and *C. krusei* candidaemia.

Trends in the susceptibility of *Candida* species BSI isolates to fluconazole over time have been assessed by both population-based and sentinel surveillance programmes. It was observed that resistance to triazoles is still not a frequent event. *C. glabrata* isolates generally exhibit bimodal susceptibility to azoles, some demonstrating frank resistance (MIC >64 µg/ml), whereas others are susceptible. *C. tropicalis* is generally susceptible to azoles and no significant increase in resistance has been observed.[7] Many new 'extended-spectrum' triazoles, both licensed and investigational, are being evaluated for efficacy against the various *Candida* species. It has been observed that ravuconazole, posaconazole and voriconazole are more active than amphotericin B, 5-fluorocytosine, itraconazole and fluconazole *in-vitro* against all *Candida* species and are possibly better agents against *C. krusei*.[17]

Generally, all *Candida* species are susceptible to amphotericin B except for some strains of *C. lusitaniae* and *C. guilliermondii*. However, recently candidaemia caused by amphotericin B-resistant strains of *C. glabrata* and *C. krusei* have also been reported.[7,18] Reports of amphotericin B resistance is of special concern because of the paucity of therapeutic alternatives in these patients. Still, amphotericin B should be used for empirical treatment in centres where the incidence of NAC candidaemia is >50%. However, excellent *in-vitro* susceptibility to the newer azoles, particularly voriconazole, can make it the alternative option in the treatment

of *C. krusei* infections.[17] For infections due to *C. glabrata*, dose-dependent responses to azoles are observed. In patients developing BT infection with severe immunosuppression or neutropenia, alternative agents are preferred.[19] Newer drugs, e.g. broad-spectrum triazoles and echinocandins may be used to treat serious *Candida* infections.[19]

In spite of increased awareness, the overall mortality due to NAC candidaemia has been reported to be around 35%-100%.[4,20] Thus, intensive search for newer therapeutic modalities as well as strict implementation of preventive strategies are required. Elimination of specific risk factors associated with particular NAC species, e.g. uncontrolled use of azoles for *C. krusei* and *C. glabrata* infections and intravascular devices for *C. parapsilosis* may be a realistic approach. General infection control measures to reduce nosocomial transmission (handwashing by healthcare workers and antisepsis) must be emphasized.

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Emerging and re-emerging infections

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Abstract: Since time immemorial, infectious diseases have been, and continue to be, the leading cause of death worldwide. The discovery of various antimicrobials kindled the hope of eradication of these diseases. However, new infections continue to be discovered, leading to increasing morbidity and mortality. Emerging infections are defined as new, re-emerging, or drug-resistant infections whose incidence in humans has increased within the past two decades or threatens to increase in the near future. The reasons for their emergence include international travel, overcrowded cities, intensive food production and new food-processing methods, poverty, sexual practices, increasing immunosuppression due to new treatment modalities and global warming.

The sudden acute respiratory syndrome (SARS) outbreak caught the attention of the media, leading to panic around the world last year, reinforcing the important lesson that we continue to remain vulnerable to unexpected microbial threats. Not only did a new infectious disease emerge, but it also spread rapidly throughout the world, in spite of our astounding technological advances or perhaps because of them. Many new infectious diseases are likely to emerge over the next 25 years unless we acquire an ecological perspective on infectious diseases rather than seeing microbes as simply an invading entity that should be blindly attacked with antibiotics or used as a tool for biological warfare.

The Institute of Medicine, USA has defined emerging infectious diseases as '*new, re-emerging, or drug-resistant infections whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the near future*'.[1]

There are some noticeable patterns in the emergence of new diseases. No person, community or country is insulated from the potential introduction of an unfamiliar microbial pathogen. The infected cases can travel around the world within the incubation period of almost any infectious disease and thereby unknowingly disseminate pathogens. The rise in international travel, overcrowded cities, intensive food production, sexual practices, poverty and global warming are some of the causes for the emergence, maintenance and spread of new infectious diseases, as well as the resurgence of older diseases such as cholera, tuberculosis and malaria.

Infectious diseases have a significant impact on the global economy. The estimates of direct and indirect costs of infectious diseases exceed US $ 120 billion annually. Major epidemics can be devastating to national economies, particularly those of developing nations. The estimated cost of the outbreak of plague in India, in 1994, was between US $ 1-2 billion. The cholera epidemic in Peru in 1991 resulted in an estimated loss of US $ 700 million.[2]

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Like other living organisms, infectious agents are subject to genetic change and evolution. This is manifested by their ability to infect new hosts, alterations in their susceptibility to antimicrobial drugs and changes in their response to host immunity. At the same time, the human host has also changed. We have adopted new behaviour, international travel has increased and new food-processing methods are being adopted, which may enhance transmission of some microbes. New diseases as well as modern medical treatment may also lead to immunosuppression and thus increase the susceptibility to pathogens.

Developments in agriculture, urbanization and deforestation have changed ecosystems and allowed the emergence of infections. Lyme disease, first identified in 1976, is spread by ticks; forest fragmentation, loss of predators and the shift of habitation closer to woodlands were the factors that led to its emergence.

Sudden acutre respiratory syndrome probably originated from the Guangdong province of China in late 2002 and came into public prominence in February 2003. By July 2003, it had been reported from 32 countries globally, affecting 8098 people with 774 deaths (9.6% case fatality) (Fig.1). The measures used for the management of cases included isolation, ribavirin, corticos-

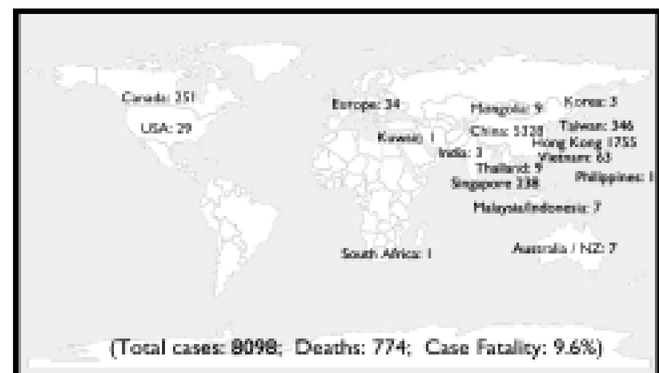

Fig 1. : SARS cases worldwide reported by WHO as of July 31, 2003; revised Setember 26, 2003. The numbers in the figure indicate cases.

teroids and mechanical ventilation.[3,4]

In the past two decades, several new human pathogens have been recognized, including those causing hepatitis C, hepatitis E and, recently, SARS. Other diseases that were thought to be under control have re-emerged, often as a result of drug resistance. Some infections that are emerging internationally are listed in Table 1, and those in India in Table 2. The major aetiological agents and infectious diseases, identified since 1983, are given in Table 3.

Table 1. Emerging infections (International)

Emerging infections	Country
Ebola haemorrhagic fever	Zaire, Gabon
Dengue haemorrhagic fever	Western hemisphere, India
Venezuelan equine encephalitis	Venezuela and Colombia
Cholera	Cape Verde
Meningococcal meningitis	Africa
Lassa fever	Sierra Leone
Streptococcus iniae infection	Canada
Variant Creutzfeldt-Jakob disease	United Kingdom, France
Escherichia coli 0157:H7 haemorrhagic colitis	Japan
Vancomycin intermediate-sensitive and resistant *Staphylococcus aureus* (VISA/VRSA)	USA

Table 2. Emerging and re-emerging infections in India

Year	Infections	Location
1990	Multidrug-resistant (ACCo) *Salmonella typhi*	Most Indian states
1991	*Vibrio cholerae* 0139	South India, West Bengal
1994	Plague	Maharashtra and Gujarat
1996	Dengue haemorrhagic fever	North India
2000	Fasciolopsiasis	Azamgarh, UP
2001	*Acinetobacter baumannii*	Manipal, Delhi
2001	*Pichia anomala*	Chandigarh
2001	Rotavirus G4P8	Kolkata
2001	Low level penicillin resistance in *Streptococcus pneumoniae*	Pondicherry, Delhi, Vellore
2002	West Nile virus infection	Maharashtra, Rajasthan, Goa, Orissa
2003	Nalidixic acid-resistant *Salmonella typhi*	Delhi, Mumbai

The human immunodeficiency virus (HIV) was first discovered in 1983, and has successfully transited from an emerging into an established infection. The acquired immunodeficiency syndrome (AIDS) epidemic claimed an estimated 3.1 million lives worldwide in 2002, and an estimated 5 million people acquired HIV in 2002, bringing the estimated number of people globally infected with the virus to 42 million. With the current disease burden, HIV will emerge as the largest cause of adult mortality in this decade, along with an additional 1 million tuberculosis (TB) cases. India, with 1.027 billion people, has an estimated HIV/AIDS infection rate estimated at 0.7% in the adult population, affecting about 3.8-4.6 million.[5,6]

The Nipah virus, which killed 100 people in Malaysia in 1999, was normally carried by the forest fruit bat and had not previously seemed to pass to humans. However, because of deforestation and agricultural techniques, the bat's normal habitat and food source were changed. This forced the bats to encroach into fruit plantations that were in close proximity to pig farms. The bats infected the pigs, which in turn infected the farmers.

Identified in 1989, hepatitis C virus is known to be the most common cause of post-transfusion hepatitis worldwide. Up to 3% of the world population is estimated to be infected, among which 170 million are chronic carriers at risk of developing liver cirrhosis and/or liver cancer. Measures to control its spread include its inclusion into routine testing of blood prior to transfusion.

Mycobaterium abscessus and other atypical mycobacterias such as *M. chelonae* and *M. fortuitum*, are responsible for a number of infections, and their incidence and reporting is increasing in the recent years as causes of soft tissue infections, chronic ear infections, bacteraemia associated with haemodialysis and peritoneal dialysis.[8]

Multidrug-resistant (MDR) *Acinetobacter baumannii* is an important cause of hospital associated infections and is known to infect patients with major burns and cancer.[9]

The increasing incidence of *Cryptosporidium* and *Aeromonas* as a cause of diarrhoea has been reported across the world. Enteroaggregative *Escherichia coli* (EAEC) is another emerging pathogen linked to acute and persistent diarrhoea in both developing and industrialized countries. Recent sporadic cases and outbreaks of a foodborne illness due to EAEC have been recognized.[10,11]

Vibrio cholerae 0139 was first detected in 1992 in India and has since spread to seven countries in Asia. The emergence of a new serotype permits the organism to continue to spread and cause disease even in populations protected by antibodies generated in response to previous exposure to other serotypes of the same organism. The upsurge in the worldwide incidence of *V. parahaemolyticus* infection in the past 8 years has been attributed to the appearance of 3 serotypes (03:K6, 04:K68 and 01:KUT) causing the first pandemic across eight countries.[12,13]

In 1994, the plague epidemic in India caused worldwide concern. The Centers for Disease Control and Prevention (CDC) developed and implemented an enhanced surveillance system to supplement the then existing regulations concerning imported plague.[14]

The World Health Organization (WHO) estimates that there are 50 million cases of dengue infection worldwide. Nearly 2500 million people are at risk for dengue. An estimated 500 000 cases of dengue haemorrhagic fever (DHF) require hospitalization every year. The first major outbreak of DHF/dengue shock syndrome (DSS) in India occurred in 1996 in and around Delhi. This was caused by dengue virus type 2, with around 8900 reported cases with a fatality rate of 4.2%.[15]

Viral conjunctivitis has also emerged as an important infection affecting large numbers in the Indian subcontinent. An outbreak of acute haemorrhagic conjunctivitis caused by enterovirus type 70 occurred in Delhi in 1996 during the rainy season, which is the season associated with most of the infectious diseases in the subcontinent.[16]

Table 3. Major etiological agents and infectious diseases identified since 1983

Year	Agent	Type	Disease
1983	Human immunodeficiency virus (HIV)	Virus	Acquired immunodeficiency syndrome
1983	*Helicobacter pylori*	Bacteria	Gastric ulcers
1985	*Enterocytozoon bieneusi*	Parasite	Persistent diarrhoea
1986	*Cyclospora cayetanensis*	Parasite	Persistent diarrhoea
1988	Human herpesvirus-6 (HHV-6)	Virus	Roseola subitum
1988	Hepatitis E virus	Virus	Enterically transmitted non-A, non-B hepatitis
1989	*Ehrlichia chaffeensis*	Bacteria	Human ehrlichiosis
1989	Hepatitis C	Virus	Parenterally transmitted non-A, non-B hepatitis
1991	Guanarito virus	Virus	Venezuelan haemorrhagic fever
1992	*Vibrio cholerae* 0139	Bacteria	New strain associated with epidemic cholera
1992	*Bartonella (Rochalimaea) henselae*	Bacteria	Cat-scratch disease; bacillary angiomatosis
1993	Sin Nombre virus	Virus	Hantavirus pulmonary syndrome
1994	Equine morbillivirus	Virus	Human pneumonia
1994	Sabia virus	Virus	Brazilian haemorrhagic fever
1995	Hepatitis G virus	Virus	Parenterally transmitted non-A, non-B hepatitis
1995	Human herpesvirus-8 (HHV-8)	Virus	Associated with Kaposi's sarcoma (AIDS)
1996	Transmissible spongiform encephalopathy (TSE)	Prion	New variant Creutzfeldt-Jacob disease
1997	Avian Influenza–type A (H5N1)	Virus	Influenza
1998	Nipah virus	Virus	Encephalitis
2003	SARS-associated coronavirus	Virus	Sudden acute respiratory syndrome

Leptospirosis is another infection emerging across northern India (Chandigarh, Delhi and Varanasi) with a reported seroprevalence varying from 9% to 43% in episodes of acute febrile illness.[17]

West Nile virus infection has emerged as an important public health problem causing epidemics in the USA, and has also been reported from western states in India.[18]

The growing threat of antimicrobial resistance is of great concern globally. It is of paramount importance that the microbiology laboratory provides accurate and timely quantitative results on antibiotic sensitivity, and the microbiologist manages the results and disseminates the information gathered.

Pathogens developing resistance to antimicrobial agents may also cause public health problems. Chloroquine-resistant *falciparum* malaria, persistence of methicillin-resistant *Staphylococcus aureus* (MRSA) and emergence of glycopeptide-resistant enterococci (GRE, earlier known as vancomycin-resistant enterococci or VRE) in hospitals, and (MDR) tuberculosis and enteric fever are clinical problems which are getting more difficult to manage.

Staph aureus is the most frequently isolated Gram-positive pathogen and is an important cause of hospital-associated infections. MRSA has emerged as an important pathogen not only in the hospital setting but also as a cause of skin and soft tissue infections in the community. In a surveillance study at 3 centres in India, the incidence of MRSA was found to be around 32%, varying from 27% to 47%.[19] The emergence of GRE, vancomycin intermediate-sensitive and resistant *Staph aureus* (VISA/VRSA) have added to the problem of treating Gram-positive infections. Similarly, extended spectrum beta-lactamases (ESBL) and device-associated infections due to staphylococci and *Candida* have compounded the problem of treating MDR Gram-negative infections, particularly in intensive care settings.

Salmonella typhi is endemic in developing countries with an estimated incidence of 33 million cases every year. The occurrence of MDR isolates of *S. typhi* along with the emergence of decreased susceptibility to quinolones (especially ciprofloxacin) is an important harbinger of increasing treatment failures in enteric fever. Nalidixic acid-resistant *S. typhi* with decreased susceptibility to ciprofloxacin has been responsible for outbreaks in Tajikistan and Viet Nam. Despite the low level of resistance to ciprofloxacin, treatment failures are increasingly being reported and have led to a demand for revised breakpoints for quinolones against *Salmonella*.[20,21]

Microorganisms have survived ecological change for millions of years because of their rapid rate of replication, mutation, and genetic recombination and exchange, and they will continue to do so as the global ecology changes. Surveillance is the key to recognize new or emerging infectious diseases, and to track the prevalence of more established ones. A well-designed, well-implemented surveillance programme can detect unusual clusters of disease, document the geographical and demographical spread of an outbreak, and estimate the magnitude of the problem. It can also help to describe the natural history of a disease, identify factors responsible for its emergence, facilitate laboratory and epidemiological research, and assess the success of specific intervention efforts.

The microbiology laboratory plays a critical role in recognizing new, emerging and re-emerging infectious diseases by identifying specific causes for the disease syndromes seen by clinicians, and by reporting new or unusual pathogens that they encounter. The laboratory may also serve as a key surveillance point for information gathering and dissemination, as is the case for antimicrobial resistance data. Appropriate specimen collection involves an attending medical staff that is knowledgeable about the samples to be obtained and availability of proper containers, especially if the specimens are to be transported outside the hospital or clinic setting.

Future challenges certainly include antimicrobial-resistant infections, the threat of another influenza pandemic, and the likeli-

hood of increasing problems with DHF and the risk of urban yellow fever in new areas. The global HIV epidemic will put a large number of people at risk for currently recognized and new opportunistic infections. The roles of hepatitis B and C viruses in chronic liver disease and hepatocellular carcinoma, of human papillomaviruses in cervical cancer, and of *Helicobacter pylori* infection in peptic ulcer disease and gastric cancer are now well-established. It is quite likely that more chronic diseases may be found to have an infectious aetiology.

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Drug Profile

DUTERSTERIDE

Dutersteride, 4 azasteroid is a selective and potent inhibitor of type 1 and 2 isoforms of 5x reductase. This drug is used in the treatment of BPH (Benign prostatic hyperplasia) current medical treatments of BPH include the use of after X-adrenoceptor antagnonists and 5x reductase inhibitors to relieve symptoms and improve urinary flow. How X-adrenoceptor antagonists acts directly on smooth muscle to decrease muscle tone; 5X redutase inhibitors decrease the size of the prostate.

Mechanism of action : Dutasteride is a dual 5X reductase inhibitor. The enzyme 5X reductase is central to the conversion of testosterone to DHT. Daily doses of dutasteride result in a dose dependent reduction of serum DHT that is greater than finasteride.

Pharmacokinetics : Following single dose of 0.5 mg, peak serum concentration occurs within 1-3 hours. It is well absorbed, with bioavailability of approximately 60%. It is highly bound to plasma proteins (>99.5%). The volume of distribution is large (app. 300-5001). Drug is extensively metabolised in the liver by the human cytochrome P450, isoenzyme-CVP3A4 & CYP3A5. Single doses of <5.0 mg are eliminated more rapidly than doses of more than 5.0 mg by both concentration-dependent and independent elimination pathways and have a half life of 3-9 days. Dutasteride and it metabolities are mainly excreted in faces.

- *Pharmacokinetics in special patient groups* Dutasteride is contraindicated for use in children and adolescents; elderly patients- no dose adjustment is required.

-In renal failure patients-No adjustment in dsage is anticipated for these patients as less than 0.1% of unchanged drug is excretedin urine.

-In hepatic impairment patients-caution should be exercised in administering to patients with mild to moderate hepatic impairment. Drug is contraindicated in patients with severe hepatic impairment.

Drug interactions : Blood concentrations of dutersteride may increase in the presence of inhibitors of CYP3A4 such as ritonavir, ketoconazole, verapamil, diltiazime, ciprofloxacin. There are no pharmacokinetic or pharmacodynamic interactions between dutersteride and tamsulosin, terazosin, warfarin, digoxin and cholestyramine.

Indications and Usage - Dutasteride is indicated for the treatment of symptomatic benign prostatic hyperplasia (BPH) in men to i) improve symptoms, and (ii) reduce the risk of acute urinary retention (iii) reduce the risk of the need for BPH related surgery.

Contraindication - The drug is contraindicated for use in women and children and in patients with known hypersensitivity to drug.

Warnings - Dutasteride is absorbed through the skins; therefore women who are pregnant or may be pregnant should not handle because of the possibility of absorption of dutasteride and the potential risk of a congenital anomaly in the male fetus.

Men being treated with dutasteride should not donate blood until a least months have passed following their last dose.

Dosage and administration : The recommended dose of Dutasteride is 0.5 mg daily orally. The capsules should be swallowed whole, can be given with or without food.

Effect on PSA-PSA levels decrease following dutasteride treatment. To interpret PSA value in a man treated with dutasteride for 6 month or more, the PSA value should be doubled for comparison with normal values in untreated man.

Interpretation of TORCH

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Abstract: Diseases in expectant mothers and newborns, like long-term disabilities, should be prevented by establishing an early diagnosis, evaluating and implementating adequate therapy. Some maternal infections, especially those during early gestation, can result in foetal loss or malformation because the ability of the foetus to resist infectious organisms is limited and the foetal immunological system is unable to prevent the dissemination of these organisms to the various tissues. One group of microbial agents generally known as TORCH can cause remarkably similar manifestations. The increasing need to diagnose these infections has fuelled and supported the rapid improvement in antigen and antibody tests. All seroassays are only surrogate markers of the disease. The presence any positive pathogen-specific IgM in the maternal serum should call for additional confirmatory testing in a reference laboratory before undertaking any intervention. Direct antigen testing or multiple testing would seem to be appropriate for confirmation. All these assays need to be correlated clinically to avoid misinterpretation of results and minimize the anxiety of patients, especially if termination of pregnancy is being considered.

Introduction

Maternal viral and protozoal infections *contribute enormously* to childhood morbidity. Primary TORCH infections include infections associated with *Toxoplasma*, Other organisms (Parvovirus, Human Immunodeficiency virus, Epstein Barr virus, Human Herpesvirus 6 and 8, Varicella, *T. pallidum*, enterovirus), rubella, cytomegalovirus (CMV) and herpes simplex virus (HSV) type 1. These infections in a pregnant woman can lead to severe foetal anomalies or even foetal loss. Highly sensitive and specific tests are available for the diagnosis of TORCH infections. Individual TORCH tests based on the clinical presentation and history of the patient may be useful. Because evidence of foetal infection on initial screening raises the spectre of complicated and dangerous foetal diagnosis (cordocentesis) or elective abortion, accurate testing and its proper interpretation are crucial to achieve a reduction in the number of foetuses who are unnecessarily injured by such techniques.

Toxoplasmosis

Toxoplasmosis is caused by *T. gondii*, a unicellular protozoan parasite found worldwide. Serological prevalence data indicate that toxoplasmosis is one of the common infections of humans throughout the world. The seroprevalence of *T. gondii* among various patient groups in India has been reported to be between 6% and 57%.[1,2,3]

Maternal acute toxoplasmosis during pregnancy has been implicated in spontaneous abortion, stillbirth and premature births. Congenital toxoplasmosis occurs when a woman gets infected during pregnancy, or, more rarely, if she is immunocompromised and a previously acquired infection is reactivated. The incidence of vertical transmission ranges from 11% in the first trimester to 90% in the late third trimester, with an overall transmission rate of approximately 50%.[4] There may be no sequelae of congenital toxoplasmosis, or sequelae may develop or be evident after birth. The clinical features include chorioretinitis, strabismus, blindness, epilepsy, psychomotor or mental retardation, anaemia, jaundice, rash, encephalitis, microcephaly, intracranial calcification, hydrocephalus, diarrhoea, hypothermia and non specific illness.[5] The isolation of *T. gondii*, detection of *T. gondii* antigen in tissues, blood, amniotic fluid, cerebrospinal fluid (CSF) and antibodies for *T. gondii* (IgG, IgM and IgA), and detection of *T. gondii* DNA by molecular assays may be used for the diagnosis of toxoplasmosis. When Toxoplasma infection is suspected during or before pregnancy, the diagnosis is primarily based on serology.

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Interpretation of *Toxoplasma* serology in pregnancy

The diagnosis of acute *T. gondii* infection in most cases requires demonstration of a rise in the IgM or IgG antibody titres (OD values or IU/ml) in serial serum samples drawn 3 weeks apart tested in parallel (either seroconversion from a negative to a positive or its significant rise from a low to a higher value).[5] Because the diagnosis is frequently considered relatively late in the course of the disease, the antibody levels might already have achieved their peak values at the time the first sample is obtained. It is, therefore, often difficult to discriminate between infections acquired recently (possibly during pregnancy) or in the distant past. Thus, the initial serum sample must be obtained as early as possible during gestation. An algorithm for the serodiagnosis of toxoplasmosis during pregnancy is shown in Fig 1.[6]

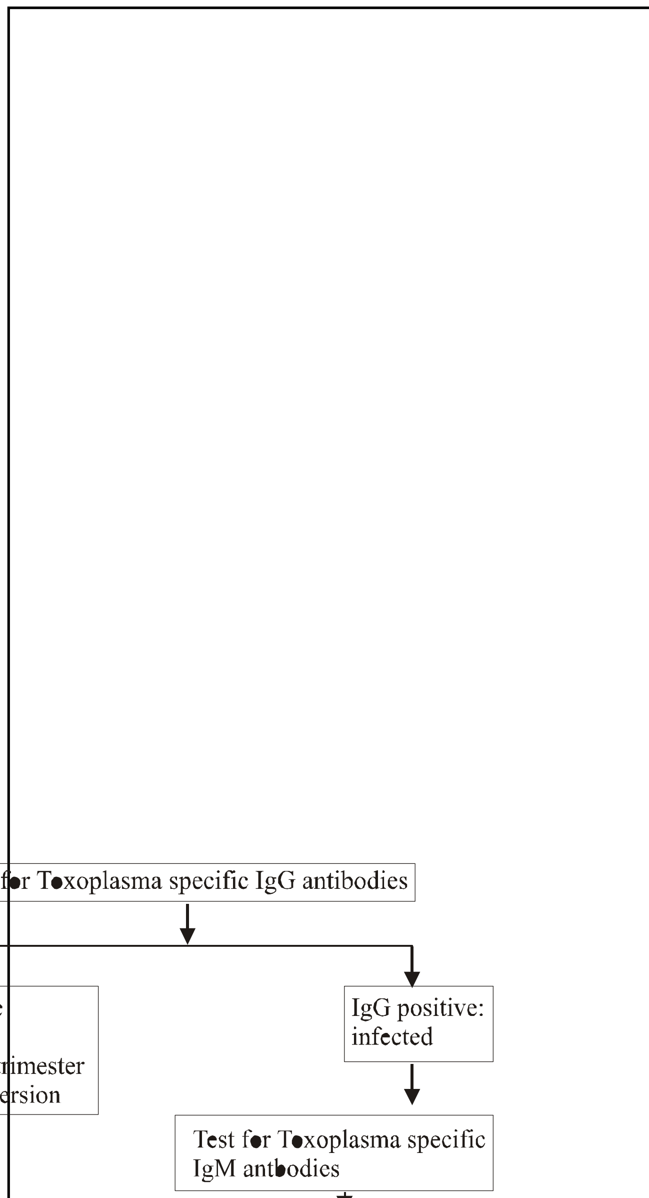

Fig. 1 Diagnostic algorithm for *Toxoplasma* serology in pregnant women.

Initial screening of maternal serum involves testing for IgG and IgM antibodies. A lack of both essentially excludes active infection, but identifies the patient as being at risk for acquisition of primary infection and hence, in need of instruction about primary prevention and retesting during each trimester to rule out seroconversion. The presence of IgG antibodies in the absence of IgM antibodies in the first two trimesters almost always indicates maternal exposure to infection with essentially no risk to the foetus (the exceptions are immunodeficient patients). In the third trimester, a negative IgM test is most likely to be consistent with a chronic maternal infection, but does not exclude the possibility of an acute infection acquired early in pregnancy. This is especially true in those patients who exhibit a rapid decline in their IgM titres during the acute phase of infection. In these cases, other serological tests (e.g. IgG avidity) may be used. Hence, if a pregnant woman is tested in the second or third trimester rather than the first trimester and is found IgG positive but IgM negative, it is advisable to perform an IgG avidity test. High-avidity IgG indicates that the infection was acquired more than 4 months ago and low avidity may be indicative of recent infection. However, the avidity test is not confirmatory for recent infection.

A negative IgM essentially excludes recent infection, but a positive IgM result is difficult to interpret without fallacies because Toxoplasma specific IgM antibodies may be detected by ELISA as late as 6-12 months and rarely 18 months after acute infection.[5] A major problem with Toxoplasma-specific IgM testing is the lack of specificity. Two situations are frequently seen. First, positive IgM but negative IgG, and second, both IgG and IgM positive in the serum. In the first case, a follow-up blood sample of the patient should be drawn 3 weeks after the initial collection and both samples tested simultaneously. In case of acute infection, the second specimen drawn 3 weeks later may have high positive IgG and IgM antibodies. If the IgG remains negative and IgM is positive in both the specimens with not much difference in values, the IgM result should be considered false-positive and the patient as not infected. In the second situation, avidity testing followed by prenatal molecular assays are recommended. In fact, all serology test results with equivocal IgG or IgM or positive IgM should be verified at a reference laboratory (e.g.Toxoplasma Reference Laboratory, Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi).[6]

Prenatal diagnosis for foetal infection is advised in a pregnant woman when a diagnosis of acute infection is suspected clinically. Acute infection is confirmed by the isolation of *T. gondii* or amplification of its DNA in the blood or body fluids. Polymerase chain reaction (PCR) performed on the amniotic fluid for the detection of *T. gondii*-specific DNA at 18 weeks of gestation is a sensitive, rapid and specific assay compared with conventional serodiagnostic procedures done on the foetal blood.[7]

The treatment of an acutely infected pregnant woman does not eliminate, but may decrease, the incidence of foetal infection. Spiramycin is the drug of choice and sulfadiazine can be used as an alternative with proper precautions at term. As spiramycin does not reliably cross the placenta, if foetal infection is documented, daily administration of sulfadiazine (4 g), pyrimethamine (25 g) and folinic acid (5-15 mg) is recommended as an alternative to the termination of pregnancy where abortion is illegal or when the woman wishes to continue the pregnancy.[8]

Cytomegalovirus infection

Cytomegalovirus infection in humans has a varied presentation, ranging from no disease in normal hosts; congenital CMV syndrome in neonates, which is frequently

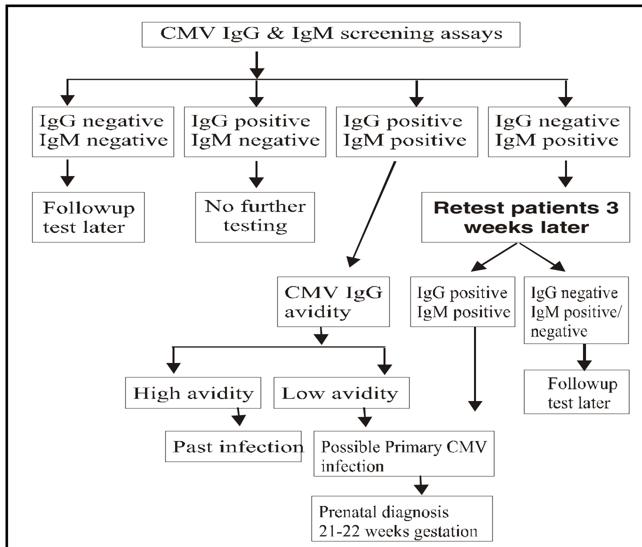

Fig. 2: Diagnostic algorithm for CMV serology in pregnant women

fatal; to the infectious mononucleosis syndrome in young adults. Primary infection is seen in seronegative patients who have never been infected with CMV. Secondary infection is the activation of latent infection or reinfection in a seropositive person. Intrauterine CMV infection has been detected in 0.2%-2.5% of cases and is the most commonly identified cause of congenital infection. Serological surveys indicate that in India, 20%-94% of pregnant women are seropositive for CMV.[9,10] Most of them have no symptoms and very few have a disease resembling mononucleosis. It is the foetus that may be at risk of congenital CMV infection following primary CMV infection (40%). Intrauterine transmission of non primary CMV is rare (0.2%-1%). It is in this context that prenatal risk assessment of pregnancy at 21-22 weeks of gestation, potentially complicated by CMV, can be accurately estimated using molecular assays, e.g. nucleic acid sequence-based (NASBA) amplification assay .

Fewer than 5% of congenitally infected infants develop symptoms during the neonatal period. Possible manifestations range from severe disease with intrauterine growth retardation, jaundice, hepatosplenomegaly, petechiae, central nervous system (CNS) abnormalities and chorioretinitis, to a more limited involvement.

Interpretation of cytomegalovirus serology in pregnancy

Severe infections with CMV in pregnant women are generally asymptomatic or accompanied by symptoms non-specific for CMV, so serological testing for CMV antibodies is usually the first test ordered by the physician. The main challenge for diagnostic laboratories in screening pregnant women for CMV specific IgM during non-primary infections is latency of the virus or cross-reactivity of CMV (also known as human herpesvirus 5) with the herpes group of viruses. The approach to these problems requires a diagnostic algorithm based on screening pregnant women with a CMV-specific IgG assay and a sensitive IgM capture assay, followed by further testing of CMV IgM-and IgG-positive specimens with a CMV IgG avidity assay (Fig. 2).

If the tests for CMV IgG and IgM antibodies are negative, it indicates that there is no active infection and the patient has to be tested later as she may be at risk for acquiring the infection in future. If IgG alone is positive, it suggest that the patient is immune and no further testing is required. If both IgG and IgM are positive, a CMV IgG avidity test may be done which measures the functional binding affinity of the IgG class of antibodies in response to infection. During the first few weeks of primary infection, IgG antibodies with a low avidity are produced whose avidity increases over time. This maturation of antibody avidity over time can be used at the diagnostic level to discriminate between primary and non-primary infections.[11] Detection of low avidity in specimens from pregnant women generally indicates that primary CMV infection has occurred within the past 18- 20 weeks, whereas detection of high-avidity CMV IgG excludes primary infection.[12] However, in as may as 35%-40% cases, low-avidity IgG response may also be seen in infections of more than 4 months duration.

Avidity tests can be performed only on IgG-and IgM-positive specimens, so the main problem occurs when only the CMV IgM result is positive. Since the rise of CMV IgM and IgG titres occurs almost simultaneously in response to primary CMV infection in immunocompetent individuals, it is recommended that another specimen be obtained within 3 weeks and test for CMV IgG and IgM antibodies. If the IgG test remains negative, the first CMV IgM result is taken as false-positive which can be due to viral cross-reactivity in CMV IgM assays.

If primary CMV infection in a pregnant woman is documented, further testing should be done to determine if actual transmission of the virus has occurred. As stated earlier, these antibody assays are surrogate markers of the disease and need to be proven further. Detection of viral DNA by molecular assays has now replaced cell culture as the 'gold standard' for the detection of CMV. Recent studies have shown that a high viral load determined by quantitative PCR in the amniotic fluid correlates with CMV disease in the foetus and newborn.[13] Determination of CMV pp67 mRNA using NASBA has also been found to be useful in determining the reactivation of this disease[14]. There are limited treatment options for CMV infection in pregnancy. Current recommendations limit the use of ganciclovir in pregnancy to severe (life- or sight-threatening) maternal CMV infections.

Rubella virus

Rubella (German measles) acquired in the first 12 weeks of pregnancy is associated with a 90% risk of congenital malformations. The seroprevalence of rubella in Indian pregnant woman has been reported to be between 74% and 95%.[10,15] The typical features of congenital rubella syndrome are cataract, intrauterine growth retardation,

thrombocytopenia, purpura, patent ductus arteriosus, osteitis and hearing impairment. The only reliable evidence of acute rubella infection is the presence of rubella-specific IgM antibody, demonstration of a significant rise in IgG antibody from paired acute and convalescent sera, or a positive viral culture for rubella that remains the 'gold standard' for diagnosis. Although rubella infection is definitively diagnosed by isolation of the virus in tissue culture, viral cultures are labour-intensive, and therefore not carried out in many laboratories and the generally not used for routine diagnosis of rubella. Hence, serology is the most common method of confirming the diagnosis of rubella. Neonatal rubella infection can be diagnosed reliably by using a cord blood sample for IgM rubella assay.

Interpretation of rubella serology in pregnancy

The immune status of a pregnant woman is determined by routine antenatal screening for rubella-specific IgG antibody, and detection of levels >10 IU/ml implies immunity following vaccination or infection before pregnancy. The diagnosis of infection is usually made by the detection of rubella-specific IgM. Although commercial assays are available, they vary in format, sensitivity and specificity. Furthermore, rubella-specific IgM may be present a year or more after natural infection or vaccination and after subclinial reinfection.[16] False-positive results may also be due to cross-reacting IgM antibodies or rheumatoid factor as in other IgM-based serologies, so the results of rubella IgM assays in pregnant women should always be confirmed in a reference laboratory and interpreted with caution. Any history of rash or contact, previous rubella immune status and history of vaccination should be taken into consideration. Also, measurement of rubella IgG avidity may help to determine the presence of primary infection, reinfection or approximate time of infection in an asymptomatic pregnant woman.[17]

Herpes simplex virus

Herpes simplex virus is a DNA virus belonging to the family *Herpesviridae*. Two biologically distinct serotypes have been identified - HSV-1 and HSV-2. The clinical manifestations of primary HSV-1 infection include gingivostomatitis, conjunctivitis, keratitis, herpetic whitlow and encephalitis. The classic presentation of primary HSV-2 infection is herpes genitalis, an infection characterized by the appearance of extensive, bilaterally distributed lesions in the genital area, by fever, inguinal lymphadenopathy and dysuria. Approximately 85% of cases of primary genital HSV are caused by HSV-2, with the remaining cases being caused by HSV-1. The acquisition of genital herpes during pregnancy has been associated with spontaneous abortion, prematurity, and congenital and neonatal herpes.The majority of neonatal infections occur during the peripartum period, but some also occur *in utero* or postnatally. Neonatal HSV infection is almost invariably symptomatic and frequently fatal. Babies may present with localized disease of the skin, eyes and mouth, encephalitis with or without skin involvement or disseminated disease. It has been reported that the seroprevalence of HSV in Indian pregnant women is 70%.[9]

There is considerable difference of opinion regarding routine screening of pregnant women for HSV. An estimated 35%-80% of infants with neonatal herpes are born to women with no known history of genital herpes or physical signs of infection at delivery. Therefore, the screening of asymptomatic pregnant women has the potential of identifying unrecognized active HSV infections.[18] To identify newly acquired infection in HSV-seronegative women, repeat testing in late pregnancy should be carried out. In those who are HSV-seropositive, a concern for transmission to the infant is likely to result in administration of antiviral drugs to the mother or in a caesarean section; the effectiveness of neither of which in controlling HSV transmission has been substantially demonstrated.[19] Though serological testing for HSV in the latter half of pregnancy could identify susceptible women, so that serological testing of their partners and appropriate counselling as to the risk of acquiring genital herpes could be undertaken, the effectiveness of such counselling has not been demonstrated.

Risk and cost-benefit analysis are needed to assess HSV type specific serological screening of pregnant women. After one such evaluation, it was concluded that screening for maternal type-specific HSV antibodies is not beneficial in preventing neonatal herpes.[20] Therefore, routine serological screening for genital herpes in asymptomatic pregnant women remains debatable. Viral culture is the most sensitive method for the laboratory diagnosis of HSV. It also allows typing of the viral isolate whereas no single seroassay is available that can reliably differentiate between HSV-1 and 2. Moreover, the above and below the navel concept of HSV-1 and 2 is also not absolute. An equal number of infections with HSV-1 below the navel have been documented.

TORCH testing in HIV-infected women

This issue remains confusing, even more so than it is in non-HIV infected gravidae. Patients who are newly diagnosed as being HIV infected and referred for prenatal care should be tested for the TORCH group of infections and *T. gondii*-specific IgG antibodies. If positive, test for IgM antibodies to rule out acute *T. gondii* infection should be carried out. Patients who test negative do not require further testing until after the pregnancy unless they are severely immunocompromised or show signs and symptoms of toxoplasmosis. IgG antibodies to CMV should be obtained in or HIV-infected gravida who is at high risk of CMV infection, i.e. patients with CD4+ counts less than 100/c- mm. There is no value in routinely testing prenatally for evidence of HSV infection.[21]

Conclusion

There have been tremendous advances in direct antigen detections and the sensitivity and specificity of assays detecting TORCH antibodies. It is the interpretation of the results in individual cases that need to be made carefully. The relatively poor degree of reliability can lead to unnecessary obstetric interventions or elective termination of pregnancy. Any positive pathogen-specific IgM in the maternal serum should be subjected to additional confirmatory testing in a reputed research laboratory before any intervention is carried out. No termination of pregnancy should be recommended only on the basis of a single antibody test. Antenatal diagnosis must be attempted by way of virus isolation or its antigen detection using molecular assays. Limitations of IgM serology by way of false-positive and negative results cannot be undermined; however, cord blood samples are preferred for IgM serology. At the same time, undue importance cannot be given to low-avidity IgG antibodies. Seroassays cannot be viewed in isolation, therefore, the results need to be correlated clinically to avoid their misinterpretation and minimize the anxiety of patients, especially if termination of pregnancy is being considered.

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Literature Review

Compiled by Dr. DDS Kulpati

Inhibitory effect of Nicotine on Experimental Hypersensitivity Pneumonitis (HP) on Vivo and in Vitro. *Blanchet, Mr, Assayag E1 and Cormier Y. Am J. Respir Crit Care Med 2004;169:903-909.*

The nicotine, which is a major component of cigarette smoke, has immunomodulatory and antifibrotic effects. In fact, it inhibits lymphocye proliferation, interleukin (IL), IB, tumour necrosis factor (TNF) IL-6 and IL-12 production by macrophages and fibroblast proliferation. Interestingly certain inflammatory diseases, such as sarcoidosis and ulcertative colitis are less frequent in smokers than in non-smokers and cigarette smoking protects against radiation pneumonitis. When exposed to an environment that can cause hypersensitivity pnecumouitis, smokers have lower level of specific antibodies to causative antigen. On the other hand when HP occurs in smokers, it promotes an insidious and more chronic form of the disease and worsens the clinical outcome.

In this study HP was inducted mice that were treated with nicotine either intraperitonally (IP) (0.5 to 2.0 mg/kg/day or intransasally (IN) (0.025 to 2.0 mg/kg/day) both IP & IN - treated animals had fever bronchoatveolar larae total cells and lymphocytes and a decreased lung-tissue inflammation IFN - but not interleukiv - 10-m RNA expression was reduced in lung tissual of 2.0 mg/kg. IN - treated animals. To test the effect of nicotine on alveaear macrphages, AMJ2-C11 cells were treated with nicotine and stimulated with LPS or saccha ro-polyspora rectivigula (SR) a causative agent of HP.

Nicotine reduced TNF release & TNF, interleukine-10, & IFNY-m RNA expression after stimulation and decreased CD-80 expression by 55% in LPS stimulated cells and by 41% in SR-stimulated cells. It was concluded that nicotine could be at least impart, responsible for the protection observed in smokers against HP. The inhibitory effect of nicotine on alveolare - macrophases could be one of the mechanisms involved.

The Bactericidal activity of Moxyfloxacin in patients with pulmonary tuberculosis. Gosline, RED, Viso, LO, San N E et al. *Am J Respir Crit Care Med. 2003;16:1342-40*

Flouroquinolines, which inhibit, DNA-gyrase, are highly active against, Myabacterim Tuberculosis, including strains resistant to first line drugs. The minimum inhibitory conc. of moxifloxacin, is four fold lower than that of levofloxacin. It has the greatest sterilizing activity. The combination of rifampin, pyrazinamide, and moxifloxacin had substantially greater sterilizing activity compared with the standard regimen.

In this study, patients in whom acid-fast bacilli smear-positive tuberculosis was newly diagnosed, were randamized to receive 400mg moxifloxacin, 300mg isoniazid, or 600mg rifampicin daily for 5 days. Bactericidal activity was estimated by the time taken to kill 50% of viable bacille (Vt 50) and the fall in sputum viable count during first 2 days designated as the early bactericidal activity (EBA). The mean Vt50 of moxifiloxacin was 0.88 days (95% confidence interval) and the EBA was 53 (95% CI). For the isoniazi group the (Vt50) was 0.40 days (95% CL) and the mean EBA was.77. For rifampin, the mean Vt50 was .71 days (85cl) and the mean EBA was .28 (5%CI). Using EBA method isonizid was significantly more effective than rifampin ($p<0.01$) but not moxifloxacin. Using Vt50 method isoniazid was more effective than both rifampin and moxifloxacin.

Moxifloxacin has an activity similar to rifampin in human subjects with pulmonary tuberculosis, suggesting that it should undergo further assessment as a part of a short course regimen for the treatment of drug resistant tuberculosis

JIMSA 2004: 17(3)

Endocarditis: A clinical microbiologist's viewpoint

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Abstract: Endocarditis is an infective condition that poses a major challenge to cardiologists, cardiac surgeons, infectious disease physicians and microbiologists alike. Despite substantial improvements in the diagnosis and management of this infective condition, it is still associated with a significant morbidity and mortality. This article reviews the epidemiological features, clinical presentation, diagnostic methodology, microbiology, treatment and prevention of infective endocarditis and highlights the differences seen between India and the West. An attempt has been made to cover the relevant aspects of this not so infrequent infective condition that is associated with a considerable morbidity and mortality.

Epidemilogical Features

The incidence of infective endocarditis (IE) is difficult to determine, yet most western studies place it at approximately 2-6 per 100 000 person-years. However, in India there is a complete lack of data regarding the exact incidence of this disease.

In the West, the mean age of patients with IE has gradually increased, *the median age* was 30-40 years during the pre-antibiotic era and is 47-69 years now.[1,2] Among patients with endocarditis associated with injection-drug use, there is a trend towards younger people being affected. Though studies are few, in India the young population is mainly affected by IE, the mean age being 24 years with 90% aged <40 years as seen in a large referral hospital in northern India.[3] In India, as in the West, males are more often affected than females.[3,7] Whether they are adults or children, the male to female ratio is 2.5:1 in India versus an average of 1.7:1 in the West.

In India, rheumatic heart disease has been found to be the most frequent underlying heart lesion in IE patients (even up to 60%).[3,5] This again is in contrast to that seen in the West, where mitral valve prolapse is the commonest cardiovascular abnormality predisposing to IE.[6] Congenital heart disease ranks second with ranges from 27.3% to 33% seen in various Indian studies.[3] Intravenous drug abuse is another important predisposing condition in the West but is rare in the Indian setting.[8,9]

Clinical Manifestations

Fever is the most common presentation but may be absent (5% of the cases) or minimal in several situations, especially in *the setting of* congestive heart failure, chronic renal or liver failure, severe debility, previous antimicrobial therapy or IE caused by less virulent organisms.[6] Persistent fever during antimicrobial therapys though relatively infrequent, is an ominous sign. Prolonged fever (≥2 weeks duration) is associated with the following:

i) Specific aetiologic agents-*Staphylococcus aureus*, Gram-negative bacilli, fungi, culture-negative endocarditis; and
ii) Microvascular phenomena, embolization of major vessels, intracardiac (e.g. myocardial) abscess, peripheral complications, tissue infarction, pulmonary emboli, drug reactions, a need for cardiac surgery and *a* higher mortality rate.

The clinical features of prosthetic valve endocarditis (PVE) are essentially similar to those of native valve endocarditis (NVE). However, there is a higher frequency of new or changing regurgitant murmurs, congestive heart failure, persistent fever in spite of optimal antimicrobial therapy, and new electrocardiographic conduction disturbances, than those seen in NVE. Nosocomial infective endocarditis, on the other hand, usually has an acute onset and signs of endocarditis are infrequent.

Diagnosis

The diagnosis of IE is based on a combination of clinical, laboratory and echocardiographic data. Non-specific laboratory parameters may be abnormal but none is diagnostic. Anaemia, leucocytosis, thrombocytopenia, abnormal urinalysis results; *and an* elevated erythrocyte sedimentation rate and C-reactive protein level may all be present.

Over the years, various diagnostic criteria for IE have been proposed, such as the Beth Israel criteria in 1982 and the Duke criteria in 1994.[10] These criteria combine factors predisposing patients to the development of IE, the blood culture isolate and persistence of bacteraemia and echocardiographic findings along with other clinical and laboratory information. A modified version of the Duke criteria has recently been proposed (Table 1).[11]

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Table 1. Proposed criteria for the diagnosis of infective endocarditis

Criteria	Description
Major criteria a) Microbiological	Typical microorganism isolated from two separate blood cultures: viridans streptococci, *Streptococcus bovis*, HACEK group, *Staphylococcus aureus*, or community-acquired enterococcal bacteraemia without a primary focus or Microorganism consistent with IE isolated from persistently positive blood cultures or Single positive blood culture for *Coxiella burnetii* or phase I IgG antibody titre to *C. burnetii* >1:800
(b) Evidence of endocardial involvement	New valvular regurgitation (increase or change in pre-existing murmur not sufficient) or Positive echocardiogram (transoesophageal echocardiogram recommended in patients who have a prosthetic valve, who are rated as having at least possible IE by clinical criteria, or who have complicated IE)
Minor criteria	Predisposition to IE that includes certain cardiac conditions and injection-drug use Fever >38°C (100.4° F) Vascular phenomena Immunological phenomena Microbiological findings
Diagnosis	Definite endocarditis Two major criteria, or One major and three minor criteria, or Five minor criteria Possible endocarditis One major and one minor criteria, or *Three* minor criteria

HACEK: *Haemophilus* species (*H. parainfluenzae, H. aphrophilus*, and *H. paraphrophilus*), *Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens* and *Kingella kingae.*

Blood culture

The single most important laboratory test of diagnostic significance to both the clinician and the clinical microbiologist is the blood culture.[12] The site chosen for venepuncture is important. Femoral sites or sites with dermatological disease will often yield a higher rate of contamination. It must be remembered that arterial blood does not provide a higher yield than venous blood.

Adequate cleansing of the selected site is done with 70% isopropyl or ethyl alcohol applied concentrically, starting at the centre. The skin should be allowed to dry. This is followed by a second cleansing with an iodophor or tincture of iodine over the same area. The disinfectant should be allowed to dry completely. In iodine allergy, a double application of alcohol should be used for skin disinfection. Sterile gloves should be used throughout the procedure. Blood cultures should be obtained preferably by fresh venepunctures, not through indwelling intravascular devices to minimize contamination. Changing needles after venepuncture and before inoculation of blood into the culture media has not shown decrease in the rate of contamination.

It is believed that the best time to obtain blood for culture is just before the onset of chills or fever spikes. However, the continuous nature of most bacteraemia in IE renders timing less important.[13]

In acute endocarditis, two to three blood samples should be obtained quickly within 5 minutes of each other prior to starting antibiotic therapy. On the other hand, in subacute endocarditis, several (3 or more) blood samples should be obtained 30 minutes to 1 hour apart over several (24) hours, to establish a specific and conclusive microbiological diagnosis. Multiple blood cultures are necessary to demonstrate the presence of continuous bacteraemia, differentiate between contamination and true bacteraemia, and to know if the patient has received antibiotics in the preceding 2 weeks.

At least 10 ml (in adults) and 1-5 ml (in infants and children) of blood should be cultured in more than one type of blood culture medium, e.g. 1 in trypticase soy (or brain-heart infusion) broth and 1 in thioglycolate broth, to increase the chances of recovery of certain microorganisms. The new commercial media are also effective with the additional ability to counteract or neutralize the inhibitory effect of antibiotics (BACTEC or BacT Alert). If there is a high suspicion of IE due to anaerobes, then one anaerobic bottle should be included among the total 2-6 bottles inoculated from the samples drawn.

Inspection for macroscopic growth should be done daily and routine subcultures done on days 1, 3 and 5. Modern commercial blood culture systems demonstrate recovery of clinically significant microorganisms within 5 days. The older manual blood culture systems required at least 2-3 weeks of incubation. However, it is probably wiser to incubate these bottles for 3 weeks so as to maximize the chances of positive results.

Blood cultures may be negative in 2.5%-31% of cases, i.e. the so-called blood culture-negative IE. Previous antibiotic therapy, right-sided, mural or prosthetic valve endocarditis and slow-growing or fastidious organisms are responsible for most of these cases.

If blood cultures from patients remain negative after 5-7 days of incubation, one must consider prolonged incubation and the plating of subcultures on a more enriched medium.

ECHOCARDIOGRAPHY

Echocardiograms have correctly identified vegetations on all valves even in culture-negative cases. However, transthoracic echocardiography (TTE) may be inadequate in up to 20% of adult patients owing to obesity, chronic obstructive pulmonary disease or chest wall deformities; the overall

sensitivity is variable (<50% to >90% positive). It is of great value in assessing local complications of IE, especially those surrounding the aortic valve. Transoesophageal echocardiography is more sensitive and cost-effective than conventional TTE in the detection of intracardiac vegetations (approximately 95% and 60%-65%, respectively), particularly in the setting of prosthetic valves.

Microbiology

Many studies have shown that streptococci, especially viridans streptococci, are responsible for the largest proportion of cases.[4,5,7,14] Other studies have found staphylococci to be more frequent.[3,6] Which of these is more frequent depends on whether native valve endocarditis occurs in addicts or non-addicts. A similar situation exists in early versus late PVE.[15]

Endocarditis due to enteric Gram-negative bacteria is uncommon though the incidence is increasing: even in India. *Salmonella*, *Enterobacter*, *Citrobacter*, *Escherichia coli, Klebsiella*, *Serratia marcescens, Proteus*, *Pseudomonas*, and *Providencia*: have all been implicated.[3,8,9] Persistent bacteraemia is common even with high levels of antimicrobial activity and the prognosis is poor. In the early postoperative period after prosthetic valve replacement, sustained Gram-negative bacillary bacteraemia does not necessarily imply IE, and other foci of infection (sternal wound, pneumonia, urinary tract, intravenous catheters, etc.) should be carefully sought. Bacteraemia persisting for days before treatment or for ≥72 hours after the removal of an infected catheter and initiation of treatment, especially in patients with abnormal heart valves or prosthetic valves, suggests the development of nosocomial infective endocarditis.

Other Gram-negative bacteria such as *Neisseria*, *Haemophilus*, *Actinobacillus* spp. *Cardiobacterium* spp., *Eikenella* spp. and *Kingella* spp. have also been implicated in IE. Gram-positive bacilli *(Corynebacterium)* and anaerobic organisms, though not common, have also been reported. Fungal endocarditis is common in patients who have received prolonged antibiotic therapy, are on indwelling intravascular devices, or have undergone reconstructive cardiovascular surgery.[14,15] Narcotic addicts are also prone to this form of endocarditis. Many species of *Candida* and *Aspergillus* have been implicated.[15] These cases are usually difficult to treat and the prognosis is frequently poor.

Table 2 lists some common pathogens causing IE in various clinical situations and Table 3 lists the common pathogens found in various categories of patients in decreasing order of importance.

Table 2. Common pathogens in various clinical situations

Common pathogens	Clinical situations
Staphylococcus aureus	Intravenous drug abuse, infected intravascular catheter
Enterococci	Manipulation of genitourinary tract
Streptococcus bovis	Elderly, gastrointestinal malignancy and colonic polyps
Streptococcus mutans	Dental caries
Streptococcus pneumoniae	Alcoholics
Streptococcus mitis	Drug addicts
Gram-negative bacilli	Drug addicts, prosthetic valve recipients and patients with cirrhosis
HACEK	Pre-existing valvular disease, drug addicts and dental procedures
Fungi	Drug addicts, reconstructive cardiovascular surgery, prolonged intravenous or antibiotic therapy

Table 3. Common pathogens in various categories of patients

Category	Common pathogens
Neonates	*Staphylococcus aureus*, CNS, group B streptococci, occasionally GNB
Older children	Streptococci, *S. aureus*
IVDA	*Staphylococcus aureus*, *Pseudomonas*, other GNB, HACEK group, fungi
NVE, PVE >12 months after surgery	MSSA, streptococci, GNB, fungi
PVE <2 months, between 2-12 months after surgery	CNS, MRSA, GNB
Nosocomial endocarditis	*Staphylococcus aureus*, CNS, enterococci, streptococci, *Candida* spp., GNB

CNS: Coagulase-negative staphylococci; NVE: native value endocarditis; PVE: prosthetic valve endocarditis; MSSA: methicillin-sensitive *Staph.* aureus; MRSA: methicillin - resistant *Staph* aureus HACEK (See table1.)

Prophylaxis

The American Heart Association (AHA) has formulated extensive guidelines for the prevention of bacterial endocarditis.[16] Though definitive data are lacking, of the various preventive measures that could be employed, appropriate administration of antibiotics before procedures expected to produce bacteraemia has received the *most* attention. The AHA lists the procedures requiring or not requiring endocarditis prophylaxis. Specific recommendations for the use of antibiotics for prophylaxis are also included and can be obtained from standard references.[16]

Compliations

The complications of endocarditis are well known. These may be cardiac, embolic, neurological or renal. The most prevalent complications are congestive heart failure, paravalvular abscess formation and embolism (especially stroke). In addition, endocarditis may be complicated by septic arthritis, vertebral osteomyelitis, pericarditis, metastatic abscesses, and an array of renal problems ranging from immune complex glomerulonephritis to renal abscesses. Further, complications as a result of medical treatment of endocarditis can result in ototoxicity and nephrotoxicity, skin rashes and serum sickness.[17]

Table 4. Usual antimicrobial therapy for various causes of infective endocarditis

Pathogen	Native valve endocarditis (NVE)		Prosthetic valve endocarditis (PVE)	
	Antimicrobial therapy	Comments	Antimicrobial therapy	Comments
Penicillin susceptible viridans Streptococci, *Streptococcus bovis,* and other streptococci (MIC of penicillin ≤ 0.1 µg/ml)	Penicillin G 10-20 million units i.v./day in 6 equally divided doses for 4 weeks or Ceftriaxone 1 g i.v. q 12 hour for 4 weeks If immediate hypersensitivity to beta-lactam antibiotics use Vancomycin 30 mg/kg/day i.v. (total dose <2 g/day)	2 week regimen of Penicillin G (or Ceftriaxone) in the doses as mentioned with Gentamicin 1 mg/kg i.m. or i.v. is optional. Exceptions– myocardial abscess, extracardiac foci of infection, PVE	Penicillin G 10-20 million units i.v./day for 6 weeks and Gentamicin 1 mg/kg i.m. or i.v. for 2 weeks If immediate hypersensitivity to beta-lactam antibiotics use Vancomycin 30mg/kg/day i.v. (total dose ≤ 2g/day)	Shorter duration of treatment with an aminoglycoside (2 weeks) is usually appropriate for PVE due to Penicillin susceptible viridans Streptococci, S. bovis, or other streptococci with MIC of Penicillin ≤ 0.1 µg/ml
Relatively penicillin-resistant streptococci (MIC of penicillin >0.1-0.5 µg/ml)	Penicillin G 20 million units i.v./day in 6 equally divided doses for 4 weeks and Gentamicin 1 mg/kg i.m. or i.v. for 2 weeks		Penicillin G 20 million units i.v./ day in 6 equally divided doses for 6 weeks and Gentamicin 1 mg/kg i.m. or i.v. for 4 weeks	
Streptococcus species (MIC of penicillin >0.5 µg/ml), *Enterococcus* species, or *Abiotrophia* species	Penicillin G 20-30 million units i.v./day for 4-6 weeks or Ampicillin 12 g i.v. in 6 equally divided doses/day for 4-6 weeks with Gentamicin 1 mg/kg i.m. or i.v. (single dose of 80 mg) q 8 hour for 4-6 weeks	Six weeks of therapy is recommended for patients with symptoms lasting longer than 3 months, myocardial abscess, or selected other complications	Penicillin G 20-30 million units i.v./day for 6 weeks or Ampicillin* 12 g i.v. in 6 equally divided doses/day for 6 weeks with Gentamicin 1 mg/kg/ i.m. or i.v. (single dose of 80 mg) q 8 hours for 6 weeks.	
Methicillin-susceptible staphylococci	Cloxacillin 2 g i.v. q 4 hour for 4-6 weeks with or without Gentamicin 1 mg/kg/i.m. or i.v. (single dose ≤ 80 mg) q 8 hour for 3-5 days of therapy	In the few patients infected with a Pencillin susceptible *Staphylococcus*, Penicillin G may be used instead of Cloxacillin	Cloxacillin 2 g i.v. q 4 hour for ≥6 weeks with Rifampicin 300 mg orally q 8 hour for ≥6 weeks with Gentamicin 1mg/kg i.m. or i.v. (single dose ≤80 mg) q 8 hour for 2 weeks	Wise to delay initiation of Rifampicin for 1 or 2 days, until therapy with two other effective antistaphylococcal drugs has been initiated
Methicillin-resistant staphylococci	Vancomycin 30 mg/kg/day i.v. (total dose ≤2g/day) for 4-6 weeks with or without Gentamicin 1 mg/kg i.m. or i.v. (single dose ≤80 mg) q 8 hour for first 3-5 days of therapy		Vancomycin 30 mg/kg/day i.v. (total dose ≤2 g/day) for ≥6 weeks with Rifampicin 300 mg orally q 8 hour for ≥6 weeks and Gentamicin 1 mg/kg/i.m. or i.v. (single dose ≤80 mg) q 8 hour for 2 weeks	*Staphylococcus,* resistant to Gentamicin, an alternative third agent should be chosen on the basis of *in vitro* susceptibility testing
Right-sided staphylococcal NVE in selected patients	Cloxacillin 2 g i.v. q 4 hour for 2 weeks with Gentamicin 1 mg/kg/i.m. or i.v. (single dose <80 mg) q 8 hour for 2 weeks	Exclusions to short course therapy include any associated cardiac or extracardiac complications, persistence of fever for ≥7 days, infection with HIV and probably patients with vegetations greater that 1-2 cm according to echocardiography		
HACEK organisms	Ampicillin 2 g i.v. q 4 hour or Ceftriaxone 1 g i.v. q 12 hour for 4 weeks with Gentamicin 1 mg/kg i.v. q 8 hour (peak serum level 4-6 µg/ml) for 4 weeks		Ampicillin 2 g i.v. q 4 hour or ceftriaxone 1 g i.v. q 12 hour for >6 weeks with Gentamicin 1 mg/kg i.v. q 8 hour (peak serum level 4-6 µg/ml) for ≥6 weeks	
Gram-negative bacilli (some examples) *Pseudomonas*	Piperacillin (3 g i.v. q 4 hour), Ceftazidime (2 g i.v. q 8 hour) or Imipenem (0.5–1 g i.v. q 6 hour) and Tobramycin 1.7 mg/kg q 8 hour for 6 weeks	Further changes according to antimicrobial susceptibility. Always give the maximum permissible dose	Piperacillin (3 g i.v. q 4 hour), Ceftazidime (2g i.v. q 8 hour) or imipenem (0.5 – 1 g i.v. q 6 hour) and Tobramycin 1.7 mg /kg q 8 hour for 6 weeks	Further changes according to antimicrobial susceptibility. Always give the maximum permissible dose
Enterobacteriaceae	Cefotaxime (2 g i.v. q 4 hour) or Imipenem ((0.5–1 g i.v. q 6 hour) or Aztreonam (2 g i.v. q 6 hour) + Gentamicin 1mg/kg i.m. or i.v. q 8 hour		Cefotaxime (2 g i.v. q 4 hour) or Imipenem ((0.5 – 1 g IV q 6 hour) or Aztreonam (2 g i.v. q 6 hour) + Gentamicin 1mg /kg i.m. or i.v. q 8 hours	
Fungal	Amphotericin B 1mg/kg/day i.v. (total dose 2.0-2.5g) for 6-8 weeks with or without Flucytosine 150 mg/kg/day orally in 4 divided doses for 6-8 weeks		Amphotericin B 1 mg/kg/day i.v. (total dose 2.0 –2.5 g) for 6-8 weeks with or without Flucytosine 150 mg/kg/day orally in 4 divided doses for 6-8 weeks	
Culture-negative	Penicillin 20-30 million units i.v./day/Ampicillin 12 g i.v. in 6 equally divided doses/day or Vancomycin 30 mg/kg/day i.v. (total dose ≤2 g/day) and Gentamicin 1 mg/kg i.m. or i.v. (single dose ≤80 mg) q8 hours	This empirical therapy should be guided by clinical response. Duration of therapy is 4-6 weeks for NVE, 6-8 weeks for PVE. If fungal endocarditis is suspected, treat accordingly	Vancomycin 30 mg/kg/day i.v. (total dose ≤ 2g/d/day) + Gentamicin 1 mg/kg i.m. or i.v.(single dose ≤80 mg) q 8 hour + Ceftriaxone 1g i.v. q 12 hour/Cefotaxime 2g i.v. q 4 hour	If endocarditis 12 months after valve replacement, Ceftriaxone/Cefotaxime for 4-6 weeks for NVE and 6-8 weeks for PVE. If fungal endocarditis is suspected, treat accordingly

Adapted from Mylonakis E, Calderwood SB[6] and Bansal RC[15]
Note: Antibiotic doses should be modified appropriately in patients with renal or hepatic dysfunctions
HIV : Human immunodeficiency virus

CARDIAC COMPLICATIONS

Congestive heart failure is the most common cause of death and the most compelling indication for surgery in patients with endocarditis. The usual cause of heart failure in patients with endocarditis is valvular insufficiency caused by infection-induced valvular destruction. Fragments of valvular vegetations may occasionally embolize into the coronary arteries and cause acute myocardial infarction and subsequent heart failure. Patients with aortic valve endocarditis are at risk for *rapidly developing* progressive heart failure or pulmonary oedema and require emergency surgery. Congestive heart failure is less frequent in patients with mitral valve endocarditis.

Paravalvular abscesses occur in patients with PVE, more commonly in patients with mechanical PVE than with bioprosthetic valve endocarditis. In NVE, paravalvular abscesses are more likely to form fistulae or aneurysms in patients with mitral valve involvement than with the aortic valve. However, abscess formation is associated with death rates >75% unless surgical intervention is carried out.

NEUROLOGICAL COMPLICATIONS

Strokes following embolic events contribute significantly to mortality and long-term sequelae due to IE. The rate of embolic events decreases rapidly after the initiation of effective antibiotic therapy.

Intracranial mycotic aneurysms most commonly involve the middle cerebral arteries, especially distally. A mycotic aneurysm complicating endocarditis can present as stroke or subarachnoid haemorrhage. Some aneurysms leak slowly before rupture and produce headache and mild meningeal irritation in some patients, whereas in others there are no premonitory signs or symptoms before sudden intracranial haemorrhage.

EMBOLIC COMPLICATIONS

Systemic emboli, besides involving the central nervous system, most commonly involve the spleen, kidney, liver and the iliac or mesenteric arteries. Splenic as well as other metastatic abscesses can be a cause of prolonged fever, and splenomegaly may be absent.

RENAL COMPLICATIONS

Renal complications are particularly frequent in patients with IE due to *Staph.aureus*. They may manifest as haematoma, glomerulonephiutis or renal infarction, and are due to embolic and immune complex-mediated processes.

Treatment

ANTIMICROBIAL THERAPY

Successful therapy involves prolonged parenteral administration of bactericidal antimicrobial agent(s) with specific activity against the causative organism (Table 4). Delay in therapy can result in further valvular damage and abscess formation. However, if the patient is haemodynamically stable as in subacute endocarditis, it is safe to wait for 2-3 days or more to ensure adequate collection of blood for isolation of the causative organisms. Both minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of different possible antibiotics to the organism should be determined.

The causative organism should be retained for future studies and the need to revise therapy. After initiation of therapy, blood cultures should be repeated during the course of treatment till they are negative. If blood cultures are persistently positive, this may necessitate further action-changing the dose of the antibiotic, adding another antibiotic or changing it, or even considering surgery. Other laboratory tests, including complete blood count, serum creatinine and liver enzyme levels should also be carried out during the course of therapy.

SURGICAL THERAPY

Congestive heart failure is the strongest indication for surgery in.[6] Infection that is refractory to medical therapy, i.e. lack of improvement inspite of more than 1 week of antibiotics or lack of blood sterility within a week also needs surgical attention.[13] Surgery is also indicated if endocarditis is due to certain pathogens such as *Pseudomonas aeruginosa, Brucella* spp., *Coxiella burnetii*, *Candida* spp., other fungi and probably enterococci.

In PVE, if there is an early-onset, progressive infection, haemodynamic deterioration or a relapse after adequate medical therapy, then these cases need surgical evaluation. Antimicrobial treatment alone is indicated in late-onset PVE (>12 months after implantation of a prosthesis), infection by viridans streptococci, HACEK organisms or enterococci, with no evidence of perivalvular extension of infection.

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IMSA News

IMSA Chapter Activities (July to Sept 2004) :

Delhi Chapter

25.08.2004 : Lt. Col. Dr. A.K. Singhal, Dr. B.K. Dkaun *'Osteo Arthitis'.*

16.09.2004 : Dr. S.M. Kaul *'Malaria and Dengue'.*

24.09.2004 : Dr. Neeru Aggarwal *'Recurrent Urinary Tract Infection in Women'.*

: Dr. Ashok Kumar *'Basti Sevika as 'Ambassador of Health'.*

26.09.2004 : Dr. H.K. Chopra, Dr. I.P.S. Kalra *'Spirituality and Medicine'.*

Tamil Naidu Chapter

10.07.2004 : Dr. Bagyam Raghavan *'Imaging in Breast Disease - A Multimodality Appraoch'.*

08.08.2004 : Dr. Palanisamy *'Treatment of Heart Failure'.*

12.09.2004 : Dr. Manoharan *'Surgical Aspects of Tetrology of Fallots'.*

Election of Fellows / Members

Fellows elected on 11-7-2004

Dr. Chella David PHD (Honorary) *USA*
Dr. Nicole Sandhu *USA*
Dr. Gurpreet Sandhu *USA*
Dr. M. Duggirala *USA*
Dr. Roshni Abraham *USA*
Dr. Suresh Kotagal *USA*
Dr. sareth Pinnamaneni *New York*
Dr. Sridevi Pinnamanent *New York*
Dr. Anupama Sharma *USA (Florida)*
Dr. Sheetal Singh *Jammu Tawi, India*
Dr. Kirti Sure Verma *New Delhi*
Dr. N. Vetrivel *Chennai*
Dr. M. Wali *New Delhi*

Members elected on 11-7-2004

Dr. Ashok Kumar Patel *USA*
Dr. Kartik Ghosh *USA*
Dr. Amit Ghosh *USA*
Dr. Charanjit Rihal *USA*
Dr. Nisha Manek *USA*
Dr. Jaswinder Singh *USA*
Dr. Baljeet Kaur *USA*

Members elected on 25-8-2004

Dr. A. Radhakrishna *Tamil Nadu*
Dr. C.S. Balachandran *Tamil Nadu*
Dr. V. Anandi *Tamil Nadu*
Dr. P.T. Thamizharasu *Pondicherry*
Dr. S. Murugan *Chidambaram*
Dr. A.R. Annamalai *Chidambaram*
Dr. A.R. Annamalai *Chidambaram Tamil Nadu*
Dr. P. Viswanathan *(Tiruchi) Tamil Nadu*
Dr. Chitturi Surya Prakash *Tamil Nadu*
Dr. D. Rajakumar *Tamil Nadu*
Dr. G.N. Pitchchaiya *Chennai*
Dr. (Col) M.A.S. Warrier *Chennai*
Dr. Murugesan *Tamil Nadu*
Dr. R. Muthukumaran *Tamil Nadu*
Dr. Shashi Kant Singla *New Delhi*

Fellows elected on 25-8-2004

Dr. Parijat Chandra *New Delhi*

IMSACON-2005

Venue : JAIPUR (Rajasthan) **Dates :** 23-10-2005 to 25-10-2005 **Theme :** EMERGING HEALTH CHALLENGES

Please send your papers to Dr. S. Panicker, Organising Secretary or Dr. I.P.S. Kalra, Secretary General, IMSA. Kindly keep these dates free for attending the **Conference** with your spouse and children. For details please contact:

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Obituary

Dr. Pratibha Ranjan Dutt born on March 25 1911 in Kolkata completed his MMBS in 1936 earning a Distinction in the field of Mid-wifery; he obtained his Diploma in Public Health from Calcutta in 1941. He began his service career with the Medical Corps of the Indian Army He joined the Directorate General of Health services in 1954 and retired as Deputy Director General Health Services (Public Health), Government of India in 1969. He was then with the NIHAE for two years; later joined the Grndhigram Institute of Health and Family Welfare as its Director and was on its Board of Trustees.

He became a fellow of the International Medical Sciences Academy in 1987. He has written several books in the field of Primary health Care and his last book was in three volumes in 1996 which he dedicated to the Gandhigram Institute of Health and Family Welfare in Tamil Nadu. Dr. P.R. Dutt dedicated his life to the Rural Health Services in India and was deeply concerned with the welfare of the rural women and children.

JIMSA 2004: 17(3)

Genitourinary Tuberculosis: Diagnostic challenges and therapy

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Abstract: Urinary tract infections are among the commonest bacterial infections. Genitourinary tuberculosis must be suspected in patients with long-standing urinary symptoms and sterile pyuria. There are myriad presentations of this disease and there is intermittent shedding of tubercle bacilli in the urine in small numbers. Diagnosis by staining for acid-fast bacilli and mycobacterial culture may not always be rewarding. The use of molecular assays, computed tomography and magnetic resonance imaging may aid in the diagnosis. Treatment with antituberculous drugs is usually effective; however, some patients may require surgery.

Introduction

Urinary tract infections (UTI) are *among* the commonest bacterial infections encountered worldwide. Approximately 10% of humans have UTI at some time during their lives.[1] UTI is an inflammatory response of the urothelium to bacterial invasion, which is usually associated with bacteriuria (bacteria in the urine) and pyuria (presence of eight or more leucocytes cmm on microscopic examination of uncentrifuged urine). Depending upon the anatomical location of the infection, these can be either an upper UTI (ureter and kidney) or lower UTI (bladder, urethra, prostate and epididymis). UTI can also be classified as uncomplicated or complicated. Uncomplicated infections occur in healthy females and occasionally in infants and young males without any structural or neurological dysfunction. They readily respond to antibiotics to which the aetiological agent is susceptible. Complicated infections occur in both the sexes, who may have certain risk factors including underlying disease (diabetes, sickle cell anaemia), stones in the kidney structural or functional abnormalities of the urinary tract and indwelling urinary catheters. Complicated infections are more difficult to treat and have a greater morbidity and mortality than uncomplicated ones.

Types of urinary tract infections

There are four major types of UTI: urethritis, cystitis, acute urethral syndrome and pyelonephritis.

Urethritis : The symptoms include dysuria and frequency. Common causative organisms include *Chlamydia trachomatis, Neisseria gonorrhoeae* and *Trichomonas vaginalis*.

Cystitis : Patients with cystitis present with dysuria, frequency, urgency and suprapubic pain. There may be tenderness over the bladder area due to mucosal inflammation of the bladder. Systemic signs are usually not present.

Acute urethral syndrome : Patients with this syndrome are young, sexually active women, who experience dysuria, frequency and urgency, but yield fewer than 10^5 colony forming units/ml in their urine. Most of these patients have pyuria.

Pyelonephritis : Infection of the kidney parenchyma, pelvis, calyces, usually caused by bacterial infection, is called pyelonephritis. The clinical presentation includes fever, flank pain, frequency, urgency and dysuria. Systemic signs of vomiting, diarrhoea, chills, tachycardia and abdominal pain may be present. Forty per cent of these patients will be bacteraemic.

Patients with vague, long-standing urinary symptoms and sterile pyuria for which there is no obvious cause should be suspected to have genitourinary tuberculosis (GUTB).

GENITOURINARY TUBERCULOSIS

Tuberculosis (TB) has been observed in humans for more than 7000 years and continues to remain one of the world's most deadly infectious diseases. The World Health Organization (WHO) has declared TB to be a global emergency with approximately 3 million people dying each year, the majority of whom live in the developing countries.[2] In the western world, GUTB develops in 8%-10% of patients with pulmonary TB, compared with 15%-20% in developing countries.[3] GUTB is estimated to comprise almost 30% of non pulmonary TB.[4] The incidence of female genital TB is almost 10.3% in India.[5]

PATHOGENESIS

During the initial primary pulmonary infection, the microorganism *Mycobacterium tuberculosis* multiplies and evokes an inflammatory reaction. Progressive renal TB results from haematogenous seeding of the renal cortex to form small caseous foci that spread to the medulla, and a progressive caseous ulcerative lesion develops. Involvement of the adjacent renal papillae follows and spread to the lower urinary tract ensues.[6] The healing process results in fibrous tissue and deposition of calcium salts. The fibrous tissue involving the drainage system may cause strictures in the calyceal system or at the *pelviureteral* junction (PUJ) or ureter. Bladder

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involvement is usually secondary to renal TB. Infection starts around one or another ureteral orifice with inflammation. The inflammation is replaced by fibrosis that starts around the orifice, which contracts and can produce a stricture or become withdrawn and rigid, assuming a golf hole appearance. TB of the testis, epididymis, penis, scrotum and prostate is almost always secondary to infection of the kidney.

In women with genital TB, the fallopian tube is involved in more than 90% of cases. Endometrial involvement is secondary to tubal involvement.[7] The ovaries, cervix, vulva and vagina may also be involved.

CLINICAL FEATURES

The predisposing factors GUTB include immune suppression, exposure to an active case of TB, diabetes mellitus, chronic renal failure, malignancy and organ transplantation. Males are more often affected than females (male to female ratio 2:1). The manifestations of GUTB can be variable (Table 1).[8]

Table 1. Clinical manifestations of GUTB.

Organs involved	Clinical manifestations
Kidney	Frequency, pyuria, haematuria, colic, renal failure, flank pain
Ureters	Ureteral colic
Urinary bladder	Pyuria, frequency, dysuria, urgency, suprapubic pain
Prostate and urethra	Urethritis, prostatitis
Male and female reproductive system	Infertility
Penis	Penile ulcers
Scrotum	Scrotal mass
Female genital tract	Menstrual disturbances: menorrhagia or secondary amenorrhoea, pelvic pain, vaginal discharge and abdominal masses

DIAGNOSIS

Microscopic examination of the urine usually reveals pyuria and red blood cells. Sterile pyuria is the classic urinary finding. Secondary bacterial infection is seen in 20% of cases and may lead to misdiagnosis.

Mycobacteria are shed intermittently in the urine and are present in small numbers. Hence, it is essential to evaluate 3-5 early whole morning urine samples. After thorough cleaning of the genitalia, the first early morning whole sample of the urine should be collected in sterile containers. Each specimen of urine should be inoculated as soon as possible after collection, because the longer the urine remains in contact with organisms, the less likely they are to remain viable.[9] Urine samples from each kidney can be separately collected by ureteric catheterization, which helps localize the infection. A minimum of 50 to 100 ml of whole morning urine is centrifuged (4000 *g* for 30 min), and the deposit is used for staining and culture. A prostatic massage may increase the yield of acid fast bacilli (AFB) in the seminal fluid. Uterine curettage, cervical biopsy or laparoscopic biopsy is used for the diagnosis of female genital TB. Endometrial curetting is performed shortly before menstruation.

The two commonly used stains are the Ziehl-Neelsen and Auramine-Rhodamine stains, the latter being a more sensitive[10] and valuable technique (Table 2).

Table 2. Comparison of Zich-Neelsen stain and direct fluorescence[11]

Sample	Positivity (%)	Direct fluorescence positivity (%)
Respiratory samples	60	74
Pus, tissue	43	87
Urine	40	48
Overall positivity	50	70

CULTURE

Culture still remains the 'gold standard' for the diagnosis of mycobacterial infections. This is more sensitive than staining techniques, and allows for exact identification of the mycobacterial species and its sensitivity pattern. The urine sediment obtained after centrifugation is first decontaminated with 4% sodium hydroxide and then inoculated on the desired culture media (Table 3). For maximal recovery, one liquid and one solid media are recommended. Conventional culture techniques take 4-6 weeks for growth while the automated methods take 10-14 days. In general, the rates of recovery of mycobacteria do not differ considerably among the different automated systems and are higher for solid media.[12]

Table 3. Conventional and automated culture methods

Conventional Methods		
Egg-based solid media	Lowenstein Jensen medium	
Agar-based media	Middlebrook 7H10, 7H11 medium	
Broth media	Middlebrook 7H9, Dubos Tween albumin broth	
Automated/semiautomated		
Biphasic medium	Septichek system	Becton-Dickinson
Radiometric	BACTEC 460 system	Becton-Dickinson
Non-radiometric	MB/BacT system	BioMerieux
Fluorescence	MGIT, BACTEC 9000MB	Becton-Dickinson

Due to the paucibacillary nature of the disease, smears are often negative and cultures are positive in 10%-90% of the patients.[8] In a 10-year retrospective study by Mortier *et al.*[13], 7200 midstream morning urine specimens obtained from 2814 patients with a presumptive diagnosis of tuberculosis were inoculated onto Lowenstein-Jensen medium. Only 33 patients (1.2%) yielded positive cultures for *M. tuberculosis*. Of these 33

patients, 22 presented with pulmonary tuberculosis and 7 patients had tuberculosis limited to the genitourinary tract as diagnosed by clinical signs and symptoms.

IDENTIFICATION

It is important to differentiate organisms belonging to the *M. tuberculosis* complex from non-tuberculous mycobacteria, since the treatment modalities differ between these two groups. Species identification is done by conventional biochemical tests.[14] The NAP (p-nitro–α-acetylamino–b-hydroxy propiophenone) test, high-performance liquid chromatography, gas liquid chromatography and nucleic acid probes (Accuprobe- Genprobe) can be used to identify isolates from culture.

MOLECULAR METHODS

Nucleic acid assays (NAA) include the *Mycobacterium tuberculosis* direct (MTD) test, AMPLICOR MTB assay, nucleic acid sequence based assay (NASBA), transcription-mediated assay (TMA), strand displacement assay (SDA), and the ligase chain reaction (LCR). However, two NAA tests, the MTD (Genprobe BioMerieux) and AMPLICOR (Roche), have been cleared by the US Food and Drug Administration (FDA) for direct use in smear positive respiratory samples. Genprobe has also been approved for smear negative samples. NASBA and TMA detect rRNA in the samples, which is more abundant than DNA; this also gives added information about the viability of the organism. The inability of polymerase chain reaction (PCR) to differentiate between live and dead organisms renders it inappropriate for monitoring patients while on therapy. Moussa *et. al.*[15] found PCR to be a highly sensitive (95%) and specific (98%) tool for the rapid diagnosis of GUTB. Fontana *et al*[16] performed the MTD on 95 patients, comprising 35 subjects with a high index of suspicion for GUTB and 60 subjects with evidence of non-mycobacterial disease. Assuming culture as the reference standard, the sensitivity, specificity, positive and negative predictive value of MTD were 100%, 91.93%, 86.84% and 100%, respectively.

SEROLOGY

In India, a positive tuberculin test is of not much value in our environment where nearly half the population has been exposed to TB and neonatal BCG is actively promoted. The results of seroassays must not be taken as the sole evidence of presence or absence of tubercular disease. There is no single seroassay having a reasonable amount of sensitivity and specificity.[10]

ANTIMICROBIAL SUSCEPTIBILITY

The Centers for Disease Control (CDC) recommends that all initial isolates from patients should be subjected to drug susceptibility testing, which should be repeated if the patient remains cultures positive after 3 months of receiving adequate therapy or if the patient does not respond to therapy.[17] Conventional susceptibility test methods include the resistance ratio and the proportion method, the results of which are obtained after 3 weeks. The rapid testing systems include the radiometric BACTEC 460 system, MGIT, MB/BacT, 3D system and the ESP culture system. These systems allow the sensitivity results to be available within 15 days. Various molecular techniques for detection of the *kat G* and *rpo B* gene are also used to predict resistance.

HISTOPATHOLOGY

Histopathological examination of the tissue obtained by biopsy may show the typical granuloma formation along with Langhans giant cells (Fig. 1). Diagnostic cystoscopy does not have much role in GUTB and biopsies of the bladder do not reveal tuberculosis in most of the cases unless the disease is far advanced.

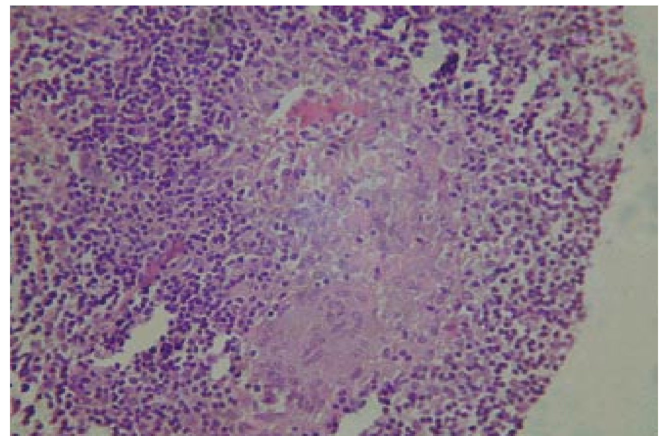

Fig.1. Photomicrograph (100X) of the endometrium showing typical granuloma formation

RADIOGRAPHY

The earliest radiographic findings of GUTB may demonstrate changes in the minor renal calyces with loss of sharpness and blunting.[8] Renal calcification may develop in 7%-14% of patients.

Ultrasound is done in all cases. If ultrasound shows any back pressure effects, then an intravenous urography is done to further define the problem and document the renal function Computed tomography (CT) scan (Fig. 2) is markedly superior in demonstrating parenchymal abnormalities caused by renal infection and delineating the extent of disease.[18] CT Scan also gives the functional status of the affected kidney. Magnetic resonance imaging helps to differentiate macronodular tubercular lesions from other mass lesions.[19] Retrograde pyelography is only necessary to define the ureters and proximal dilatation, if that has not been demonstrated by intravenous pyelography (IVP) and is always done before the procedure of double J stenting. Sometimes a selective sampling of urine is required, and ureteric catheterization can be done. Angiography is useful when focal lesions mimic a primary renal

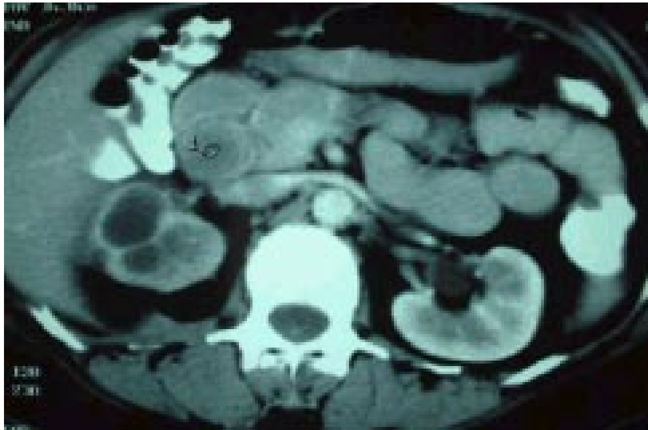

Fig.2. Contrast-enhanced computerized tomography (CECT) scan showing multiple abscesses caused by tuberculosis in the right kidney

mass or when partial nephrectomy is planned. Angiography also shows obliterated interlobar arteries and avascular lesions. Renal nuclear scan findings are nonspecific, but they can be used to assess the function of the kidney and monitor the effects of the therapy.

In a study of 35 patients with proven GUTB from India,[20] 94% had a positive PCR, 37% had a positive urine culture and 91% had abnormal radiographic findings. Bladder biopsies were positive in 46% of patients.

Management

The aims of management are to treat the active disease and preserve the maximal amount of renal tissue. Certain aspects of GUTB make it likely to respond well to anti-TB chemotherapy.[3] Fewer organisms are involved in the renal than in the pulmonary form of the disease. Isoniazid (INH) and rifampicin pass freely into the renal cavities in high concentrations and all these drugs reach adequate concentrations in the kidney, ureter, bladder and prostate. Two regimen plans can be used. The first regimen includes 2 months of daily INH, rifampicin, pyrazinamide and ethambutol (EHRZ), followed by twice a week INH and rifampicin for 4 months. The second regimen uses INH, rifampicin, ethambutol or pyrazinamide daily for 2-3 months followed by INH and rifampicin twice a week for 6-7 months.[21] Rifabutin can be substituted for rifampicin in human immunodeficiency virus (HIV)-positive patients. Follow up is done at 3, 6 and 12 months after finishing the course of chemotherapy. At each review, 3 consecutive early morning specimens of urine are examined.

The use of Corticosteroid is indicated in conjunction with antitubercular therapy to decrease fibrotic changes. Surgery continues to play an important but limited role in the management of GUTB.[22] Nephrectomy is indicated if the kidney is non-functioning, whether or not there is calcification. Reconstructive surgery is done in GUTB for ureteric strictures, most common at the ureterovesical junction, at the PUJ and rarely the middle third of the ureter. Double J stenting is required in cases of ureteric narrowing and it is often possible to avoid open surgery. Augmentation cystoplasty is required for small capacity bladders.

The treatment of female genital TB is EHRZ for 2 months followed by INH and rifampicin for 7 months.[23] The patients are examined monthly and after six months of therapy, endometrial curettage speimems are examined histopathologically and bacteriologically. If negative, the treatment is continued for another 3 months. Surgery is indicated when there is persistence or increase in the pelvic masses after a six-month course of antitubercular therapy.

Our experience with GUTB

One hundred and eighty-two patients (92 men and 90 women; between 3 and 76 years of age) were diagnosed as cases of GUTB on the basis of clinical examination, laboratory and radiological findings at Sir Ganga Ram Hospital. Seventy-eight per cent of the patients were above 31 years of age. The presenting symptoms of these patients are shown in Fig. 3. The other features included suprapubic pain (3%), genital pain (1.6%), bilateral *epididymo-orchitis* (1%), haemospermia (2%), cough (2%), infertility (1%), backache (1%), alternate diarrhoea and constipation (1%), and constipation (1%).

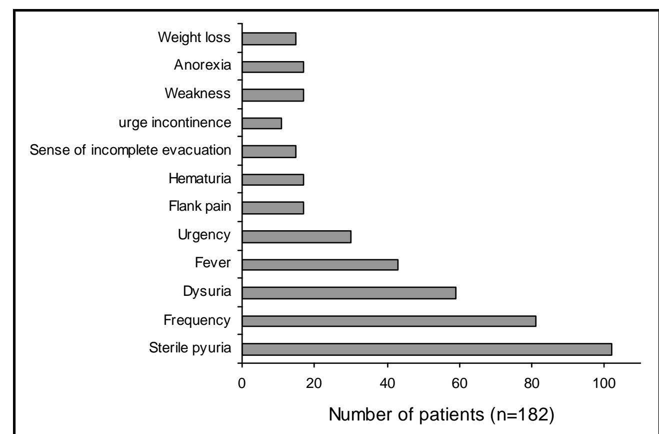

Fig. 3. Common presenting symptoms seen in patients with GUTB at Sir Ganga Ram Hospital.

Direct fluorescence staining was positive in 34 out of 40 patients; AFB culture in 7 out of 145. PCR of the urine for TB was positive in 142 patients out of 150 patients and NASBA was positive in 10 out of 15 patients. However, 'gold standard' by way of culture positivity was not achieved in all 182 cases diagnosed clinically as cases of GUTB. It appears that NAA may sooner or later replace culture as the 'gold standard' in the diagnosis of GUTB. The radiological findings observed in our patients are given in Table 4.

Table 4. Radiological findings of patients at SGRH

Diagnostic modality	Findings
Ultrasound (182)	31 hydronephrotic changes
Intravenous urography (IVU) (31)	14 ureteric narrowing
computed tomography (CT) scan	7 mass lesions
Associated stones	6

Figures in parenthesis indicate number of cases.

PITFALLS IN DIAGNOSIS

There is intermittent shedding of mycobacteria in the urine; hence 3-5 samples are required to be processed immediately after collection. The patient should not have received antibiotics 48 hours prior to sending the sample for culture. In GUTB, the number of organisms present in the urine may be too small to be detected by smear and culture. Some urine samples also have inhibitors which make interpretation of molecular assays difficult.[24] The currently available NAA should be used along with culture methods and each test should be interpreted along with the clinical and radiological findings of the patients, since culture positivity in GUTB remains poor.

All 182 patients were treated with a six-month course of short-term chemotherapy (2EHRZ + 4HR). Nephrectomy was done in 5 cases, double J stenting in 12 and augmentation cystoplasty in 3 cases. Twelve patients had received steroids in addition to ATT and had stenting done. There was recurrence of the disease in 1 patient only and complete clinical recovery in *the* rest of the 181 patients. Two patients did not complete the course of therapy; of these one required nephrectomy at a later date. There appears to be a good correlation between NAA positivity and clinical recovery.

Summary

Patients with long-standing urinary symptoms and sterile pyuria should be investigated for GUTB. The manifestations of GUTB can be variable and cause a variety of clinical patterns. Mycobacterial culture and staining, radiological investigations and molecular assays may all aid in the diagnosis. Short-course chemotherapy with EHRZ is effective. Some patients may need surgical intervention. Genital TB usually presents as infertility in females. GUTB is difficult to diagnose and all cases must be investigated thoroughly. The role of molecular assays cannot be overemphasized; however, these assays cannot be taken as the sole evidence of disease.

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Molecular tools in infectious diseases

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Abstract: Molecular tools have been found to be extremely useful in the diagnosis of infectious diseases. Molecular diagnosis is very important and practical in the case of those infectious agents that are difficult to detect, identify or test for susceptibility with conventional methods. Since the target nucleic acids may be present in a very small amount in the clinical samples, many types of nucleic acid testing techniques for the early detection of infectious agents have been developed, e.g. nucleic acid probes, signal amplification and target/ nucleic acid amplification techniques. Availability of the Food and Drug Administration (FDA) approved commercial test kits have improved the testing conditions. These tests have also been successfully used to detect specific antimicrobial drug resistance genes in many organisms.

Introduction

Worldwide infectious diseases (ID) are a major threat to humankind. The diagnosis of ID is still based on the old diagnostic techniques, i.e. detection of aetiological microorganisms depending upon their phenotypic characteristics, such as morphology and biochemical characterization, etc.[1] These techniques are expensive and time consuming, and may even lose their relevance by the time definite tests are completed. For the monitoring and treatment of ID, especially those caused by fastidious microorganisms such as *Mycobactericum tuberculosis*, *Chlamydia, Listeria, Mycoplasma,* etc. and hard to detect viruses such as human immunodeficiency virus (HIV) and arboviruses, more rapid, accurate and advanced diagnostic tests are required. Automation in diagnostic microbiology has improved the methods of microbial diagnosis but it still has certain limitations, especially in cases of small sample size, low-degree bacteraemia, past infection, drug-resistant strains, patients already on treatment, etc. Immunological assays have played an important role in the diagnosis of ID. However, in case of a window period, these may escape detection before seroconversion, making these assays unreliable substitutes for culture methods and biochemical identification.[2,3] Therefore with the advent of better diagnostic technology, it was, thought that the development of diagnostic techniques based on the genetic make up of aetiological agents would overcome the above lacunae in the field of microbial diagnosis.

Types of molecular tools

The new molecular techniques are primarily based on the detection of nucleic acids (DNA/RNA) of the target organism. Nucleic acid amplification (NAA) has introduced new avenues for the detection, identification and characterization of pathogenic organisms in the field of clinical microbiology. The three main types of nucleic acid testing techniques are: (i) nucleic acid probes, (ii) signal amplification and (iii) NAA.

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NUCLEIC ACID PROBES

This is the simplest of three techniques. It can distinguish between two or more species, determine a particular strain within a given species, or identify differences between genes.[2] It is helpful in the early diagnosis and management of tuberculosis. DNA probes may have an advantage, especially for the detection of certain viruses, e.g. human papillomavirus (HPV), hepatitis B virus (HBV), Epstein-Barr virus (EBV), etc. Hybridization techniques employing nucleic acid probes help in quick diagnosis and early management of infections, especially those caused by organisms with fastidious growth requirements or difficult to culture, e.g. *Mycobacteria, Legionella, Mycoplasma, Borrelia burgdorferi,* HIV and other sexually transmitted diseases. Nucleic acid probes have also been tested successfully in malaria. A specific probe can detect as little as 10 pg of purified *DNA of Plasmodium falciparum* or 1 ng of DNA of *P. falciparum* in blood.[4] Detection of DNA probes for fungal infections, e.g. *Histoplasma capsulatum, Blastomyces dermatidis*, *Cryptococcus neoformans* along with culture confirmation has many advantages over conventional means of identification.[5] Almost all known organisms can be detected using this technique.

SIGNAL AMPLIFICATION TECHNIQUES

There are mainly two types of such kits available commercially.

(1) Branched DNA (bDNA): The bDNA probe technique designed by Chiron Corporation is the commonly used signal amplification method.[6] Several studies have shown its sensitivity to be in the range of 10^3 to 10^5 DNA molecules. bDNA assays have been developed for HIV-1, hepatitis C virus (HCV) and cytomegalovirus (CMV) for which specific

Table 1. Various amplification techniques

Amplification Method	Nucleic acid target	Detection Method	Commercial Source	Different kits available for
Polymerase chain reaction (PCR)	DNA and RNA	Enzyme immunoassay (EIA)	Roche Diagnostics	HIV-1(monitor*),HIV-1(amplicor) HCV*, *M. tuberculosis*, *C. trachomatis**, *N. gonorrhoeae*, CMV, HTLV-1/2, etc.
Nucleic acid sequence based amplification (NASBA) assay	RNA	Electrochemilum-inescence (ECL)	BioMerieux, formerly Organon Teknika	HIV-1, HIV-1QT, CMV pp67, basic kits for: *M. tuberculosis*, *Toxoplasma,* rubella, dengue fever, malaria, etc. (interactive website)
Transcription-mediated amplification (TMA)	RNA and DNA	Hybridization protection assay (HPA-ECL)	Gene-Probe Incorporate	*M.tuberculosis**, *C. trachomatis**
Ligase chain reaction (LCR)	DNA	EIA	Abbot Laboratories	*C. trachomatis**, *N. gonorrhoeae**
Strand displacement amplification (SDA)	DNA		Chiron Corporation	*C. trachomatis*, *N. gonorrhoeae*
Branched DNA (bDNA)	DNA and RNA	EIA	Becton Dickinson and Company	HIV-1, HCV, hepatitis B

PCR: Polymerase chain reaction; HIV-1: Human immunodeficiency virus type 1; HCV: Hepatitis C Virus; CMV: cytomegalovirus; HTLV-I/II: Human T-lymphocyte virus I or II. *FDA approved

antiviral therapies are available.[2] Thus, bDNA provides quantitative detection over a range of several orders of magnitude. It is also useful for monitoring therapeutic response to α-interferon (hepatitis B and C), azidothymidine (HIV) and ganciclovir (CMV).

(b) Digene-hybrid capture system: This is another assay based on signal amplification, which uses a solution hybridization antibody capture assay and chemiluminescent detection.[6] Here the target DNA hybridizes with an RNA probe. This system is useful for the detection of HPV infection in samples such as cervical biopsy and cervical swabs. The disadvantage of this method is that it is comparatively less sensitive than amplification methods and has a detection limit of $5x10^2$ to $2x10^5$ nucleic acid target molecules.

NUCLEIC ACID AMPLIFICATION METHODS

These are *in vitro* methods of enzymatic amplification of a target molecule (DNA) to levels at which it can be readily detected. These systems are capable of simultaneously identifying a pathogen and providing a replica of the target sequence which can be further characterized. There are many types of NAA techniques, e.g polymerase chain reaction (PCR), transcription, based amplification, ligase chain reaction, strand displacement amplification, etc.

Polymerase chain reaction :

PCR is an *in vitro* method for replicating a target DNA sequence, so that its amount is increased exponentially. PCR can amplify single DNA copy to a million copies within a few hours. It can be used to selectively target sequences that are present in low abundance in a background of genomic DNA. This feature makes it potentially useful for the diagnosis of pathogens present in small numbers. PCR is a widely used technique because of its simplicity and flexibility. One of the best examples is the detection of proviral sequences of HIV type 1 (HIV–1) with a low prevalence in human mononuclear cells for virus specific sequence.

Several comparative studies of the PCR and conventional culture detection of HIV-1 in the peripheral blood of infected individuals have confirmed the sensitivity of nucleic acid-based techniques. At the same time, PCR is faster, less expensive and potentially less hazardous than culture. In the past few years, the availability of PCR reagents in kit form has enabled the synthesis of oligonucleotides at a low cost and the tremendous increase in availability of nucleotide sequence data have increased the application of PCR technology in the diagnosis of ID.

One of the most interesting applications of PCR has been the analysis of archived paraffin embedded tissue specimens[7] and mucous specimens. Roche Diagnostics System Incorporated (USA) has developed PCR-based kits for the diagnosis of various infectious diseases (Table 1). In addition to this, many other DNA amplification-based techniques have been developed and applied for the detection of microbial infection, identification of clinical isolates and strain subtyping.[1] These tests are valuable for identifying cultured and non-cultivable organisms. PCR is also designed for rapid identification and determination of species/sub-species of *M. tuberculosis*.

In addition to the amplification of a target DNA sequence by a target PCR procedure, several specialized types of PCR have been developed for specific applications. Some of them are relevant to diagnostics (Table 2). Multiplex PCR has been shown to be a valuable method for the identification of various viruses such as HIV-1 and-2, human T lymphotropic virus-1 and -2 (HTLV), Herpes simplex virus (HSV)-1 and -2 , CMV, EBV, etc. and many bacteria, e.g. *Neisseria gonorrhoeae, C. trachomatis, Haemophilus influenzae, Streptococcus pneumoniae, Mycoplasma* spp., *Campylobacter coli, Ureaplasma, etc.* and parasites such as *Giardia lamblia, Cryptosporidium parvum, Leishmania* spp. etc.[8]

Real-time PCR :

It is capable of screening genetic activity within hours, using a minimal amount of sample material and can detect a single molecule of DNA or RNA.[9] Another advantage of real-time

Table 2 . Types of polymerase chain reaction (PCR) methods available and their applications

Amplification method	No. Primers	Target	Applications
PCR	Two	DNA	HIV-1, HCV, CMV, *C.trachomatis*, *Neisseria gonorrhoeae*, HTLV-I/II, enterovirus, *Mycobacterium tuberculosis*
Nested PCR	One	DNA	Designed mainly to increase the sensitivity of PCR tests
Reverse transcriptase (RT) PCR	Two or more (multiple)	RNA	RNA viruses, detection of viable *Mycobacterium* , monitoring antimicrobial therapy
Multiplex PCR	One	DNA/RNA	Detects more than one organism in a single specimen. More helpful in human & cancer genetics
Arbitrarily primed PCR	One	DNA	To differentiate strains of various species, and subtypes in a serotype, mainly for epidemiological work
Broad-range PCR	One	DNA/RNA	Rapid bacterial identification
Expression cassette PCR	One	DNA	Used for the generation of proteins with N and/or C terminal
Quantitative PCR	One	DNA/RNA	Used in HCV, HIV-1, CMV, etc. monitor kits for viral quantification
Membrane-bound PCR	One	DNA	Useful in the cases where there is a small amount of DNA
PCR		DNA/RNA	Improves end point analysis in PCR assays and also prevents contamination due to closed tube detection

HIV-1: Human immunodeficiency virus type 1; HCV: hepatitis C virus; CMV: cytomegalovirus; HTLV-I/II: human T-lymphotropic virus I or II.

PCR is that the PCR tubes do not need to be opened after the amplification reaction is complete. This prevents contamination by PCR products and reduces false-positive results.

Transcription-based amplification

It is a non-PCR target amplification system based on amplification by *in vitro* transcription. There are mainly two types of transcription-based amplification tests; NASBA (nucleic acid sequence-based amplification) assay and TMA (transcription-mediated amplification).

(i) Nucleic acid sequence-based amplification assay

Nucleic acid sequence-based amplification (NASBA) assay amplifies RNA in a DNA background.[10] The NASBA (bioMerieux) system called NucliSens is used for measuring the load of CMV and HIV besides approximately 72 other applications, both viral and bacterial. In our laboratory we use NASBA for the detection of CMV (pp67, mRNA) and have found it very useful for the diagnosis of active CMV disease, especially in renal transplant cases[11]. This assay detects messenger RNAs coding for matrix tegument protein pp67 of CMV that is only expressed during viral replication. The detection of CMV pp67 mRNA indicates whether the patient is suffering from an active infection or not. In tuberculosis, the problem of detecting non-viable organisms by PCR can be overcome by detecting mRNA instead of DNA. NASBA is also helpful in assessing bacterial viability by detecting RNA. It is very helpful in differentiating between active and past infections. Basic kits of NASBA are also available for tuberculosis, toxoplasmosis, malaria, dengue, enterovirus, etc. (Table 1). We use it for tuberculosis, HCV, malaria and dengue. The NASBA kit developed for quantitative testing of HIV-1 has shown promising results. A completely automated system called NucliSens is available as a work station for handling bulk samples. It does all the steps of NASBA in a single tube with its robotic action and completes the assay in 3 hours.

(ii) Transcription-mediated amplification

A transcription-mediated amplification (TMA)-based assay has been introduced in the market as Gene-Probe (BioMerieux) for the detection of *M. tuberculosis* in smear-positive as well as negative sputum specimens and also for *C. trachomatis* infection. A TMA assay for the quantification of HIV-1 RNA was recently shown to be more sensitive than RT-PCR or bDNA.[12] This is the only assay that is approved by the US Food and Drug Administration (FDA) for direct detection of *M. tuberculosis* from both smear-positive as well as-negative sputum samples.

Ligase chain reaction

Ligase chain reaction (LCR) is a probe-based amplification system**.** Several studies of FDA-approved LCR diagnostic tests for *C. trachomatis* infection have shown comparable clinical sensitivity to PCR.[13] A combination LCR kit for the detection of both *C. trachomatis* and *N. gonorrhoeae* has been introduced by Abbot Laboratories. Although convenient and readily automated, one potential drawback of LCR is the difficult inactivation of post-amplification products.

Strand displacement amplification

A semi-automated system (Probetech ET, Becton Dickinson) to carry out strand displacement amplification (SDA) with real-time fluorescence detection was introduced for the detection of *C. trachomatis and N. gonorrhoeae.* This system has been shown to be of equivalent sensitivity and specificity to LCR.[14]

APPLICATIONS OF MOLECULAR TOOLS

Molecular techniques have revolutionized the epidemiological investigations in ID. Besides this, the application of these techniques in the clinical microbiology laboratory are: - (i) prompt and accurate diagnosis of microorganisms, (ii) identification of unusual bacteria, (iii) disease monitoring through organism quantification, (iv) detection of antimicrobial drug resistance genes in many organisms e.g.

mec A resistance gene in methicillin-resistant staphylococci, (v) detection of epidemics, (vi) subtyping of microorganisms, etc. The most important application of 16S rRNA sequencing is the identification of previously unrecognized and new species and their molecular epidemiology.[13,15]

Future prospects

Introduction of automation in these tests can overcome most of the problems associated with the first-generation assays. In particular, automating the sample preparation only can eliminate most of the contamination problems. Gene-Probe Incorporate has developed a fully automated instrument for the detection of NAA called Tigiris. These second-generation instruments are fully automated. They can process 500 tests in 8 hours and the VITROS system developed by Johnson and Johnson can detect an infectious agent in less than 2 hours. A triplex TMA assay for screening blood for simultaneous detection of HIV-1, HCV and HBV currently under trial in the USA, has been found useful in identifying pre-seroconversion infectious blood units. Despite their cost-effectiveness, these tests are expected to become the standard techniques in transfusion medicine. The introduction of microelectrochemical system-based microfluids (lab-on-a-chip) for DNA analysis is also very promising for the simultaneous identification and genotypic determination of drug-resistant microorganisms. Scientists are trying to make a universal drug resistance chip that will contain oligonucleotide arrays specific for all the known drug resistance genes on a single chip. DNA nanotechnology, molecular beacons, invasive cleavage assays, rolling circular amplification, peptide nucleic acid technology, data bank, online applications and ordering facilities such as Eurogenetics Clinical Investigation, Life Technologies, etc. are some of the upcoming technologies, which will prove to be some of the milestones in the development of DNA diagnostics. What is required at the moment is the availability of universal standards & methods for quality control (QC) of NAA. Until QC for various NAA tests is in place it may not be easy to interpret or extrapolate the results of various laboratories conducting these assays.

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Images in Medicine
Lichen Serufulos orum : A tuberculoid

KAMLENDER SINGH

Department of Dermatology, Sir Ganga Ram Hosptial, New Delhi

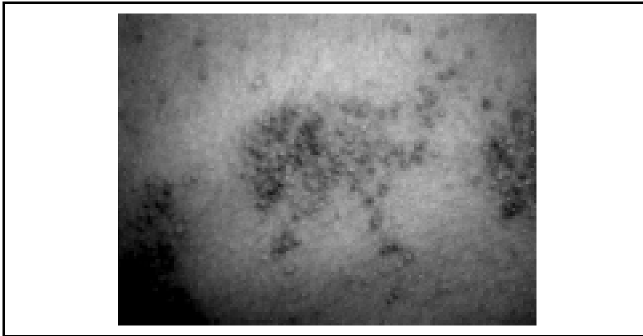

Fig.1. Flat-topped papules seen all over the body, mainly the chest, back and abdomen

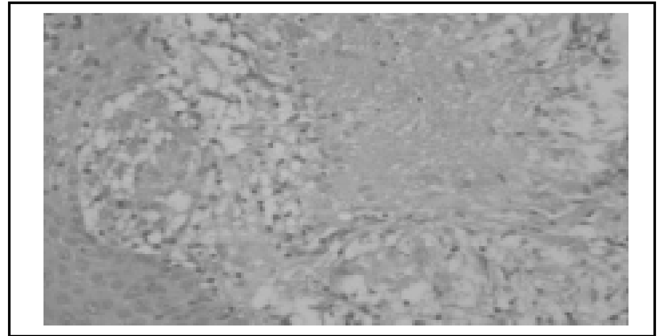

Fig.3. Epithelioid cells, lymphocytes and stray Langhan's giant cells on H&E staining (400X).

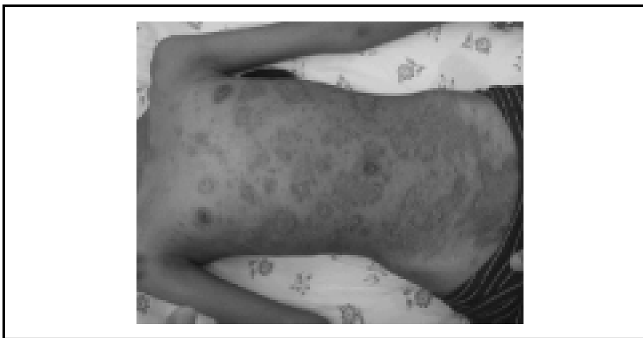

Fig. 2. Papules with central clearing

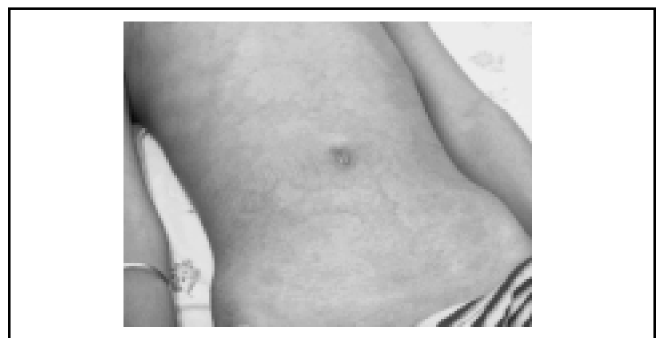

Fig. 4. Lesions regressed within two cycles of therapy.

A 13-year old, thin girl had numerous 2-3 mm sized, pale or dusky red, firm, flat-topped papules, mainly follicular and some-non follicular, mostly grouped, as well as discrete and confluent. Some papules had horny spines or fine scales (Fig. 1). Plaques formed from coalescence were 0.5-4 cm in size, discoid to bizarre in shape, dry, rough, scaly and dusky red in colour. There was some apparent clearing in the central region (Fig. 2). Lesions were present mainly over the abdomen, chest and back. Few, smaller lesions were scattered over the limbs. The lesions started over the left pelvic region about 2 months earlier. Occasionaly, she had itching. There was nothing relevant on systemic examination and no lymphadenopathy. However, the patient had fever for about a month which was of low grade, occurred usually at night accompanied with a slight chill. There was no cough with or without expectoration, but there was loss of appetite and weight.

The Mantoux test showed a zone of induration measuring 12 mm. Histologically, granulomas were found in the superficial dermis abutting and infiltrating the epidermis, and in mid dermis where some were periappendageal. These were composed of epithelioid cells, lymphocytes and stray Langhans giant cells. Caseation necrosis was seen in an occasional granuloma (Fig. 3).

During the initial phase of treatment with multivitamins, cetrizine and local steroid cream, the rash continued to spread rapidly. Once the diagnosis of lichen scrofulosorum was established, rifampicin 250 mg, isoniazid 75 mg, ethambutol 600 mg, pyrazinamide 500 mg and multivitamins were given daily orally, while coconut oil as an emollient was applied twice a day. In less than 2 weeks, skin lesions regressed considerably. Most of the papules reduced to half the original size, became pale and smooth without any scales (Fig. 4). At the end of 6 weeks of treatment, the fever had settled, appetite was almost back to normal and there were a few 1-2 mm sized pale, smooth, flat papules on the skin, which were scattered sparsely over the trunk and limbs.

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JIMSA 2004: 17(3)

LETTER TO THE EDITOR

To,

The Editor
International Medicine Science Academy
National Medical Library Building,
Ansari Nagar, Ring Road, New Delhi-29

Sir,

I congratulate Dr. K. Jagadeesan President, IMSA, Editor Dr. P.D. Gulati and Guest Editor Dr. Syed Amin Tabish for focusing on the concept of 'Health of the Nation' in the special issue of The Journal of International Medical Sciences Academy (JIMSA) which I feel is one of the most prestigious magazines of this globe. In Gujarat too, rightly so, Shri N. Modi, the honourable Chief Minister, based on similar concept of globalization of medicine and promotion of medical tourism, had recently inaugurated an exhibition on 'The National Heritage Expo 2004,' where he has genuinely encouraged this new chapter of medical tourism.

The under signed-a fellow of IMSA has also launched his 'Global Concept of PUC (Pollution Under Check) for mankind, its early detection and management and detoxification by the scientific, ethical and integrated approach by integrating Internal Medicine to Environmental and Nutritional Medicine the lesser known, newer and more dynamic of sciences which can globally cut down pollution effects on mankind as a whole; this is the call of the day with increasing pollution and deteriorating public health standards, especially in the metropolis towns all over the globe.

The author has incorporated his 'Global Nutritional and Environmental Medicine' programmes in the recently conducted National institutional Week in the erstwhile princely estate of Balasinor in Khede district and has planned to extend it over the years 2004-2005-2006 during which he plans to educate thousands of patients, public and doctors, para medicos in Delhi, Gujarat under the banner name of 'The environ ®' a registered global concept of Pollution Effects and Management'.

I have been seeing your site *www.jimsaonline.com* and congratulate you for the same.

Sd/-

(Dr. B.J. Singh)
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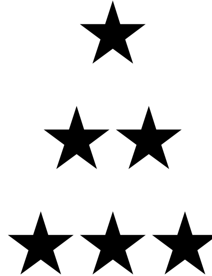

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